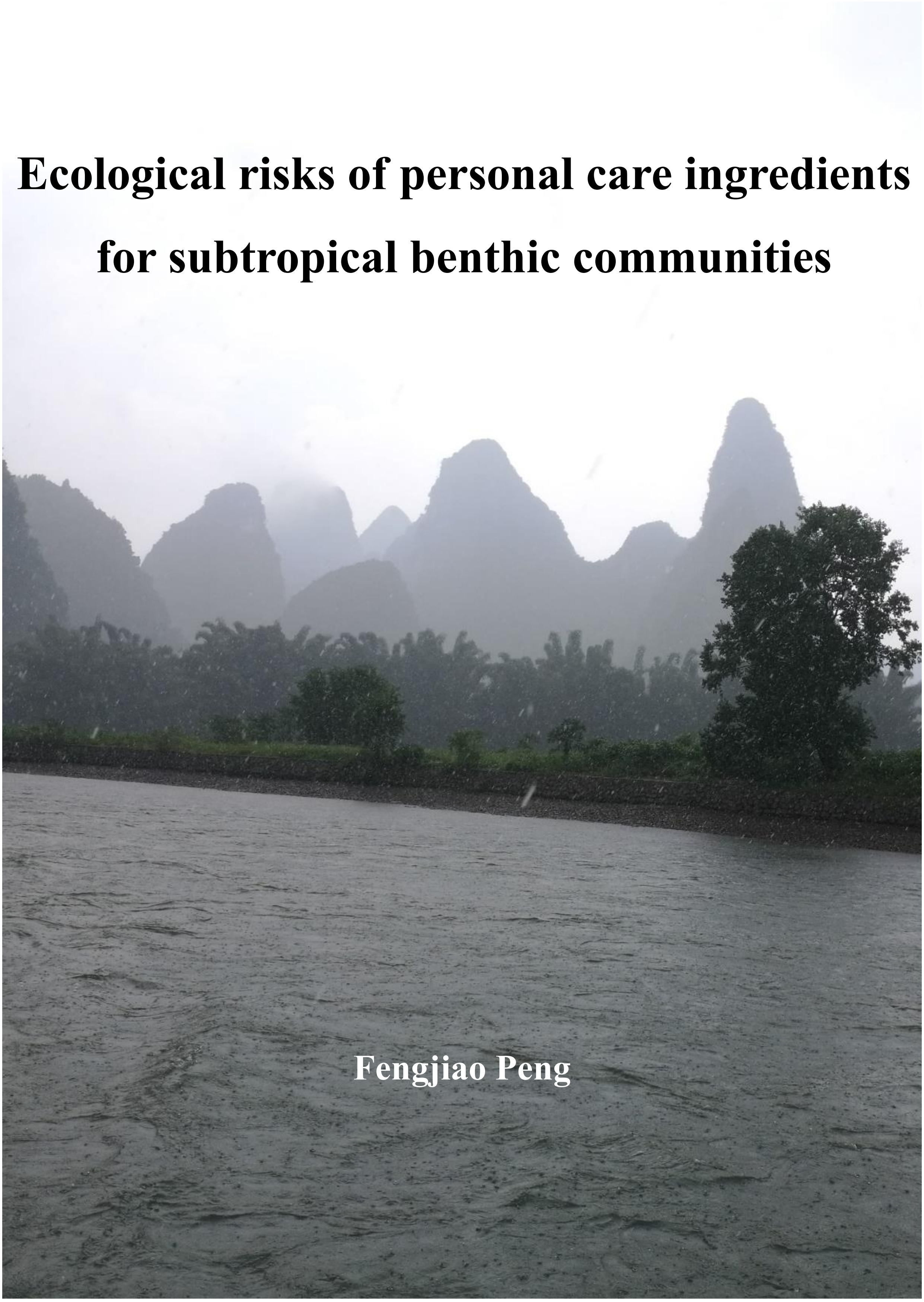
Ecological risks of personal care ingredients for subtropical benthic communities



Fengjiao Peng

Proposition

1. Benthic macroinvertebrates should be incorporated when assessing the sediment toxicity of chemicals to benthic microbial community.

[this thesis]

2. Bioaccumulation in benthic macroinvertebrates can be an important fate pathway for hydrophobic chemicals in the environment.

[this thesis]

3. Understanding the (eco)toxicity and persistence of biotransformation products from emerging organic compounds is important for environment risk assessment.

4. The careless attitude towards environmental pollution may benefit the economic development in developing countries, but will harm the quality of life of people in the long run.

5. In megacities in China, it is required to improve the ecosystem quality in the urban rivers.

6. English as the universal language in academic research benefits western researchers.

7. Research interest is produced all along the PhD period rather than only at the beginning of your PhD.

Propositions belonging to the thesis entitled:

“Ecological risks of personal care ingredients for subtropical benthic communities”

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Wageningen, 27 August 2018

Ecological risks of personal care ingredients for subtropical benthic communities

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Ecological risks of personal care ingredients for subtropical benthic communities

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Thesis
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Contents

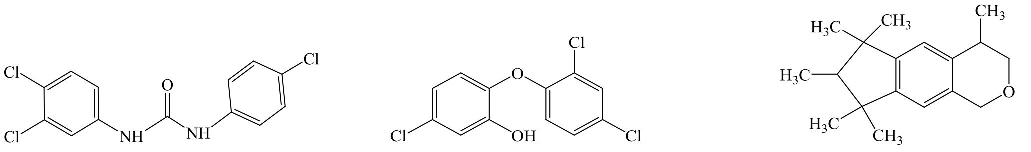
Chapter 1 General introduction

1.1 Personal care products

Personal care products (PCPs) are a group of organic chemicals contained in different products widely used in human daily life (e.g., soaps, detergents, perfumes, toothpaste, shampoo, sunscreens) and are generally directed at altering odor, appearance, touch, or taste, yet do not display significant biochemical activity (Daughton and Ternes 1999; Díaz-Cruz and Barceló, 2015). PCPs comprise diverse groups of organic compounds, namely, antimicrobial agents (e.g., triclosan and triclocarban), synthetic musks (i.e., polycyclic musks and nitro musks), insect repellants (e.g., N,N-diethyl-m-toluamide), preservatives (e.g., Parabens), and sunscreen UV filters (e.g., benzophenone-3 and 4-methyl-benzilidine-camphor) (Daughton and Ternes, 1999; Liu and Wong, 2013). After use, PCPs and their metabolites are washed down the drain and discharged into wastewater treatment plants (WWTPs) or directly released into aquatic environments (Ying and Kookana, 2007; Chalew and Halden, 2009). However, WWTPs are not always capable of removing micropollutants such as PCPs. Due to the extensive usage, continuous release and incomplete removal in WWTPs, PCPs have become ubiquitous contaminants in the aquatic environment throughout the world (Díaz-Cruz and Barceló, 2015; Homem et al., 2015; Wang, et al., 2016). Furthermore, PCPs have received growing attention in recent years as emerging contaminants for their possible threats to aquatic ecosystems and human health (Liu and Wong, 2013).

1.2 Target compounds in this study

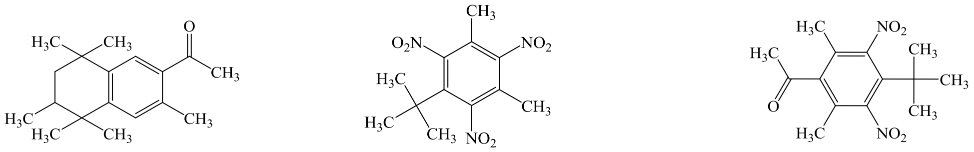
Based on the hydrophobicity, source, usage, detection frequency and concentration level in the aquatic environment, we selected 2 classes of PCPs as target compounds, namely, two antimicrobial agents (triclosan (TCS) and triclocarban (TCC)) and four synthetic musks (galaxolide (HHCB), toxalide (AHTN), musk xylene (MX) and musk ketone (MK)), to investigate their occurrence and ecological risks in urban rivers of Guangzhou (South China). Their chemical structures are given in Figure 1.



Triclocarban (TCC)

Triclosan (TCS)

Galaxolide (HHCB)



Tonalide (AHTN)

Musk xylene (MX)

Musk ketone (MK)

Figure 1. Chemical structures of the target compounds.

1.3 Environmental fate and behavior of PCPs

PCPs in the environment originate mainly from domestic wastewater discharging. Possible sources are comprised of 1) PCPs used in human daily activities (e.g., bathing, swimming, disinfection and makeup) enter WWTPs with the domestic wastewater or are directly released into natural waters; 2) residual PCPs in WWTPs enter farmland with sludge application or sewage irrigation, and afterwards enter natural waters via terrestrial runoff; and 3) wastewater leaching from the WWTPs and drainage pipes enter the groundwater or rivers with runoff.

Fig. 2 describes the origins and fate of PCPs in the environment. After entering groundwater and rivers, hydrophobic PCPs mainly end up in the sediment. Consequently, the sediments may accumulate hydrophobic organic contaminants and further serve as a reservoir of contaminants for bioaccumulation and trophic transfer, which might cause significant contamination to the overlying water and loss of desirable species (Burton, 2002).

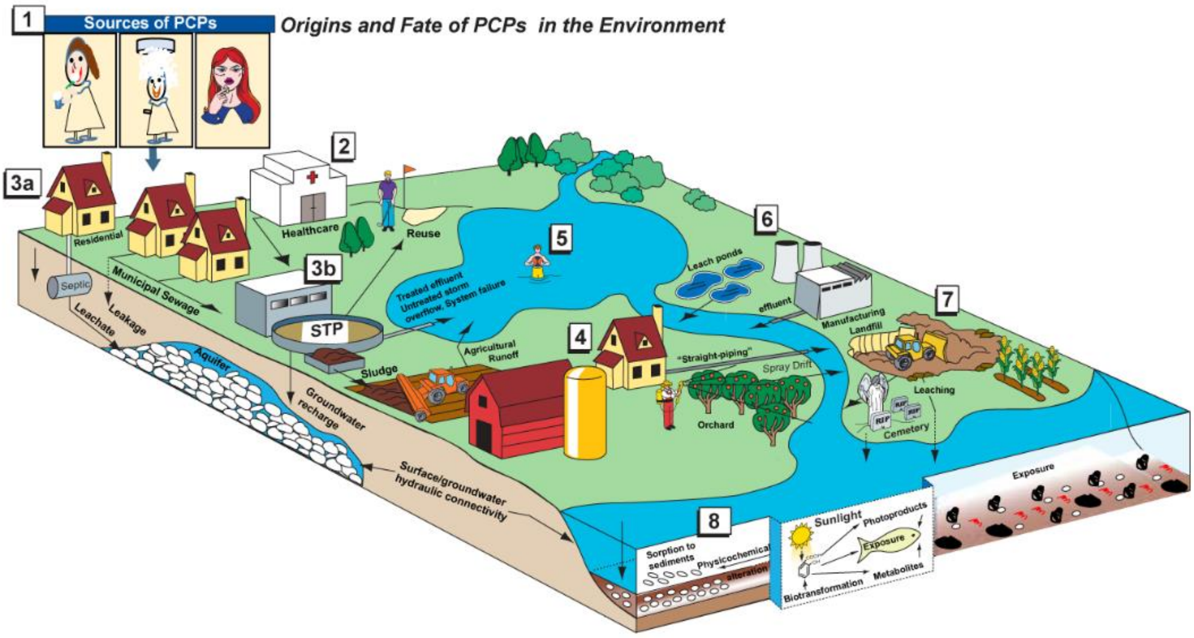


Figure 2. Origins and fate of personal care products in the environment. This graph is re-edited according to USEPA, the original graph can be downloaded from http://www.epa.gov/ppcp/pdf/drawing.pdf.

PCPs, trace environmental contaminants in waters, sediments, and sewage sludge, are unregulated in China and many other countries. Generally, sorption, biodegradation, chemical transformation and volatilization are their possible removal mechanisms in WWTPs (Lozano et al., 2013; Homem et al., 2015; Healy et al., 2017). As the target compounds in the present study have high octanol-water partition coefficients (log $K_{OW} > 4.5$) (USEPA, 2012) and low water solubility, they are expected to accumulate in the sludge.

Indeed, TCC and TCS are mainly removed from WWTPs through sorption and settling of solids (Heidler et al., 2006; Heidler andHalden, 2007; Lozano et al., 2013). The removal efficiencies ranged from 46-93% and > 97% for TCS and TCC, respectively (Bendz et al., 2005; Heidler et al., 2006; Nakada et al., 2006; Lozano et al., 2013). Unlike TCC, TCS can be biodegraded under aerobic conditions in WWTPs, with methyl-triclosan (Me-TCS) as the main biodegradation product (Lozano et al., 2013). However, Me-TCS is more hydrophobic and more persistent than TCS (Lozano et al., 2012). Recently, TCC and TCS have been reported in the sludge of a WWTP in the US with levels of 13.1±0.9 and 20.3±0.9 µg/g dw, respectively (Lozano et al., 2013). In WWTPs effluents, the concentrations of TCS were in the range of < limit of qualification (LOQ) to a few µg/L (e.g., McAvoy et al., 2002; Reiss et al., 2002; Loraine and Pettigrove, 2006; Ying et al., 2007; Trenholm et al., 2008; González-Mariño et al., 2009; Lozano et al., 2013), whereas TCC were in the range of < LOQ to 0.2 µg/L (e.g., Weigel et al., 2004; Halden and Paull, 2005; Trenholm et al., 2008; González-Mariño et al., 2009; Wick et al., 2010; Yu et al., 2011). Due to their incomplete removal in WWTPs, the residual PCPs end up in the receiving rivers. Indeed, TCS and TCC have been frequently detected in various environmental compartments, such as surface water, sediment, aquatic organisms, soil, indoor dust, and even humans (Coogan and Point, 2008; Cha et al., 2009; Higgins et al., 2011; Bedoux et al., 2012; Hartmann et al., 2016; Yin et al., 2016; Peng et al., 2017). Currently, the maximum reported concentrations for TCS were 5.16 µg/L and 1.33 µg/g dw in the surface water and sediment, respectively (Ramaswamy et al., 2011; Zhao et al., 2010a). The maximum reported concentrations for TCC were 6.75 µg/L and 2.63 µg/g dw in the surface water and sediment, respectively (Halden and Paull, 2005; Zhao et al., 2010a). Furthermore, TCS and TCC are among top 10 most commonly detected organic wastewater compounds for frequency and concentration levels (Kolpin et al., 2002; Halden and Paull, 2005; Brausch et al., 2011).

Synthetic musks is a group of compounds with a chemical structure that is not readily biodegradable (Gatermann et al., 1998; Osemwengie and Steinberg, 2001), and their removal in WWTPs occurs mainly through sorption onto sludge particles (Simonich, 2005). Thus, WWTPs have been regarded as the largest contributing source of environmental contamination for musks (García-Jares et al., 2002; Homem et al., 2015). The overall removal efficiencies were in the range of 36-63%, 34-76%, > 60% and about 99% for HHCB, AHTN, MK and MX, respectively (Homem et al., 2015). HHCB and AHTN were frequently detected in WWTPs (>98% samples) with concentration ranging from 0.011-33.54 µg/L and 0.003-703.68 µg/g dw in effluents and sludge, respectively (e.g., Reiner et al., 2007; Chen et al., 2007; Gomez et al., 2005; Guo et al., 2010; Liu et al., 2014). Although nitro musks have been phased out from the market and their use is prohibited in Europe (EU, 2009), MX and MK were still detected in the last decade with concentrations in the range of 0-0.67 µg/L in effluents and 0-2.5 µg/g dw in sludge of WWTPs from many countries, such as China (Liu et al., 2014), Spain (Vallecillos et al., 2014) and USA (Chase et al., 2012). Similar to antimicrobial agents, polycyclic musks (e.g., HHCB and AHTN) have been detected in air (Peck and Hornbuckle, 2004; McDonough et al., 2016), surface waters (e.g., Reiner and Kannan, 2011; Peng et al., 2017), groundwater (Osenbrück et al., 2007), sediments (Reiner and Kannan, 2011; Peng et al., 2017), wildlife (Nakata et al., 2007; Reiner and Kannan, 2011; Subedi et al., 2012; Lange et al., 2015), human adipose fat (Kannan et al., 2005) and human breast milk (Kang et al., 2010; Yin et al., 2012). For example, HHCB and AHTN were found in human adipose fat (New York City, USA) at concentrations ranging from 0.012-0.798 µg/g lipid weight (lw) and <0.005-0.134 µg/g lw, respectively (Kannan et al., 2005). Due to the lipophilic property (log Kow = 5.9 and 5.7 for HHCB and AHTN, respectively) (Balk and Ford, 1999a), HHCB and AHTN have been shown to accumulate in fish tissue collected from surface waters receiving effluent discharges in Germany, with a maximum concentration of 12 and 0.45 µg/g lw, respectively (Subedi et al., 2012).

1.4 Aquatic toxicity of selected PCPs

The aquatic toxicity data of antimicrobial agents and synthetic musks are given in Table 1. The toxicity of TCS and TCC in aqueous solutions towards a variety of organisms including microorganisms, algae, crustacean, fish, plants and benthic organisms have been well studied, even at different climate regions (e.g., Chalew and Halden, 2009; USEPA, 2009; Bedoux et al., 2012; Tamura et al., 2013; Khatikarn et al., 2016). Overall, algae were found to be the most sensitive species (Orvos et al., 2002; Chalew and Halden, 2009), followed by crustacean (Chalew and Halden, 2009; Zhang et al., 2016a), fish (Bedoux et al., 2012; Tamura et al., 2013), microorganisms (Chalew and Halden, 2009) and benthic organisms (Khatikarn et al., 2016). Furthermore, there was no significant difference in TCS toxicity between temperate and tropical species (Khatikarn et al., 2016). However, all these toxicity tests were based on single species and aqueous phase, we thus still do not know how these chemicals impact diverse communities in a setting that represents the environment where exposure is expected, i.e. the sediment phase.

Compared to TCS and TCC, synthetic musks showed much lower toxicity to aquatic organisms. For example, in 72-h studies with green algae (*Pseudokirchneriella subcapitata*), the no observed effect concentrations (NOEC) were 374 µg/L for AHTN and 201 µg/L for HHCB (Balk and Ford, 1999b). However, a recent study reported that HHCB and AHTN could induce lipid peroxidation, protein carbonylation and primary genetic damage in the zebra mussel at environmentally relevant concentrations (Parolini et al., 2015). Given their current high usage volumes, there are concerns regarding environmental exposure and toxic effects of polycyclic musks (Sun et al., 2014).

1.5 Benthic macroinvertebrates and microbial community

Sediment serves as a sink for a battery of hydrophobic contaminants in aquatic environment, which may deteriorate sediment quality especially in rivers draining through large cities. However, neither sediment quality guideline nor information on sediment toxicity data of hydrophobic PCPs was available, we therfore do not know if sediment-associated hydrophobic PCPs would affect aquatic organisms at the reported maximum environmental concentration, especially sediment associated organisms, such as benthic invertebrates and microbes.

Naididae (Oligochaeta) and larval Chironomidae (Insecta) are widely distributed sediment-dwellers that are often abundant in contaminated sediments due to their tolerance to a range of pollutants (Rae, 1989; Carew et al., 2007; Vivien et al., 2015). As such, these organisms are commonly used as biological indicators of water quality (Milbrink, 1973; Winner, 1980). They live in close contact with the sediment, ingesting sediment particles and egesting them on the sediment surface in the form of pseudofeces (Mum et al., 1983; Kaster et al., 1984; Widenfalk et al., 2008; Dafoe et al., 2011). Since hydrophobic PCPs will strongly bind to the benthic and suspended sediment (USEPA, 2014), sediment-dwellers may suffer more stress from sediment-associated PCPs relative to pelagic organisms. As sediment-dwellers can absorb hydrophobic contaminants through feeding on pore water, overlying water and sediment particles (Selck et al. 2012; Widenfalk et al., 2008; Diepens et al. 2014), these contaminants may thus go through accumulation and degradation in their bodies. Also, the bioturbation caused by sediment-dwellers at the water-sediment interface may accelerate the microbial degradation of sediment-associated contaminants (Madsen et al., 1997; Kristensen and Holmer, 2001).Therefore, sediment-dwellers may significantly influence the fate of sediment-associated PCPs in the environment. However, little is known about the interactions between sediment-dwellers and sediment-associated PCPs in more realistic systems.

Indigenous microorganisms in the sediments can play a vital role in the transformation and mineralization of inorganic and organic contaminants in wastewater discharges such as nitrogen, organic carbon, and trace emerging organic contaminants (Fredrickson et al., 1989; Li et al., 2016). In turn, nutrients and contamination could significantly influence the composition of microbial community (Staley et al., 2014; Ibekwe et al., 2016). Furthermore, bioturbation activities of benthic macroinvertebrates can influence the biogeochemical properties (e.g., redox potential), biogeochemical processes including nitrification, denitrification and sulfate reduction, and microbial community composition in sediments (Mermillod-Blondin et al., 2004; Nogaro et al., 2014; Zeng et al., 2014). Given the importance of aquatic sediments in the aquatic ecosystem (Wall, 2004), it is imperative to understand the interactions between hydrophobic contaminants such as PCPs, benthic macroinvertebrates community and microbial community for establishment of sediment quality criteria, fate and sediment risk assessment. However, such knowledge is currently limited.

1.6 Study area

Based on the large consumption of PCPs and current contamination state in Guangzhou (South China) (Peng et al., 2008; Zhao et al., 2010a; 2013), it was chosen as a case study to investigate fate and effects of personal care ingredients in subtropical sediments by considering the importance of sediment-dwellers in PCPs degradation. Guangzhou is a highly urbanized megacity in southern China with a population of 13.1 million (http://www.guangzhou.gov.cn/). Due to rapid economic development and urbanization, urban drainage pipe network is well developed in Chinese megacities, such as Guangzhou (http://www.gzepb.gov.cn/). Currently, 10204 kilometers of municipal drainage pipe network has been installed, and 48 WWTPs have been installed and is in use in Guangzhou. The treated domestic sewage amount reached 4.99 million tons/day. 93% and 48% of the produced domestic sewage in urban areas and rural area enter into the WWTPs, respectively (http://www.gzepb.gov.cn/). Therefore, a small proportion of the produced domestic sewage in urban areas is still directly released into urban rivers (i.e., does not undergo wastewater treatment), which has become a serious environmental problem and has attracted growing concern of the Chinese government (Zhang et al., 2016b).

1.7 Objectives

This thesis comprises several studies that collectively contribute to increase our knowledge on the distribution, fate, and environmental risks posed by personal care ingredients in the subtropical region of China, with an emphasis on the evaluation of the risks posed by triclosan and galaxolide for subtropical benthic communities.

The specific research objectives of this thesis are:

1. To assess the occurrence and distribution of hydrophobic PCPs in subtropical sediments.
2. To assess the ecological risks of personal care ingredients for subtropical biological communities under different exposure scenarios.
3. To examine the fate and effects of personal care ingredients in water-sediment systems with and without the presence of sediment-dwellers.
4. To identify the contributions of sediment-dwellers to the dissipation of sediment-associated PCPs.

1.8 Outline

This thesis starts with a chemical monitoring program of emerging organic chemicals in urban rivers to identify the key hydrophobic PCPs in the subtropical region of China. In **chapter 2**, we use Guangzhou as a case study to investigate the contamination profiles of emerging organic chemicals and assess their ecological risks to aquatic organisms in subtropical urban rivers in China. Specifically, we assess the general water quality in these urban rivers of Guangzhou based on measurements of nutrients and other parameters, and measure concentration levels of the selected PCP compounds: i) in a gradient from, presumed, un-contaminated and low-density populated rural area to, presumed, highly contaminated and high-density populated urban area, ii) between seasons (wet and dry season). In this study, we also compare geographic contaminant level variability by relating our findings to published literature values in China and worldwide. Furthermore, we perform a screening-level ecological risk assessment for surface water and sediment by relating the measured environmental concentrations (MECs) in surface water or estimated concentrations in the sediment pore water with published toxicity data for water exposure.

Chapter 3 reports the results of a biological monitoring program focusing on benthic macroinvertebrate and microbial communities to identify the key benthic macroinvertebrate and microbial taxa indicative for the pollution in urban, subtropical rivers. In this chapter, we use Guangzhou as a case study to investigate the distribution patterns of benthic macroinvertebrates and microbial community in sediments. Besides, we reveal environmental variables (i.e., the selected emerging organic chemicals, nutrients and heavy metals) that may explain the differences in taxon composition among rivers. Furthermore, we also identify traits associated with these differences using a novel multivariate technique.

The next three **Chapters** (**4**, **5** and **6**) focus on the interactions between benthic macroinvertebrates, bacteria and sediment-associated hydrophobic PCPs (i.e., TCS and HHCB) using microcosms mimicking shallow subtropical pond systems exposed to hydrophobic PCPs contamination. The microcosm setup comprises PCPs spiked sediment, un-spiked water, one primary producer (algae) and 4 primary consumers (Daphnia, snails, midges and a benthic worm) collected from Guangzhou. We quantify the fate of sediment-associated PCPs in indoor benthic microcosms with and without addition of a benthic community, and examine the effects of sediment-associated PCPs on organisms' survival and growth and bacterial communities.

Chapter 7 investigates the bioaccumulation and biotransformation of hydrophobic PCPs in a sediment-dweller in water/sediment systems. We quantified the fate of the two most commonly used and detected hydrophobic PCPs, triclosan and galaxolide and examined their biotransformation in the sediment-dweller *Limnodrilus hoffmeisteri*.

In **Chapter 8**, a general discussion and conclusions are provided, which summarizes all important findings of this thesis and place them into a broader perspective on understanding the interactions between PCPs, benthic macroinvertebrates and the microbial community. All research objectives are addressed and discussed, while recommendations are provided for further research that investigates the effects of sediment-associated hydrophobic contaminants on benthic communities.

Table 1. Summary of the aquatic eco-toxicity data of antimicrobials and typical synthetic musks.

Compound	Species	Trophic group	Duration, Effect, Endpoint	Value (µg/L)	Reference
TCC	*Pseudokirchneriella*	Algae	72 h, Growth inhibition, NOEC	< 10	(Yang et al. 2008)
	Pseudokirchneriella	Algae	72 h, Growth inhibition, LOEC	10	(Yang et al. 2008)
	Pseudokirchneriella subcapitata	Algae	72 h, Growth inhibition, NOEC	5.7	(Tamura et al. 2013)
	Ceriodaphnia Dubia	Crustacean	8 d, Reproduction NOEC	1.9	(Tamura et al. 2013)
	Mysidopsis bahia	Crustacean	28 d, Reproduction, NOEC	0.062	(USEPA, 2009)
	Danio rerio	Fish	9 d, Hatching, Survival, NOEC	24	(Tamura et al. 2013)
	Pimephales promelas	Fish	35 d, Mortality, NOEC	5	(USEPA, 2009)
TCS	*Scenedesmus subspicatus*	Algae	96 h, Biomass, NOEC	0.69	(Orvos et al. 2002)
	Pseudokirchneriella	Algae	72 h, Growth inhibition, NOEC	0.2	(Yang et al. 2008)
	Pseudokirchneriella subcapitata	Algae	4 d, Growth, NOEC	8.3	(Harada et al. 2008)
	Scenedesmus subspicatus	Algae	72 h, Growth rate, NOEC	0.5	(Orvos et al. 2002)
	Dunaliella tertiolecta	Algae	96 h, Cell density, NOEC	3.55	(DeLorenzo and Fleming, 2008)
	Pseudokirchneriella subcapitata	Algae	72 h, Growth inhibition, NOEC	0.53	(Tamura et al. 2013)
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	40	(Orvos et al. 2002)
	Daphnia magna	Crustacean	21 d, Survival, NOEC	200	(Orvos et al. 2002)
	Ceriodaphnia dubia	Crustacean	7 d, Survival, NOEC	50	(Orvos et al. 2002)
	Ceriodaphnia dubia	Crustacean	7 d, Survival, NOEC	339	(Orvos et al. 2002)
	Ceriodaphnia dubia	Crustacean	8 d, Reproduction, NOEC	30	(Tamura et al. 2013)
	Ceriodaphnia dubia	Crustacean	7 d, Reproduction, NOEC	6	(Orvos et al. 2002)
	Ceriodaphnia dubia	Crustacean	7 d, Reproduction, NOEC	182	(Orvos et al. 2002)
	Oncorhynchus mykiss	Fish	35 d, Survival, NOEC	34.1	(Orvos et al. 2002)
	Danio rerio	Fish	9 d, Hatching, Survival, NOEC	26	(Tamura et al. 2013)
	Oncorhynchus mykiss	Fish	35 d, Survival, LOEC	71.3	(Orvos et al. 2002)

Table 1. (Continued).

Compound	Species	Trophic group	Duration, Effect, Endpoint	Value (µg/L)	Reference
HHCB	*Pseudokirchneriella subcapitata*	Algae	72 h, Growth, Biomass, NOEC	201	(Balk and Ford, 1999b)
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	111	(Balk and Ford, 1999b)
	Lepomis macrochirus	Fish	21 d, Growth, NOEC	93	(Balk and Ford, 1999b)
	Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	1,000	(Carlsson and Norrgren, 2004)
	Pimephales promelas	Fish	32 d, Larval growth, NOEC	68	(Balk and Ford, 1999b)
	Folsomia candida	Insecta	28 d, Reproduction, NOEC	45,000	(Balk and Ford, 1999b)
AHTN	*Pseudokirchneriella subcapitata*	Algae	72 h, Growth, Biomass, NOEC	374	(Balk and Ford, 1999b)
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	196	(Balk and Ford, 1999b)
	Lepomis macrochirus	Fish	21 d, Growth, NOEC	89	(Balk and Ford, 1999b)
	Pimephales promelas	Fish	36 d, Larval growth, NOEC	62.5	(Balk and Ford, 1999b)
	Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	10	(Carlsson and Norrgren, 2004)
	Brachydanio rerio	Fish	21 d, Growth, NOEC	35	(Balk and Ford, 1999b)
	Folsomia candida	Insecta	28 d, Reproduction, NOEC	45,000	(Balk and Ford, 1999b)
MX	*Scenedesmus subspicatus*	Algae	72 h, Biomass, NOEC	> sol. (150)	(Schramm et al. 1996b)
	Selenastrum capricornutum	Algae	5 d, Fluorescence, NOEC	> 5,600	(Balk et al. 2004)
	Daphnia magna	Crustacean	48 h, Swim activity, NOEC	> sol. (150)	(Schramm et al. 1996)
	Daphnia magna	Crustacean	48 h, Swimming behaviour, NOEC	320	(Balk et al. 2004)
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	56	(Balk et al. 2004)
	Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	10	(Carlsson and Norrgren, 2004)
	Brachydanio rerio	Fish	14 d, Growth, NOEC	< 100	(Balk et al. 2004)
MK	*Selenastrum capricornutum*	Algae	72 h, Growth, NOEC	88	(Balk et al. 2004)
	Scenedesmus subspicatus	Algae	72 h, Biomass, NOEC	>sol. (460)	(Schramm et al. 1996)
	Acartia tonsa	Crustacean	5 d, Survival, NOEC	800	(Wollenberger et al. 2003
	Daphnia magna	Crustacean	48 h, Swim activity, NOEC	>sol. (460)	(Schramm et al. 1996)
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	169	(Balk et al. 2004)
	Acartia tonsa	Crustacean	5 d, Survival, NOEC	800	(Wollenberger et al. 2003
	Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	3.3	(Carlsson and Norrgren, 2004)
	Onchorynchus mykiss	Fish	21 d, Growth, NOEC	125	(Balk et al. 2004)

Chapter 2

Occurrence and ecological risk assessment of emerging organic chemicals in urban rivers: Guangzhou as a case study in China

Feng-jiao Peng, Chang-gui Pan, Min Zhang, Nai-sheng Zhang, Ronja Windfeld, Daniel Salvito, Henriette Selck, Paul J. Van den Brink, Guang-Guo Ying

Abstract

Urban rivers may receive contamination from various sources including point sources like domestic sewage and nonpoint sources (e.g., runoff), resulting in contamination with various chemicals. This study investigated the occurrence of emerging organic contaminants (3 endocrine disrupting compounds (EDCs), and 17 pharmaceuticals and personal care products (PPCPs)) in six urban rivers of a representative subtropical city, Guangzhou (southern China). Our results showed that EDCs and personal care products were frequently detected in the water phase and sediment phase. 4-nonylphenol (4-NP) was the most predominant compound with the highest concentration of 5,050 ng/L in the water phase and 14,400 ng/g dry weight (dw) in the sediment. Generally, higher total concentrations of EDCs and PPCPs were detected in the four urban streams compared to the main stream Zhujiang River and the Liuxi River at the suburb area. A screening-level risk assessment showed that 4-nonylphenol and triclosan (TCS) pose potential risks to aquatic organisms in most sampling sites. For individual taxa, 4-NP may pose risks to various groups of aquatic organisms, while TCS only might pose high risks to algae.

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1. Introduction

Guangzhou is a highly urbanized megacity in southern China with a population of 13.1 million (http://www.guangzhou.gov.cn/). Due to rapid economic development and urbanization, urban drainage pipe network is well developed in Chinese megacities, such as Guangzhou (http://www.gzepb.gov.cn/). Currently, 10204 kilometres of municipal drainage pipe network has been installed, and 48 WWTPs have been installed and is in use in Guangzhou. The treated domestic sewage amount to 4.99 million tons/day. 93% and 48% of the produced domestic sewage in urban areas and rural area enter into the WWTPs, respectively (http://www.gzepb.gov.cn/). Therefore, a proportion of the produced domestic sewage in urban areas is still directly released into urban rivers (i.e., does not undergo wastewater treatment), which has become a serious environmental problem and has attracted growing concern of the Chinese government (Zhang et al., 2016b). The Zhujiang River, which has the second highest flow rate in China, receives input from hundreds of tributaries in Guangzhou (Zhang et al., 2008). Particularly in megacities like Guangzhou, the majority of these tributaries receive discharges of treated and untreated wastewaters from surrounding residential areas resulting in increased levels of both nutrients and contaminants in the receiving waters. Lastly, the Chinese government has implemented a system to characterize water quality based on nutrient levels (i.e, five-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total organic carbon (TOC), total phosphorous (TP), total nitrogen (TN), and ammonia-nitrogen (NH_3-N)), referred to as the Environmental Quality Standards for Surface Water (EQS, GB 3838-2002).

Regarding contaminants, endocrine disrupting chemicals (EDCs), and pharmaceuticals and personal care products (PPCPs) have raised significant concerns due to their wide applications in consumer products, bioaccumulation ability and potential adverse effects to the environment. In recent years, EDC and PPCP contamination have been widely detected in different environmental compartments, such as wastewater effluents (e.g., Zhao et al., 2009; Lange et al., 2015), surface water (e.g., Zhao et al., 2010b; 2010b; Klosterhaus et al., 2013; Salgueiro-González et al., 2015a), sediment (Zeng et al., 2008; Klosterhaus et al., 2013; Chen et al., 2014a) and aquatic organisms (Lee et al., 2014; Yang et al., 2014; Salgueiro-González et al., 2015b), with concentrations ranging from ng/L to μg/L in aqueous matrices and ng/g to μg/g in solid matrices and organisms.

Among EDCs, 4-t-octylphenol (4-t-OP), 4-nonylphenol (4-NP) and Bisphenol A (BPA) represents three of the most reported phenolic xenoestrogen compounds in China (Ying et al., 2002; Zhao et al., 2009). 4-t-OP and 4-NP are degradation products of alkylphenolethoxylate surfactants while BPA is an intermediate used in certain plastics and epoxy resins (Ying et al., 2002; Asimakopoulos and Thomaidis, 2015). These three chemicals have been found in biota samples (e.g., in China) including algae, fish and mollusc (Yang et al., 2014; Gu et al., 2016), and a maximum measured concentration of 19,891 ng/g wet weight (ww) for 4-NP was found in fish samples from the estuary of the Yangtze River (Gu et al., 2016). Recently, 4-NP and BPA were reported to pose potential ecological risks to organisms in several rivers in different regions of China (Gao et al., 2014; Guo et al., 2015). However, knowledge about the ecological risks posed by most of these EDCs to aquatic organisms is still limited, especially in sediment (Diepens et al., 2014, 2016).

PPCPs constitute another important group of emerging contaminants. The production and usage volumes of PPCPs in China have been growing rapidly, resulting in China being among the top three countries with the largest consumption of PPCPs (Liu et al., 2013). After use, PPCPs are discharged into municipal wastewater treatment plants (WWTP) or directly released into aquatic environments (Ying and Kookana, 2007; Chalew and Halden, 2009). So far, most freshwater acute test performed with pharmaceuticals has shown low or negligible toxicity to aquatic organisms (Fent et al., 2006; Kim et al., 2009). However, some researchers note that a continuous release of PPCPs to riverine environments may pose potential ecological impact due to chronic exposure (Han et al., 2010; Zhang et al., 2015a). Studies on long-term effects, however, are limited and chronic effects are still not well understood for some substances.

The few studies that have investigated EDCs and PPCPs contamination in urban rivers in China have mainly focused on contamination in the water phase (Peng et al., 2008; Zhao et al., 2009, 2010b; Yang et al., 2013; Dai et al., 2015). However, no thoroughly risk assessment was performed in those studies which was based on the assessment factor (AF) method. Though the AF approach is easy to use and practical if toxicity data is limited, the PNEC estimates provided by this method exhibit great uncertainty as they are solely dependent on the minimum toxicity value and a certain factor. Instead, statistical extrapolation based on species sensitivity distributions (SSD) method gives more reliable and reasonable statistics considering that the PNEC estimates are based on an established distribution of a full of toxicity data set (Lei et al., 2012). Moreover, an increasing concentration of sediment-associated EDC and PPCPs is expected in China due to the continuous release and the physical-chemical properties of a fraction of compounds (e.g., log Kow), which leads to adsorption to particles in the water column and subsequent precipitation and accumulation in the sediment compartment. Thus benthic organisms may be at risk from these compounds (Diepens et al., 2016). Therefore, further studies to elucidate potential ecological consequences of EDC and PPCPs contamination in aquatic sediments are needed.

The objective of the current study was to 1) assess the levels of general water quality in urban rivers in Guangzhou based on measurements of nutrients, and to measure concentration levels of the selected compounds: i) in a gradient from un-contaminated and low-density populated rural area to highly contaminated and high-density populated urban area, ii) between seasons (wet and dry season); 2) compare geographic contaminant level variability by relating to published literature values in China and worldwide, and finally to 3) perform a screening-level ecological risk assessment of surface water and sediment by relating the measured environmental concentrations (MECs) in surface water or estimated concentrations in pore water with published toxicity data for water exposure.

Guangzhou and the surrounding rivers were selected as study area as this area provides a gradient in contamination and population density. The specific EDCs and PPCPs comprising three phenolic xenoestrogens, two polycyclic musks and two nitromusks, two antimicrobials and eleven acid pharmaceuticals (non-steroidal anti-inflammatory drugs and blood lipid regulators) were selected in this study as they have been studied to varying degrees both in the present study area (i.e., in surface waters and sediments collected from twelve sampling sites in six urban rivers in and surrounding Guangzhou) as well as in other economic/geographic regions.

2. Materials and methods

2.1. Chemicals and reagents

Twenty commonly used EDCs and PPCPs were selected as target compounds in the current study: 4-nonylphenol (4-NP), 4-tert-octylphenol (4-t-OP), bisphenol A (BPA), triclocarban (TCC), triclosan (TCS), galaxolide (HHCB) and tonalide (AHTN), musk xylene (MX), musk ketone (MK), clofibric acid, ketoprofen, naproxen, diclofenac, indometacin, ibuprofen, meclofenamic acid, mefenamic acid, fenoprofen, gemfibrozil and tolfenamic acid. Specific supplier sources of all the chemicals and reagents used in this study are provided in the supporting information, and the physicochemical properties of each compound is presented in Table S1 (Supplementary information). Individual stock solutions (100 mg/L) of EDCs, antimicrobials and acidic pharmaceuticals were prepared in methanol, while the fragrance material stock solutions (100 mg/L) were prepared in ethyl acetate, and stored at -18 °C until use.

2.2. Study area and sample collection

Six urban rivers including the main stream of Zhujiang River in Guangzhou were selected in this study. Shijing River, Sha River, Liede River and Chebei River are four small urban streams in the central urban area, while Liuxi River runs from the outer suburb to the central urban area of Guangzhou. The sampling map and detailed site description are shown in Fig. 1 and Table S2.

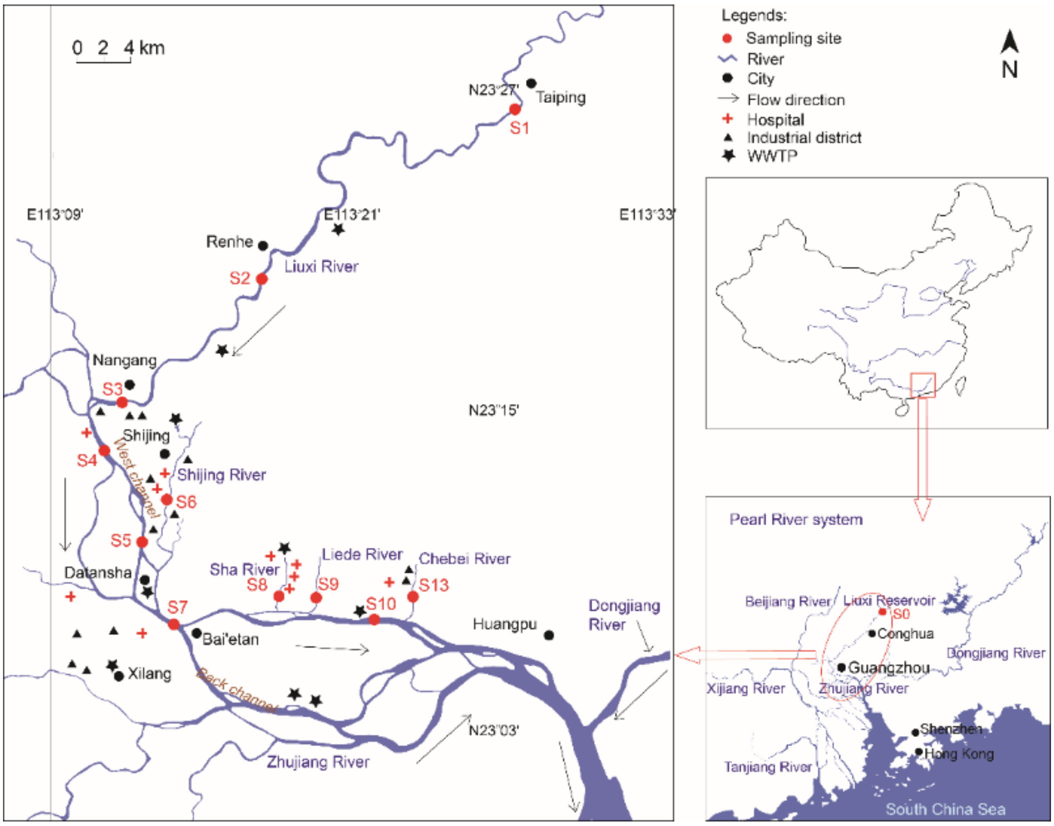

Figure 1. Map showing sampling locations in urban rivers of Guangzhou in 2015. Specifically, S0 is situated in Liuxi Reservoir which is a drinking water source in Guangzhou. S1, S2 and S3 are located in upstream, midstream and downstream of Liuxi River, respectively; S4, S5, S7 and S10 are in the main stem of Zhujiang River; S6, S8, S9 and S11 are located in Shijing River, Sha River, Liede River and Chebei River in urban region of Guangzhou, respectively.

Two sampling campaigns were conducted in 2015 (March and July), each including 12 sampling sites (S0-S11) from the upstream of Liuxi river, which is believed to have a low contamination level (Zhao et al., 2009) and a low-density population (S0), to the urban rivers receiving wastewater (S6, S8, S9 and S11). Both surface water and sediment samples were collected at each sampling site with three replicates. Surface water (10 cm below surface) samples were collected in 1-L pre-cleaned brown glass bottles, transported on ice to the laboratory, stored at 4 °C in darkness and processed within 24 h. The collected water samples were filtered using 0.7-µm glass fibre filters. The sediment (upper 5-10 cm) samples were collected by a Van Veen (area: 0.0435 m2; volume: 5 L) stainless grab sampler, and stored in 250-mL glass bottles. In addition, water samples were adjusted to a pH of 3 using 4-M H2SO4. About 50-mL MeOH was added to inhibit microbial growth, while sediment samples were preserved with sodium azide (1 g/L) on site to suppress potential microbial activity. Subsequently, sediment samples were lyophilized, homogenized, sieved through a 0.9 mm sieve, and stored at 4 °C in darkness until extraction. The pH, dissolved oxygen (DO), conductivity and water temperature were monitored in situ by a pH/DO meter (YSI-Pro2030; YSI Incorporated, Yellow Springs, OH, USA). Environmental quality parameters including COD, BOD_5, TN, NH_3-N, TP and TOC were measured following the standard methods

(Clesceri et al, 1998), while sediment particle size distribution was determined by means of laser diffractometry.

2.3. Sample extraction and instrumental analysis

Water and sediment samples were extracted and analysed following previously used methods (Chen et al., 2010, 2014b). Briefly, the water samples were extracted by solid phase extraction (SPE) using Oasis HLB SPE cartridges (500 mg, 6 mL, Waters) (Chen et al., 2010). Sediment samples for phenolic endocrine disrupting chemicals and acidic pharmaceuticals analysis were extracted by ultrasonic extraction combined with silica gel purification (Chen et al., 2010), while an Accelerated Solvent Extractor (Dionex ASE 300, USA) equipped with 34 mL stainless steel extraction cells was applied for musk fragrances extraction (Chen et al., 2014b). The target acid pharmaceuticals, TCC and TCS were determined using an Agilent 1200 high performance liquid chromatograph (Agilent, USA) coupled to an Agilent 6460 triple quadrupole mass spectrometer with electrospray ionization under negative ionization modes (HPLC-MS/MS, ESI-). EDCs and polycyclic and nitro musk fragrance materials were analysed by an Agilent 6890N gas chromatograph (Agilent, USA) connected to an Agilent 5975B MSD mass spectrometer (GC-MS) under negative chemical ionization (NCI) mode and electron-impact ionization (EI) mode, respectively. Specific procedures on extraction methods, derivatization method and instrumental analysis are shown in Supplementary Information.

2.4. Quality assurance and quality control

Strict quality assurance and quality control (QA/QC) were conducted in all analysis procedures. Details of method recoveries, limit of detection (LOD) and limit of quantification (LOQ) for each compound are given in Table S3. Field blanks were analyzed for each trip, and solvent blanks and procedural blanks were determined successively for each batch of samples in the laboratory to check background contamination. All target compounds were below the limits of detection in all blanks. The LOQ and LOD were defined as 10 times and 3 times the signal-to-noise-ratio at low spiked concentrations, respectively. Concentration data between LOD and LOQ were treated as half of LOQ in the statistical analysis.

2.5. Water quality assessment

The Chinese government assesses surface water quality based on the published “Environmental Quality Standards for Surface Water” (GB 3838-2002), identifying five classes of water bodies by their environmental functions and protection objects. Specifically, class I is applied to source waters and national nature reserves, and class II is used to the centralized surface drinking water source in the first order protection region. Class III is also applicable for use as drinking water, whereas Class IV is limited to industrial use and entertainment (e.g., landscape-water) and should not directly be in contact with human bodies. Class V is limited to be used for agricultural purposes only. These classes are based on water quality metrics such as TP, TN, and DO, and will be used to define the general quality of the water in the rivers in the current study. Therefore, based on the “Environmental quality standards for surface water” published by Chinese government and the measured water quality parameters, including pH, DO, COD, BOD_5, NH_3-N, TN and TP (Table S4 and Table S5), we used the methods of comprehensive water quality identification index (WQI) to assess the water quality of all sampling sites in 2015 (Xu, 2005a and Xu, 2005b; more background information can be found in the supporting information).

2.6. Environmental risk assessment (ERA)

The potential environmental risk of the EDCs and PPCPs detected in the surface water and sediments were assessed following the risk quotient (RQ) approach (Van Leeuwen, 2003): RQ = measured environmental concentration (MEC) / predicted no-effect concentration (PNEC). However, Zhu et al. (2016) pointed out that it is important to consider ionization and environmental pH for ionizable chemical toxicity to obtain a more realistic environmental risk assessment. Since all target compounds are ionizable chemicals in the this study, the influence of pH values on the environmental risks assessment was explored for target compounds which may ionize at the environmental pH value measure in the present study.

For the PNEC derivation, a deterministic approach or a log-logistic SSD method was used according to the European Commission Technical Guidance Document (Van Leeuwen, 2003). If enough data were available, the PNEC was based on SSD. If not, a deterministic approach was used. Specifically the PNECs were calculated using the following steps:

1. When available, an already published PNEC value based on the log-logistic SSD method was used. The quality of toxicity data explored in the SSD model construction was evaluated in respect of reliability, adequacy and relevance (Klimisch et al., 1997).

2. Secondly, when possible, PNECs were based on the HC_5 (Hazardous Concentration 5%) derived using the log-logistic distribution of SSDs. To construct an SSD curve, chronic no-observed-effect-concentration (NOEC) values of at least eight species from at least three trophic levels are required. The SSD-based PNEC was then calculated by dividing the HC_5 with an assessment factor of 3 (Van Leeuwen, 2003). A log-logistic function was conducted for SSD to calculate HC_5.

3. The PNECs based on the deterministic approach were calculated by dividing the lowest acute EC50 toxicity data or chronic NOEC values from the most sensitive species by deterministic values chosen according to the European Commission's Technical Guidance Document (Van Leeuwen, 2003), with chronic NOEC values used when available rather than acute toxicity data. When long-term NOECs from three, two or one trophic levels were available, the PNEC was calculated using an assessment factor of 10, 50 and 100, respectively. However, when only short-term $L(E)C_{50}$ values were available, an assessment factor of 1000 was used to calculate the PNEC (Van Leeuwen, 2003).

RQs of individual EDCs and PPCPs were obtained by dividing the MECs from specific stations in this study by a PNEC obtained based on effects data from the literature (Table S6). The risk classification was based on a binary criteria in which RQ < 1: “low or no risk”; RQ ≥ 1: “possible risk” (EMA, 2006) indicating further investigation may be necessary. The water exposure RQs were calculated based on the measured water concentrations, while the sediment exposure RQs were calculated based on the converted Cpore water assuming equilibrium in the system following (Van Der Kooij et al., 1991):

$$K_{OC} = \frac{C_{sediment}}{C_{water} \times f_{TOC}}; C_{pore\ water} = \frac{C_{sediment}}{K_{OC} \times f_{TOC}}$$

where Koc is the organic carbon partitioning coefficient, Csediment and Cwater are the measured concentrations in sediment and water samples, respectively; f_{TOC} is the fraction of TOC in the corresponding sediment samples (Table S5). In the present study, the median K_{OC} value (Table S7) was used when a chemical was detected in more than two-thirds of the total sampling numbers (n=24); otherwise, a K_{OC} value calculated by the EPI suite model was adopted.

However, the PNECs derived by a deterministic approach with toxicity data from the most sensitive species and PNECs derived from hazardous concentration for 5% of species (HC_5) in SSD method tend to be mostly based on algal data. As a result, these PNECs are likely to be low for the other taxonomic groups (Table S6). Therefore, the potential risk of the target compounds to each taxa (algae, freshwater fish, crustaceans, rotifers and insects) was investigated in the present study. Specifically, only chemicals showed a possible risk in at least half of samples were considered here, based on the toxicity data of the most sensitive individual species within each taxa. This was done by cumulative probability versus the measured environmental concentration data on a log scale. The fraction of sites where there are potential risks of toxic effects was calculated by dividing the number of sampling site where a compound concentration exceeded the toxicity threshold of a particular species by the total number of sampling site where this compound was detected. The toxicity threshold for each biological group was obtained by dividing the lowest NOEC values available for algae, freshwater fish, crustacean, rotifer and insect by a deterministic value of 10 (Table S6). For each chemical, the cumulative probability was calculated according to the reported method (Manly et al., 2008).

2.7. Statistical analyses

The occurrence of EDCs and PPCPs in dry season and wet season were compared using non-parametric Mann-Whitney U test, due to concentration data of some compounds did not follow a normal distribution. All statistical analyses were performed with SPSS 18.0, Sigma Plot 10.0.

3 Results and discussion

3.1 Physicochemical parameters in surface water and sediment and EQS

Surface water: pH values were similar for all sampling sites during the two seasons, with pH values around 7 to 8 (Table S4). All target pharmaceuticals showed pKa values lower than 5 (Table S1), meaning that these chemical presented as anionic form in the water phase (Table

S8). Also, for chemicals including TCC, 4-NP, 4-t-OP and BPA showed pKa values higher than 9.5, they almost existed in whole neutral form too (Table S9). However, with the pKa value of 7.9 (Table S1), TCS tend to exist in both neutral and ionized forms with neutral form as the main one (Table S9). TOC values in the surface water were generally higher in the dry season than those in the wet season at the sampling sites. Compared with the dry season, generally, sampling sites showed higher DO values and lower concentrations of all other detected parameters due to higher river flow in the wet season.

Sediment: In accordance with surface water, TOC, TP, TN and NH_3-N values in the sediment were generally higher in the dry season than those in the wet season, which could be explained by the interactions between water phase and surface sediment (Table S5). Among stations (i.e., from S0 to S11), Sand: low at S0 (4-5%) and more or less similar and higher for all other stations (38-79%); Silt: no trend among stations (rather constant); clay: highest at S0 (42-55%) and fluctuates greatly at a lower level among the remaining stations (4-15%) and no clear pattern along the river; TOC: lowest levels were detected at S1 (4-11g/kg) and highest at S6 (97-144g/kg), and the remainder varied between 15 and 93 g/kg); TP, TN and NH_3-N tended to be lowest at S0-S2.

Environmental Quality Standards (EQS): Although the Chinese government has published Environmental Quality Standards for surface water (GB 3838-2002) based on nutrients and physico-chemical parameters, currently no comparable standards are available for the sediment compartment. The results of the water quality assessment based on the categorization (I-V) during the two seasons are listed in Table S10. In general, our assessment revealed that the classes of the comprehensive water quality for all sampling sites did not differ between two seasons, except for site S1, which changed from class IV in dry season to class III in wet season. Regardless, a river is usually classified as the worse until sufficient data are available indicating it has improved sufficiently to be classified as a higher quality river. From upstream to downstream of the Liuxi River, the water quality changed from class III to class V, which indicates that water in Liuxi Reservoir (class III) can be used as drinking water according to the EQS for surface water (GB 3838-2002). The water quality belonged to class V for all sampling sites in Zhujiang River during both seasons, while sampling sites from the four urban rivers in the city center had water quality, which was worse than class V, which suggest that the water cannot be used for agricultural purposes and aquatic organisms may be affected. Therefore, the water quality in these rivers is indeed impacted, largely due to the conventional pollution. However, the poorer water quality regions often have the a parallel problem with chemical pollutants such as personal care products (Liu et al., 2015).

3.2. Occurrence of EDCs and PPCPs in surface water and sediments

Surface water compartment: Sixteen out of the 20 surveyed EDCs and PPCPs were detected above the LOQ in the water samples (Table 1, Fig. 2 and Table S1). Concentrations of four acid pharmaceuticals (fenoprofen, ketoprofen, meclofenamic acid and tolfenamic acid) were always below the LOQ. The average total concentrations of the target compounds at each sampling site in the water phase ranged from 34.4 to 8,840 ng/L in the dry season, and 32.6 to 5,100 ng/L in the wet season, suggesting that the water concentrations may be reaching a higher level during the dry season compared to the wet season. The lowest number of contaminants was detected at S0 (Liuxi reservoir) where very low concentrations of 4-NP, BPA, TCC, TCS and HHCB (only dry season) were detected during both seasons (Table S11). Among all the detected target compounds, 4-NP, BPA, TCC, TCS, HHCB, AHTN, diclofenac and ibuprofen are the most frequently detected and predominant chemicals in surface water accounting for over 90% of the total concentration, which is consistent with previous studies in the Pearl River Delta region (China) (Zhao et al., 2009), Ammer River (Germany) (Lange et al., 2015) and Minho River (Spain) (Salgueiro-González et al., 2015a, 2015b), showing their widespread distribution in the aqueous phase. In particular, the percentage of stations exceeding the concentrations of 4-NP Water Quality Criteria of Canada (1,000 ng/L; Canada, 2002) was 67% in dry season and 33% in wet season (Table S9). At present, China does not have a water quality standard for chemicals determined in this study. The highest concentration was observed for 4-NP at site S6 in the dry season (5,050 ng/L in surface water). Site S6 is located in the Shijing River and receives treated as well as a small part of untreated industrial and household discharges (Zhao et al., 2010b). Previously reported concentrations of 4-NP in surface water from the other countries are below 4,000 ng/L (Table S12) (e.g., Rocha et al., 2012; Kim et al., 2014; Salgueiro-González et al., 2015a, 2015b). Therefore, the concentrations of 4-NP in the Shijing River are considered to be very high compared to reported values in other parts of the world, suggesting a high consumption of nonylphenol ethoxylates in this region and/or by poorly operating WWTPs.The antimicrobials, TCC and TCS, were detected at all sampling sites both during wet and dry seasons, which is consistent with the extensive usage of these two chemicals in China (Zhao et al., 2013; Zhang et al., 2015b). The maximum concentrations of TCC (210 ng/L) and TCS (282 ng/L) in the water samples were higher than most corresponding reported concentration data worldwide (not detected (n.d)-75 ng/L for TCC and n.d-285 ng/L for TCS) (e.g., Kantiani et al., 2008; Kumar et al., 2010; Katz et al., 2013; Montagner et al., 2013; Gautam et al., 2014; Lv et al., 2014). Among acid pharmaceuticals, non-steroidal anti-inflammatory drugs of ibuprofen and diclofenac are the compounds with the highest detection frequencies, followed by clofibric acid and indomethacin. Compared to the reported concentrations worldwide (Table S12), the concentrations for ibuprofen (n.d-542 ng/L) in this study were similar to those determined in Mankyung River (Korea) (n.d-414 ng/L) (Kim et al., 2009), and much higher (more than a factor 10) than those reported in Europe and US. The highest concentration of diclofenac of 645 ng/L was higher than those usually reported (n.d-261 ng/L). However, a higher concentration of diclofenac (1,043 ng/L) has been reported for Aisonas River (Greece) (Stasinakis et al., 2012). Compared with those reported by Zhao et al. (2009; 2010a, b) for the Zhujiang River system sampling in 2007-2008, all compounds except diclofenac found in water from the Shijing River in this study were lower, which likely is a result of the construction of Shijing WWTP in the upstream of Shijing River in 2010 and the following decrease of direct discharge of wastewater in Shijing River nowadays. However, comparable concentrations were found for samples in Zhujiang River between the present study and that study, except that 4-NP, 4-t-OP and BPA showed higher concentrations, which is related to the increasing consumption of these chemicals in China nowadays (Liu et al., 2013).

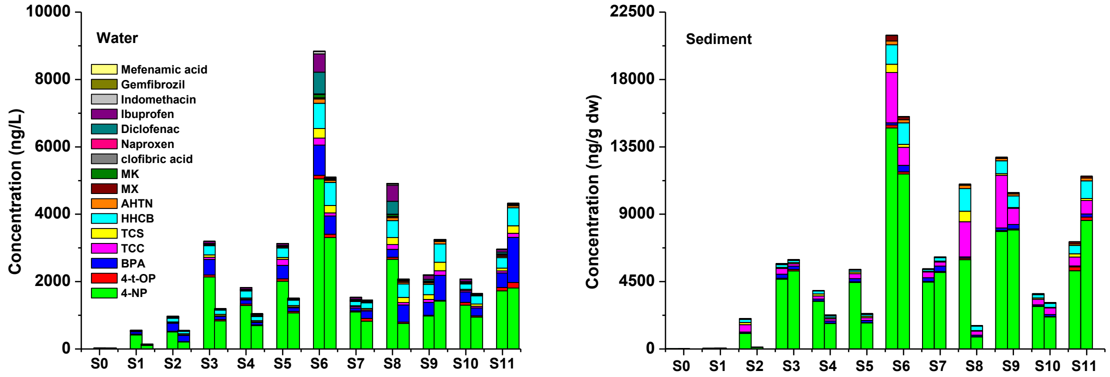

Figure 2. Concentrations of EDCs and PPCPs in surface water and sediment of urban rivers in Guangzhou of China in the two seasons. For each sampling site, dry season is on the left side while wet season is on the right side. 4-NP, 4-t-OP, BPA, TCC, TCS, HHCB, AHTN, MX and MK are abbreviations of 4-nonylphenol, 4-tert-octylphenol, bisphenol A, triclocarban, triclosan, galaxolide, tonalide, musk xylene and musk ketone.

Sediment compartment: Among the 20 target compounds, twelve target compounds were detected in the sediments (Table 1, Fig. 2 and Table S13). The total concentrations in the sediments ranged between 16.1 and 20,500 ng/g dry weight (dw) in the dry season, and varied from 27.2 to 15,500 ng/g dw in the wet season, suggesting negligible, if any, differences between the seasons ($p < 0.05$). Same as in the water phase, the lowest number of contaminants was again detected at S0 (Liuxi reservoir) where very low concentrations of 4-NP, BPA, TCC and TCS were detected during both seasons (Table S13). As expected due to their hydrophobic properties, EDCs and PCPs were the predominantly detected compounds, with 4-NP, BPA, TCC, and TCS being detected in all sediment samples (Table S1 and S13). Unlike the concentration of acid pharmaceuticals in surface water, most pharmaceuticals were not detected at the majority of sampling sites, except for clofibric acid, naproxen, diclofenac and ibuprofen. The concentrations of these detected pharmaceuticals were very low (n.d-10.6 ng/g dw) compared to other detected chemicals, which could be explained by their ionic properties resulting in a high solubility in water. Specifically, at measured pH values (around 7-8) in all sampling sites, all target acid pharmaceuticals with low pKa values (Table S1) showed high ionization (Table S8). Similar to the surface water samples, 4-NP was the most abundant compound with its maximum concentration of 14,400 ng/g at site S6 in sediment, which is higher than most reported levels from EU and US ranging between n.d and 7,000 ng/g dw (Table S14) (Klosterhaus et al., 2013; Gorga et al., 2015; Salgueiro-González et al., 2015b). However, a higher concentration of 14,500 ng/g dw for 4-NP has been observed in the

Hyeongsan River (Korea), which mostly originated from municipal activities (Kim et al., 2014). Compared with Canadian Sediment Quality Guideline (1,400 ng/g dw), 4-NP concentrations measured in most of the sites included in the present study exceeded the guideline value (Canada, 2002), in both seasons. It should be noted that 4-NP concentrations in both water samples and sediment samples from site S6 were far higher than those from the other sites and the guideline values (Canada, 2002), suggesting that industrial activity around the Shijing River is probably the major source of 4-NP. HHCB was the most frequently detected musk, with concentration range higher than recently reported concentrations in EU, US and Asia (n.d-388 ng/g dw) (Sang et al., 2012; Reiner and Kannan, 2011; Lee et al., 2014). Meanwhile, Zeng et al. (2008) have investigated polycyclic musks contamination in sediment collected from the main stream of the Zhujiang River in 2002, and the concentrations of HHCB (3.29-121 ng/g dw) and AHTN (3.62-42.5 ng/g dw) were comparable to those determined in Zhujiang River in the present study. Compared with concentrations measured in EU & US (2.89-52.0 ng/g dw for TCC and n.d-400 ng/g dw for TCS) (e.g., Cantwell et al., 2010; Kumar et al., 2010; Klosterhaus et al., 2013; Katz et al., 2013; Gorga et al., 2015), TCC and TCS had higher maximum concentrations in the present study, which could be explained by the high consumption of these chemicals in the urban area by the dense population. However, compared to data reported by Zhao et al. (2010a) in Shijing River, concentrations of TCC and TCS were lower in the present study, which should be a result of the construction of Shijing WWTP in the upstream of Shijing River in 2010, as the consumption of personal care products has been increasing since 2007 in China (http://www.chyxx.com/data/). The high concentrations of 4-NP, BPA, HHCB, TCC and TCS measured in the sediment indicated that the sediment is a major sink for hydrophobic compounds in the aquatic environment.

3.3. Seasonal variation and spatial distribution

In general, higher total contaminant concentrations were observed in samples collected in the dry season compared to those in the wet season in both sample mediums with seasonal median values of 2,690 ng/L and 6,350 ng/g in the dry season and 1860 ng/L and 4,930 ng/g in the wet season, respectively (Table 1 and Fig. 2). These results could be attributed to higher river flow in the wet season compared to the dry season. However, for individual EDCs and PPCPs, no obvious significant differences between the dry season and wet season were found for all target compounds ($p > 0.05$).

Spatial distributions of EDCs and PPCPs detected in the present study were compared among the different rivers sampled in this study. Generally, the total concentration levels of EDCs and PPCPs in both phases followed an increasing gradient from the Liuxi reservoir (S0: drinking water source), Liuxi River, Zhujiang River to urban small Rivers (Shijing River, Sha River, Liede River and Chebei River). Among all rivers, the lowest concentrations were detected at the upstream of Liuxi River (site S0-S2), followed by an increase at the stations S3-S5, S7 and S10. These latter 5 sampling sites did not seem to deviate much in total concentration between each other. The highest total concentration was detected in Shijing River (S6: runs through urban area) which flows into Zhujiang River between S5 and S7, however, the input from Shijing River to Zhujiang River did not impact the total concentration at S7, possibly caused by a high dilution in the main river. There was a tendency for higher total concentrations at sites S8, S9 and S11, likely reflecting that these 3 sampling sites are located in 3 small streams in the central urban area, which runs into the Zhujiang River. Again, there was no detectable impact of the inflow from these streams as the total concentration did not change much from S7 to S10, probably due to a high dilution in the main river. The higher total concentrations in both compartments found at site S6 (Fig. 2) in the Shijing River is probably due to this stream receiving both industrial and domestic wastewater, compared to S8, S9 and S11 which only receive domestic wastewater discharges. In addition, the concentration levels of ibuprofen and diclofenac in the water samples taken at S6 were rather high in the dry season, which may be attributed to discharges from hospitals and pharmaceutical plants around the Shijing River. As expected, the upstream of Liuxi River (S0-S1) had the lowest levels of these compounds due to the low population pressure. However, concentrations of the compounds were higher at S3 than those upstream. As shown in Fig.2, the average total concentration level of target compounds at S3 was even higher than the nearby S4 in the Zhujiang River, with 4-NP and BPA as the main contaminants, which may be caused by point sources of EDCs contamination from factories near this site. The relative low levels found at S8 in the wet season were related to the high water flow and human activity such as dredging. To our knowledge, these days, the government of Guangzhou has taken actions like river dredging to improve river environment including Sha River in the central urban area (Cheung et al., 2003). Though S10 was near the discharge port of a WWTP, concentration levels were similar to S5 and S7 located in the main stream of Zhujiang River, which could be associated with high dilution by the tidal movement in the Zhujiang River and relative high removal efficiencies of EDCs and PPCPs in that WWTP which employed unitank process and anaerobic/anoxic/oxic (A2/O) process, and/or illegally direct release of these chemicals to the Zhujiang River by the neighborhood around S5 and S7. It has been reported that A2/O process in the WWTP could remove pharmaceuticals efficiently with removal rate higher than 90% for most pharmaceuticals detected in the present study (Sime et al., 2010), and conventional activated sludge could efficiently remove phenolic compounds, antimicrobials (TCC and TCS) and polycyclic musks through sorption and biodegradation (Bester, 2005a; Drewes et al., 2005; Kupper et al., 2006; Simonich et al., 2002). Therefore, removal efficiencies of target compounds in the WWTP near the site S10 should be significant. At site S8, S9 and S11, higher concentration of BPA were found in the wet season compared with the dry season, which could be attributed to runoffs into these small rivers.

Table 1. Range, mean, median concentrations and detection frequencies of EDCs and PPCPs detected in surface water (ng/L) and sediment (ng/g dw) of urban rivers in Guangzhou in two seasons.

Compound	Surface water								Sediment							
	Dry season				Wet season				Dry season				Wet season			
	Range	Mean	Median	D.F [a]	Range	Mean	Median	D.F	Range	Mean	Median	D.F	Range	Mean	Median	D.F
4-NP	25.1-5050	1600	1300	100%	22.5-3310	1000	829	100%	10.9-14400	4440	4360	100%	17.9-11700	3760	1935	100%
4-t-OP	n.d [b] -108	52.2	49.3	92%	n.d-165	48.4	39.4	92%	n.d-261	69.8	48.7	83%	n.d-219	58.8	31.3	75%
BPA	2.30-892	302	276	100%	2.69-1340	337	199	100%	2.54-269	121	105	100%	6.47-433	173	156	100%
TCC	2.54-210	74.3	52.2	100%	2.37-135	49.4	34.1	100%	1.82-3440	978	373	100%	1.95-1210	390	228	100%
TCS	1.81-282	80.4	42.8	100%	5.06-253	93.7	39.7	100%	0.84-689	178	105	100%	0.87-199	62.0	51.0	100%
HHCB	2.70-753	254	239	100%	n.d-685	254	163	92%	n.d-1480	433	194	92%	n.d-1430	379	190	83%
AHTN	n.d-126	34.4	22.5	83%	n.d-74.0	29.3	20.7	83%	n.d-235	72.2	30.7	83%	n.d-205	59.4	30.8	75%
MX	n.d-43.8	12.8	n.d	33%	n.d-41.6	12.1	n.d	33%	n.d-376	62.8	20.4	58%	n.d-204	45.3	8.85	75%
MK	n.d-98.2	24.9	16.0	83%	n.d-18.4	7.50	4.22	83%	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Clofibric acid	n.d-19.7	4.00	1.61	83%	n.d-2.53	0.96	0.66	83%	n.d-0.79	0.30	0.25	67%	n.d-0.67	0.26	0.27	75%
Naproxen	n.d-1.71	0.83	0.92	92%	n.d-4.28	1.11	0.63	83%	n.d-0.42	n.d	n.d	25%	n.d-0.36	n.d	n.d	25%
Diclofenac	n.d-645	105	25.2	92%	n.d-20.8	8.58	7.99	83%	n.d-10.6	1.98	0.65	75%	n.d-0.99	0.46	0.43	75%
Ibuprofen	n.d-542	127	58.2	92%	n.d-32.6	16.7	17.4	92%	n.d-3.19	1.46	1.18	83%	n.d-1.02	0.37	0.38	75%
Indomethacin	n.d-69.6	11.6	2.61	83%	n.d-2.64	1.31	1.46	83%	n.d	n.d	n.d	0%	n.d-3.02	n.d	n.d	0%
Gemfibrozil	n.d-9.83	2.33	0.83	75%	n.d-0.88	0.49	0.56	83%	n.d	n.d	n.d	0%	n.d	n.d	n.d	0%
Mefenamic acid	n.d-3.55	1.07	0.45	75%	n.d-2.05	0.50	0.18	67%	n.d	n.d	n.d	0%	n.d	n.d	n.d	0%
∑Chemicals	34.4-8840	2690	2140		32.6-5100	1860	1480		16.1-20500	6350	5210		27.2-15500	4930	2730	

[a] D.F, detection frequency.

[b] n.d, not detected

3.4. Environmental risk assessment

A screening-level risk assessment was performed based on the measured concentrations and PNECs derived from literature data (Fig. 3, Table S15 and Table S16). The PNECs for 4-NP, 4-t-OP, BPA, TCC and TCS were obtained from reported literature using species sensitivity distributions (SSD) based on chronic toxicity data (Chen et al., 2014a; Gao et al., 2014; Guo et al., 2015). PNECs were derived by ourselves using the SSD concept for diclofenac and ibuprofen since sufficient chronic toxicity data were available (Table S6 and Fig S1). However, a deterministic approach was used for HHCB, AHTN MX, MK, clofibric acid, naproxen, gemfibrozil, indomethacin and mefenamic acid, since a sufficient amount of toxicity data was not available. The PNECs results are shown in Table S6. It can be seen that musks and acid pharmaceuticals detected in this study have high PNEC values varying from several μg/L to a few mg/L. Compared to their reported concentrations (Table S12), these chemicals pose a minimal risk to the aquatic environment. On the contrary, the derived PNECs based on chronic toxicity data of the EDCs and antimicrobials (TCC and TCS) were much lower, with values smaller than 1 μg/L. Referring to their environmental concentrations (Table S12), these chemicals may possibly pose a risk to some species in the aquatic environment.

The RQs ranged from 0.05 to 10.5 for 4-NP and from 0.07 to 10.8 for TCS in surface water and between 0.02 and 6.3 for 4-NP, and between 0.06 and 14.3 for TCS in sediment (Table S15 and Table S16), indicating that these two chemicals may pose risks to aquatic organisms in the studied area. Similar ranges of RQs have been reported for 4-NP and TCS in China previously (Zhao et al., 2010a; Chen and Yeh, 2010; Gao et al., 2014). Moreover, a possible risk ($RQ > 1$) was recorded in more than 75% of samples for TCS in both phases (i.e., water phase and estimated porewater) and both seasons, which could be attributed to its high toxicity with an associated PNEC value of 26.2 ng/L. The frequencies of possible risks for 4-NP in the water were 83% in the dry season and 75% in the wet season, and the frequencies were 75 % in both seasons in the sediment compartment. These results were in consistent with the water quality assessment. Similarly, Kuzmanović et al. (2016) reported that their selected organic chemicals including TCS and 4-NP posed a risk of acute effects at 42% of the sampling sites and a risk of chronic effects at all the sites (77) in the surface water from Iberian river basins (Spanish) by using the toxic unit (TU) approach. Higher RQ values were likely to be found in the Zhujiang River and the central urban rivers where poor water quality status were observed. This result suggests that it is better to combine water quality status assessment to reduce the uncertainty of environmental risk assessment for chemicals. Though the government has taken measures (e.g., improve civil awareness, build new WWTPs and make rules to regulate wastewater discharge) to reduce direct discharge of domestic wastewater and waste into urban rivers of Guangzhou, the outcome is not that satisfactory. Therefore, more work need to be done to reduce discharge of untreated sewage in the future for the government. Possible risks were only occasionally found for 4-t-OP and BPA in the urban rivers in Guangzhou (Fig. 3), which is comparable with those reported in a recent study (Salgueiro-González et al., 2015a, 2015b). Though HHCB, diclofenac and ibuprofen were occasionally detected at concentrations higher than 500 ng/L in the water phase, RQ values were in all cases below 0.1 (Table S15) because of their low toxicity. Similar RQ values were also found for HHCB and TCC in sediment. Acceptable risks of HHCB were also reported in previous studies (e.g., Federle et al., 2014; Guo et al., 2013). Zhao et al. (2010a) reported that TCC posed median to high risks in Zhujiang River system, whereas risks of TCC were low in all sampling sites in this study. This discrepancy could be explained by the different PNEC values used. In Zhao et al. (2010a), the PNEC was derived by a deterministic approach and the PNEC value was 58 ng/L, while the PNEC value of TCC applied in this study was based on more toxicity data (17) and was 11 times higher (Chen et al., 2014a).

Under the environmental pH values in the present study (around 7-8), 4-NP, 4-t-OP, BPA and TCC nearly presented as all neutral form (Table S9), indicating that their potential ionization could be ignored in the present study. Therefore, the pH values showed little influence on the environmental risk assessment for these chemicals in the present study. Though the percent of neutral form for TCS was small than the above chemicals, neutral molecule was still the main form of TCS in the present study, which is in agreement with a previous study on TCS (Zhu et al., 2016). In that study, they found that TCS almost existed in neutral form in the Chinese aquatic environment. Besides, Roberts et al. (2014) reported that neutral TCS exhibited similar toxicity under different pH levels (from 7-8). Thus, the results of environmental risk assessment for TCS based on neutral form and toxicity data at environmental related pH values are reliable. Unlike the above chemicals, all selected acid pharmaceuticals were highly ionized at pH values in the present study (Table S8), which may further affect their toxicity to organisms. Thus, the effects of pH values should be considered when perform environmental risk assessment for chemicals with low pKa values such as acid pharmaceuticals. However, given that pharmaceuticals showed rather low risks in both medium (Table S15 and Table S16), the influence of pH value on the risk assessment of pharmaceuticals was negligible in this study.

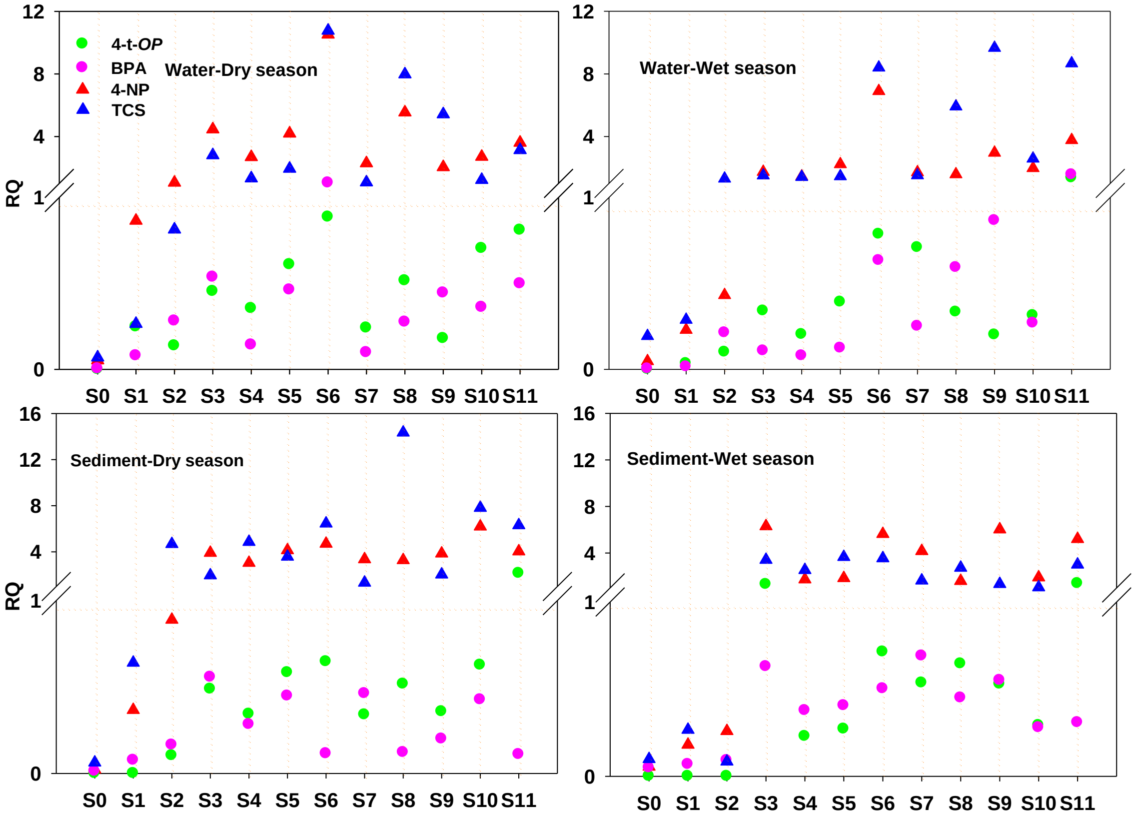

Figure 3. Calculated risk quotients (RQs) for detected chemicals in surface water and sediment samples from urban rivers in Guangzhou, excluding chemicals presenting low risks in all sampling sites. In this graph, RQ < 1 indicates low risk and RQ ≥ 1 represents a possible risk.

As half of RQs for 4-NP and TCS exceeded 1, their risks to single aquatic species of the worst-case scenario were also assessed based on their measured concentrations in surface water and sediment (Fig. 4 and Table S17). Among all the selected taxa, algae was most sensitive to 4-NP, followed by fish, rotifer, crustacean and insect. The toxicity date used here for the taxa of algae was based on the effects of 4-NP to chl-a and lipid content of diatom (Melosira varians) during a ten-day exposure (Julius et al., 2007). The author attributes the effects of 4-NP on the Melosira varians to the interaction between 4-NP and the diatom's metabolism instead of the estrogen-receptor mediation, as 4-NP is lipophilic making it particularly effective in entering the diatom cell membrane. However, other algae were more resistant to 4-NP (Ward and Boeri, 1990; Tagliabue; 1993). Algae showed threshold values most close to the PNEC values of these two chemicals. More specifically, the fraction of 4-NP causing growth inhibition in algae and reducing reproduction in fish were over 75% and over 50% in both phase in the two seasons (Table S17), respectively. In contrast, the fraction of risk to insects based on the survival of Chironomus tentans was only 8% for 4-NP, which was found in the water phase during the dry season. Unlike 4-NP, only algae were found to be at risk form to TCS with fraction values higher than 75% based on growth inhibition as an endpoint in both phases in two seasons. Similar results have been reported for TCS and 4-NP in study by Kuzmanovic et al (2016).

Therefore, the potential ecological risks and associated adverse effects of 4-NP and TCS in the urban rivers of Guangzhou may need to pay attention. It is unknown, however, whether the deterministic value of 10 used to derive threshold values based on the most sensitive species in the taxonomic groups, is too conservative or not.

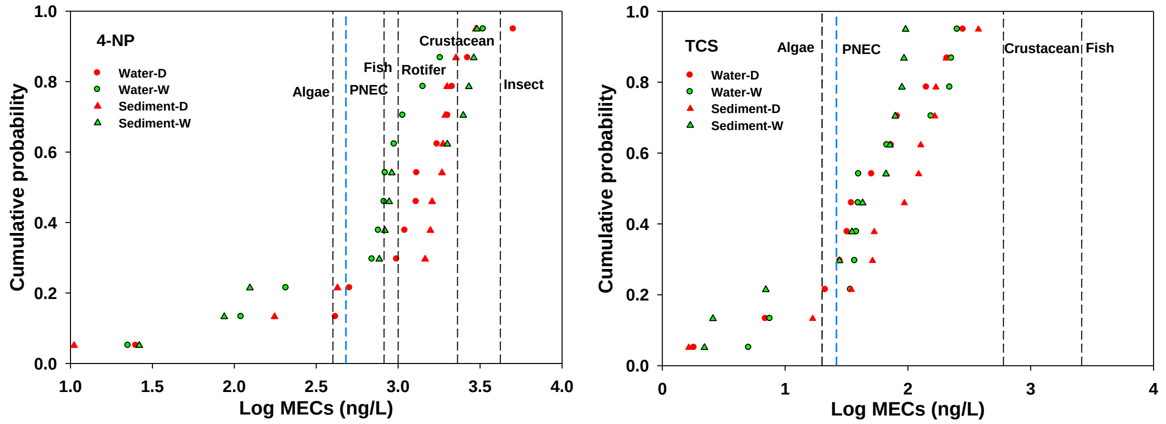

Figure 4. Probabilistic risk assessment of 4-NP and TCS in surface water and sediment in two seasons. D in the legend is the abbreviation for dry season, while W in the legend means wet season. Toxicity thresholds for 4-NP and TCS are derived from chronic toxicity data of the most sensitive species in each taxa and endpoints are shown in Table S6. Sampling sites on the left side of thresholds have no impacts on the corresponding species, while sampling sites on the right side of thresholds show toxicity to this species. MECs represents measured environmental concentrations.

4. Conclusions

The results of this study showed that 4-NP, BPA, TCC, TCS and HHCB were the most frequently detected and predominant target compounds in both water phase and sediment phase. The concentrations of 4-NP, TCC and TCS in urban rivers in the city centre of Guangzhou are relative high compared to previously reported data worldwide, which are associated with treated and untreated industrial and domestic sewage and waste discharges. Risks assessment showed that 4-NP and TCS had RQs >1 in more than 70% of samples and might pose various ecological risks to aquatic ecosystems, especially for algae. Also, it is better to consider the ionization of ionizable chemicals and water quality status when conducting environment risk assessment. Further investigations of ecotoxicological effects and potential ecological risks of these chemicals are therefore required to protect aquatic ecosystems and biodiversity in urban rivers of megacities in China.

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Supporting information

Materials and Methods

Supplier sources of all the chemicals and reagents used in this study

Standards 4-nonylphenol (4-NP), 4-N-nonylphenol (4-N-NP), triclocarban (TCC), triclosan (TCS), galaxolide (HHCB) and tonalide (AHTN), musk xylene (MX), musk ketone (MK), clofibric acid, ibuprofen, fenoprofen, diclofenac, indometacin, mefenamic acid, gemfibrozil, tolfenamic acid, ketoprofen, naproxen were purchased from Dr. Ehrenstorfer GmbH (Germany). 4-tert-octylphenol (4-*t*-OP), bisphenol A (BPA) and BPA-d16 were provided by Supelco (USA). Mecoprop and fenoprop were obtained from Riedel-deHäen (RDH, Germany). The compound 13C12-TCS was available from Cambridge Isotope Laboratories Incorporation (Massachusetts, USA), while meclofenamic acid was obtained from Sigma-Aldrich (USA). All the organic solvents used were of HPLC grade. Methanol was obtained from Merck Corporation (Germany), while ethyl acetate, hexane and dichloromethane were purchased from CNW Technologies (Shanghai, China). Oasis HLB extraction cartridges (6 mL, 500 mg) were obtained from Waters Corporation (Milford, MA, USA). The derivatization reagents pentafluorobenzoyl chloride (PFBOCl, purity > 99%) was obtained from Sigma-Aldrich (USA). Cellulose filters (30 mm) were obtained from Dionex (USA). Silica gel (80-100 mesh, 100-200 mesh; Haiyang Chemical) and silica sand (Qiangsheng Chemical) were successively ultrasonic-washed with methanol and dichloromethane 3 times and baked at 160 °C for 24 h before use, while sodium sulfate (Qiangsheng Chemical) was baked at 400 °C for 4 h and stored in a desiccator for later use.

Extraction of water samples

The water samples (1 L each) were extracted by solid phase extraction (SPE) with Oasis HLB SPE cartridges (500 mg, 6 mL), which is similar to a previous reported method (Chen et al., 2010). The collected water samples were filtered using 0.7-μm glass fiber filters, spiked with the internal standards (100 ng/L each), loaded onto cartridges preconditioned with 10 mL of methanol and 10 mL of Milli-Q water. The filtered water samples were passed through cartridges at a flow rate of 5-10 mL/min. Each sample bottle was rinsed twice with 50 mL of Milli-Q water containing 5 % methanol (v/v) and passed through the SPE cartridge. The cartridges were then dried under vacuum for 3 h. The cartridges were eluted with 3×3 mL of methanol followed by 3×2 mL of dichloromethane and 3×2 mL of ethyl acetate. The eluates were dried under a gentle nitrogen gas, re-dissolved in 1 mL of MeOH, transferred to a 2 mL amber glass vial with filtering through a 0.22 μm nylon membrane filter, and finally stored at -18 °C until analysis.

Extraction of acid pharmaceuticals, EDCs and antimicrobials in sediment

Acid pharmaceuticals, EDCs and antimicrobials in sediment samples were extracted three times by ultrasonic extraction, followed by silica gel purification (Chen et al., 2010). Briefly, 2 g of freeze-dried solid samples were spiked with 100 ng of each internal standard in a 30-mL glass centrifuge tubes, manually mixed, left in a fume hood to volatilise organic solvent, and stored in a 4 °C cold room overnight. Each replicate was then mixed with 10 mL of ethyl acetate containing 2% formic acid (v/v), sonicated for 15 min at room temperature, and then centrifuged at 1370 *g* for 10 min. The supernatants of the three times of extraction were combined and dried under a gentle nitrogen gas. Silica gel columns (18 cm×1 cmi.d.) consisted of anhydrous sodium sulfate (about 0.5 cm, on top) and 1g of silica gel were used to purify extracts. Sorbent in the silica gel columns was conditioned with 5 mL of methanol, 5 mL of ethyl acetate and 6 mL n-hexane sequentially. The dried supernatants were re-dissolved in 6 mL of n-hexane, 6 mL of ethyl acetate and 6 mL of methanol and loaded onto the silica gel columns in sequence. The first eluates in 6 mL of n-hexane were discharged, while the last two eluates in ethyl acetate and methanol were collected in glass tubes and dried under a gentle nitrogen gas. The following procedures were same as water samples.

Extraction of musks in sediment

Musk fragrances in sediment samples were extracted by an Accelerated Solvent Extractor (Dionex ASE 300) equipped with 34 mL stainless steel extraction cells (Chen et al., 2014b). Specifically, a cellulose filter was placed in the bottom of a steel extraction cell, followed by in-cell clean-up sorbents consisted of 2.0 g of silica and 2.0 g of neutral aluminum in sequence. Then 2 g of sediment sample was loaded, spiked with 100 ng of the internal standards AHTN-d_3 and Musk xylene-d_{15}, and mixed well. Cells were placed in a fume cupboard to remove the solvent, then, added with 5 g of quartz sand. The extraction solvent were acetone/dichloromethane (1:1, v/v). The extraction program was given as follows: extraction temperature (140 °C) with heat-up time of 5 min, 2 static cycles with static time of 5 min. The extracts were evaporated to about 2 mL by a rotary evaporator at 30 °C, dried under a gentle nitrogen gas and reconstituted in 1 mL of hexane. The final extract was filtered through a 0.22-μm nylon filter and transferred into a 2-mL amber glass vial and stored in -18 °C prior to analysis.

Extraction of acid pharmaceuticals, EDCs and PCPs in suspended particles

Extraction procedures for particles were similar to acid pharmaceuticals and EDCs extraction in sediment with a small modification. Specifically, after three times extraction by ethyl acetate: formic acid (50:1, v/v) solvent, two times extraction by dichloromethane: hexane (1:1, v/v) was followed and solvent volume was 10 mL for each time.

Derivatization of EDCs

The derivatization method for phenolic compounds reported by Zhao et al. (2009) was followed. Specifically, 100 μL of an extract in methanol was transferred to a 10 mL glass tube (KIMAX, USA) with polytetrafluoroethylene (PTFE) screw cap and dried under a gentle nitrogen stream. Then 2 mL of 1 M $NaHCO_3$ aqueous solution and 1 mL of 1 M NaOH aqueous solution were added into the tube and vortex mixed for 30 s. After this, 2 mL of n-hexane, 50 μL of 10%

pyridine in toluene and 50 μL of 2% PFBOCl in toluene were added in sequence, manually shaken violently for 1 min, then left at room temperature for 30 min. Then, the supernatant of n-hexane phase was transferred carefully to a 5 mL glass centrifugal tube using a glass pipette. The second time, except that only 2 mL of n-hexane was added into the 10 mL tube, other procedures were same as the first time. Then hexane mixture was dried under a gentle nitrogen stream. The final extract was re-dissolved in 100 μL of n-hexane and transferred to a 2 mL amber glass vial with a 250 μL flat-bottomed insert for analysis.

LC-MS/MS analysis

Acidic pharmaceuticals and antimicrobial agents were analyzed by an Agilent 1200 rapid resolution liquid chromatograph coupled to Agilent G6460A triple quadrupole mass spectrometer under electrospray negative ionization (ESI) mode (Chen et al., 2010). A 10 uL aliquot of each extract was injected into an Agilent SB-C18 column (3.0 mm × 100 mm ID, 1.8 μm particle size) at temperature of 40 °C with an RRLC in-line pre-column filter (4.6 mm, 0.2 μm filter), with Milli-Q water containing 0.01% acetic acid (v/v) (solvent A) and acetonitrile : methanol (1:1, v/v) (solvent B) as the mobile phase at a flow rate of 0.3 mL/min. The gradient program was given as follows: 40% B at 0 min, then increased to 50% B at 15 min, 75% B at 20 min and kept at 75% B for 2 min, then returned to the initial 40% B for column re-equilibration (5 min). The capillary was maintained at 3500 V. Dry and sheath gas flows were kept at 8 and 12 mL/min, respectively. Both dry and sheath temperatures were kept at 350 °C.

GC-MS analysis

EDCs were analysed using an Agilent 6890N GC interfaced to a 5975B MSD (GC-MS), equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific Co., USA), under chemical ionization (CI) mode. Helium (purity > 99.999%) was used as the carrier gas at a constant flowrate of 1.0 mL/min, and methane (purity > 99.999%) was applied as reaction gas with a flow rate of 2.0 mL/min in negative chemical ionization (NCI) mode. The temperature of ion source and quadrupole were both kept at 150 °C during the analysis. Injection volume is 2 μL for each samples and the injector inlet temperature is300 °C. Splitless mode was applied for injection. The column temperature was programmed as follows: from 80 °C (1 min) to 220 °C at 10 °C/min, from 220 to 260 °C at 4 °C/min, and from 260 to 300 °C (8 min) at 5 °C/min, then to the temperature 310 °C (15 min) at 20 °C/min. The MS interface temperature was maintained at 310 °C.

Musks in the extracts were measured by using an Agilent 6890 N GC interfaced to a 5975B MSD (GC-MS), equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific Co., uSA), in selected ion monitoring (SIM) mode under electron-impact ionization (EI). The temperatures for the GC-MS interface, ion source, quadrupole and injector were 280 °C, 250 °C, 150 °C and 280 °C, respectively. Carrier gas: helium (purity > 99.999%) at a constant flow of 1.0 mL/min. Injection volume: 2 μL for each sample. The GC oven temperature was programmed as follows: 80 °C for 0 min, increased to 170 °C at

15 °C/min, from 170 °C to 185 °C at 1 °C/min, then to 300 °C at a rate of 20 °C/min for 5 min.

Calculation equations of water quality identification index (WQI)

The water quality identification index (WQI) was calculated using the following equations:

$WQI = X_1 \cdot X_2$

$X_1 \cdot X_2 = \frac{1}{m} \sum (P_1' + P_2' + \ldots + P_m')$

$P_1' = X_1' . X_2'$

if the water quality is within the I-V class,

for dissolved oxygen (OD), $X_2' = \frac{\rho_{DOku} - \rho_{DO}}{\rho_{DOku} - \rho_{DOkl}} \times 10$; while for other indexes, $X_2' = \frac{\rho_i - \rho_{ikl}}{\rho_{iku} - \rho_{ikl}} \times 10$;

if the water quality is worse than the V class,

for index of OD, $X_1' . X_2' = 6 + \frac{\rho_{DO,5l} - \rho_{DO}}{\rho_{DO,5l}} \times n$; while for other indexes, $X_1' . X_2' = 6 + \frac{\rho_i - \rho_{i5u}}{\rho_{i5u}}$;

where WQI is the comprehensive water quality identification index; X_1 is the class of comprehensive water quality in water body; X_2 is the position of comprehensive water quality within variation interval of X_1 class; m is the number of individual water quality index participating in comprehensive water quality assessment; P_1', P_2' and P_m' represent the single factor water quality indexes of the first, second and m water factor, respectively; X_1' is the class of water quality for the number i water quality index; X_2' is the position of the corresponding water quality within variation interval of X_1' class; ρ_{DO} is the measured concentration of DO; ρ_{DOku} is the upper limit concentration of DO at the class k; ρ_{DOkl} is the lower limit concentration of DO at the class k; ρ_i is the measured concentration of the number i water quality index; $\rho_{ikl} \leq \rho_i \leq \rho_{iku}$; ρ_{iku} is the upper limit concentration of the number i water quality index at the class k; ρ_{ikl} is the lower limit concentration of the number i water quality index at the class k; $k = X_1'$; $\rho_{DO,5l}$ is the lower limit concentration of DO at the class V; n is a coefficient with value of 4; ρ_{i5u} is the upper limit concentration of the number i water quality index at the class V;

Table S1. Physicochemical properties of the selected compounds in this study.

Category	Compound CAS number	Molecular formula	MW	Water solubility (mg/L) [a]	log K_{ow} [a]	log K_d [b]	log K_{oc} [b]	pKa
EDCs	4-Nonylphenol (4-NP) 104-40-5	$C_{15}H_{24}O$	220.36	1.57	5.99	3.41±0.47 (n=24)	3.28±0.46 (n=24)	9.5 [e]
EDCs	4-tert-Octylphenol (4-t-OP)	$C_{14}H_{22}O$	206.33	12.6	4.12	3.09±0.28 (n=19)	3.06±0.27 (n=19)	9.5 [e]
EDCs	Bisphenol A (BPA) 80-05-7	$C_{15}H_{16}O_2$	228.29	172.7	3.64	2.72±0.50 (n=24)	2.7±0.50 (n=24)	9.73 [f]
PCPs	Galaxolide (HHCB) 1222-05-5	$C_{18}H_{26}O$	258.40	1.75	5.90	3.15±0.25 (n=25)	3.09±0.22 (n=21)	-
PCPs	Tonalide (AHTN) 1506-02-1	$C_{18}H_{26}O$	258.40	1.25	5.70	3.37±0.22 (n=19)	3.27±0.18 (n=19)	-
PCPs	Musk Xylene (MX) 81-15-2	$C_{12}H_{15}N_3O_6$	297.27	0.82	4.45	3.70±0.28 (n=7)	3.59±0.30 (n=9)	-
PCPs	Musk ketone (MK) 81-14-1	$C_{14}H_{18}N_2O_5$	294.31	1.90	4.60	NM [c]	3.40 [d]	-
PCPs	Triclocarban (TCC) 101-20-2	$C_{13}H_9Cl_3N_2O$	315.59	0.65	4.90	4.00±0.77 (n=24)	3.67±0.63 (n=24)	12.7 [g]
PCPs	Triclosan (TCS) 3380-34-5	$C_{12}H_7Cl_3O_2$	289.55	10.00	4.76	3.06±0.55 (n=24)	2.94±0.55 (n=24)	7.9 [g]
APs	Clofibric acid 882-09-7	$C_{10}H_{11}ClO_3$	214.65	582.50	2.57	2.37±0.47 (n=17)	2.37±0.47 (n=17)	3.18 [h]
APs	Ketoprofen 22071-15-4	$C_{16}H_{14}O_3$	254.29	51.00	3.12	NM [c]	2.59 [d]	4.45 [i]
APs	Naproxen 22204-53-1	$C_{14}H_{14}O_3$	230.27	15.90	3.18	2.61±0.14 (n=6)	2.61±0.14 (n=6)	4.15 [i]
APs	Fenoprofen 31879-05-7	$C_{15}H_{14}O_3$	242.28	30.13	3.90	NM [c]	2.81 [d]	4.2 [j]
APs	Dicofenac 15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.15	2.37	4.51	1.55±0.28 (n=18)	1.55±0.28 (n=18)	4.15 [i]

Table S1. (Continued).

Category	Compound CAS number	Molecular formula	MW	Water solubility (mg/L) [a]	log K_{ow} [a]	log K_d [b]	log K_{oc} [b]	pKa
APs	Indometacin 53-86-1	$C_{19}H_{16}ClNO_4$	357.80	0.94	4.27	NM [c]	2.90 [d]	4.5 [k]
APs	Ibuprofen 15687-27-1	$C_{13}H_{18}O_2$	206.29	21.00	3.97	1.31±0.36 (n=19)	1.31±0.36 (n=19)	4.91 [i]
APs	Meclofenamic acid 644-62-2	$C_{14}H_{11}Cl_2NO_2$	296.15	30.00	6.02	NM [c]	2.62 [d]	3.76 [l]
APs	Mefenamic acid 61-68-7	$C_{15}H_{15}NO_2$	241.29	20.00	5.12	NM [c]	2.41 [d]	4.2 [m]
APs	Gemfibrozil 25812-30-0	$C_{15}H_{22}O_3$	250.34	4.96	4.77	NM [c]	2.64 [d]	4.7 [n]
APs	Tolfenamic acid 13710-19-5	$C_{14}H_{11}ClNO_2$	261.71	0.78	5.17	NM [c]	2.41 [d]	4.3 [o]

[a] The water solubility and log K_{ow} values were calculated by EPI suite (USEPA, 2012).

[b] The log K_d and log K_{oc} values were calculated by concentrations and total organic carbon data detected in this study. [c] NM, not measured.

[d] The log K_{oc} values were derived from EPI suite (USEPA, 2012), due to no detection in sediment in the present study. EDCs is an abbreviation of endocrine disruptor compounds; PCPs represents personal care products; APs means acid pharmaceuticals. Notably, the log K_{oc} values were chosen from MCI method, which is more robust and be in use longer;

[e] Motoyama et al., 1999;

[f] Sambe et al., 2006;

[g] Halden et al., 2005;

[h] Packer et al., 2003

[i] SRC PhysProp Database, 2003

[j] Newton and Kluza, 1978

[k] O'Brien et al., 1984

[l] Marriner and Bogan, 1979

[m] Wolfe et al., 1976

[n] Amjadi et al., 2008

[o] Pentikäinen et al., 1982

Table S2. Basic information for each sampling site in urban rivers of Guangzhou in 2015.

Site	Geographic location (N, E)		Site location	District	Site characteristics
S0	113º28'47"	23º56'45"	Liuxi Reservoir	Conghua district	Source of Liuxi River
S1	113°28'10"	23°26'05"	Taiping	Conghua district	Upstream of Liuxi River
S2	113°17'28"	23°19'38"	Renhe	Baiyun district	Midstream of Liuxi River
S3	113°12'26"	23°14'47"	Nangang	Huangpu district	Downstream of Liuxi River
S4	113°11'07"	23°13'15"	Yagang	Baiyun district	Zhujiang River
S5	113°12'22"	23°08'15"	Shabeilijiao	Baiyun district	Zhujiang River
S6	113°13'50"	23°11'39"	Shijing River	Baiyun district	Tributary of Zhujiang River
S7	113°13'58"	23°06'22"	Baietan	Liwan district	Zhujiang River
S8	113°12'08"	23°07'10"	Sha River	Yuexiu district	Tributary of Zhujiang River
S9	113°19'43"	23°07'08"	Liede River	Tianhe district	Tributary of Zhujiang River
S10	113°21'52"	23°06'44"	Pazhou	Tianhe district	Zhujiang River, near the discharge port of a WWTP
S11	113°24'11"	23°07'12"	Chebei River	Tianhe district	Tributary of Zhujiang River

Table S3. Recoveries and detection limits of target compounds in surface water (spiked concentration of 100 ng/L) and sediment (spiked concentration of 100 ng/g) by HPLC-MS/MS or GC-MS.

Compound	Surface water			Sediment			Particles		
	Recoveries [a] (%)	LOD [b] (ng/L)	LOQ [c] (ng/L)	Recoveries (%)	LOD (ng/g)	LOQ (ng/g)	Recoveries (%)	LOD (ng/L)	LOQ (ng/L)
4-Nonylphenol	89.9 ± 1.18	0.94	2.96	113 ± 9.29	1.23	3.74	118 ± 7.16	1.18	3.06
4-tert-Octylphenol	103 ± 2.88	0.42	1.05	77.0 ± 3.74	0.30	1.00	96.4 ± 5.61	0.37	1.19
Bisphenol A	99.4 ± 4.30	0.60	1.89	104 ± 4.34	0.68	1.96	111 ± 2.48	0.63	1.85
Galaxolide	98.0 ± 3.84	0.35	1.01	109 ± 2.93	0.41	1.36	105 ± 8.97	0.43	1.41
Tonalide	122 ± 3.83	0.44	1.34	105 ± 4.28	0.49	1.52	87.1 ± 10.2	0.47	1.48
Musk Xylene	92.1 ± 6.81	1.43	4.50	104 ±5.97	1.92	5.73	124 ± 7.15	1.66	4.93
Musk ketone	70.2 ± 1.27	2.39	7.38	108 ± 1.57	3.11	9.06	84.4 ± 10.9	2.49	7.08
Triclocarban	97.4 ± 5.77	0.02	0.07	91.4 ± 3.90	0.04	0.12	99.4 ± 7.21	0.03	0.11
Triclosan	95.8 ± 4.31	0.01	0.03	96.3 ± 3.39	0.02	0.08	102 ± 2.99	0.03	0.09
Clofibric acid	96.3 ± 4.31	0.06	0.19	84.8 ± 1.09	0.07	0.23	85.8 ± 5.77	0.05	0.16
Ketoprofen	101.9 ± 0.45	0.14	0.49	84.8 ± 1.34	0.17	0.52	88.1 ± 9.74	0.11	0.35
Naproxen	92.8 ± 0.69	0.07	0.19	79.9 ± 0.71	0.08	0.22	82.6 ± 4.67	0.07	0.22
Fenoprofen	104 ± 2.67	0.10	0.26	69.9 ± 6.41	0.14	0.45	73.7 ± 5.43	0.15	0.49
Dicofenac	106 ± 5.28	0.12	0.38	87.9 ± 2.69	0.08	0.21	85.5 ± 5.99	0.11	0.34
Indometacin	87.1±0.76	0.15	0.43	96.6 ± 4.07	0.19	0.62	92.8 ± 2.38	0.17	0.52
Ibuprofen	94.0 ± 10.8	0.09	0.26	71.2 ± 3.44	0.11	0.29	79.2 ± 3.49	0.12	0.31
Meclofenamic acid	90.6 ± 4.88	0.14	0.46	88.5 ± 2.77	0.19	0.54	87.5 ± 4.16	0.21	0.65
Mefenamic acid	132 ± 4.00	0.08	0.26	113± 6.42	0.13	0.41	109± 2.07	0.15	0.47
Gemfibrozil	78.8 ± 3.93	0.08	0.21	84.2 ± 7.47	0.08	0.28	81.7 ± 1.59	0.09	0.31
Tolfenamic acid	97.6 ± 5.18	0.11	0.35	119 ± 4.15	0.16	0.46	109 ± 3.21	0.14	0.41

[a] Mean ± standard deviation (n = 3).

[b] LOD, method limit of detection.

[c] LOQ, method limit of quantitation.

Table S4. Water quality parameters of surface water in urban rivers of Guangzhou. See Table S2 for a description of the sites.

Site	Conductivity (μs/cm)		pH		DO (mg/L)		TOC (mg/L)		BOD_5 (mg/L)		COD (mg/L)		TP (mg/L)		TN (mg/L)		NH_3-N (mg/L)	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
S0	59.50	66.60	7.73	7.68	6.46	6.86	10.11	1.19	2.74	3.91	25.43	6.52	0.01	0.04	3.83	3.18	2.24	0.60
S1	207.30	102.80	7.32	7.51	4.33	6.33	7.73	6.84	5.18	5.09	14.34	17.60	0.17	0.07	4.49	4.67	1.87	0.75
S2	244.90	167.80	7.40	7.44	4.14	5.97	7.14	7.73	5.67	5.57	12.39	7.82	0.21	0.21	5.70	4.58	2.99	1.50
S3	286.70	218.30	7.23	7.18	1.09	2.00	18.43	9.51	9.19	9.88	20.21	13.04	0.42	0.24	9.06	5.14	5.53	2.09
S4	391.40	350.90	7.51	7.15	0.98	1.99	18.14	10.41	7.53	3.91	20.86	22.17	0.35	0.24	9.06	5.61	5.38	2.62
S5	420.20	363.90	7.49	7.03	1.33	1.73	24.68	11.00	7.82	5.87	28.69	46.94	0.42	0.32	8.22	9.63	4.71	3.36
S6	646.70	439.30	7.51	7.32	0.11	0.20	29.43	19.92	27.78	12.03	66.50	27.38	2.14	0.61	27.85	10.28	21.16	6.58
S7	496.60	437.20	7.70	6.91	2.32	2.20	15.76	10.41	12.32	8.41	20.21	17.60	0.47	0.40	8.22	7.66	4.34	4.49
S8	659.20	469.60	7.59	7.47	0.42	0.59	30.32	11.89	17.21	8.22	49.55	27.38	1.48	0.60	18.04	10.47	12.93	6.35
S9	576.50	458.10	7.56	7.47	0.34	0.24	25.27	22.89	16.82	18.00	37.82	41.73	0.91	0.78	12.62	14.30	8.22	8.67
S10	453.80	387.30	7.38	7.31	0.49	0.56	19.03	10.11	11.15	9.58	23.47	30.64	0.21	0.43	8.13	9.06	3.74	4.11
S11	617.30	444.50	7.51	7.39	0.15	0.30	27.05	22.89	9.19	10.56	29.99	40.42	0.72	1.04	11.68	15.70	6.73	10.47

Table S5. Parameters of sediment in urban rivers of Guangzhou. See Table S2 for a description of the sites.

Site	Particle size distribution						TOC (g/kg)		TP (g/kg)		TN (g/kg)		NH_3-N (g/kg)	
	Sand (%)		Silt (%)		Clay (%)									
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
S0	5.04	6.07	39.52	52.19	55.45	41.75	23.26	15.27	0.43	0.28	1.57	0.90	0.13	0.05
S1	79.43	70.13	16.38	25.91	4.19	3.96	4.04	11.05	0.22	0.38	0.33	0.50	0.02	0.02
S2	50.45	63.06	35.99	32.35	13.56	4.59	53.83	20.76	1.81	0.71	3.23	0.88	0.40	0.07
S3	57.66	36.72	28.38	51.40	13.96	11.88	54.64	38.78	3.38	2.11	2.84	2.11	0.41	0.21
S4	51.43	38.46	39.27	48.41	9.31	13.13	47.85	46.08	2.96	2.20	2.42	2.37	0.32	0.25
S5	50.97	40.32	40.01	50.76	9.02	8.92	49.01	44.10	2.93	2.36	2.22	1.90	0.27	0.14
S6	38.71	49.80	45.95	36.16	15.34	14.04	143.51	97.32	3.37	2.40	7.19	4.04	1.38	0.65
S7	52.58	47.90	37.40	41.62	10.03	10.48	60.91	57.64	4.62	4.32	2.13	2.33	0.26	0.32
S8	46.01	61.51	45.97	33.33	8.03	5.17	83.39	23.33	2.21	0.74	6.85	1.10	1.02	0.08
S9	52.13	51.57	34.32	36.73	13.55	11.71	93.05	61.79	2.51	1.55	5.09	2.43	0.50	0.49
S10	45.06	54.06	46.39	35.63	8.55	10.31	21.00	52.62	0.75	2.98	1.83	2.90	0.28	0.38
S11	48.56	55.80	43.47	31.16	7.97	13.04	59.10	77.34	2.92	2.20	3.11	4.04	0.49	0.50

Table S6. Summary of the aquatic ecotoxicity data and the derivation of PNEC values for the detected target compounds in urban rivers of Guangzhou.

Compound	Species	Trophic group	Duration, Effect, Endpoint	Value (μg/L)	Reference	Assessment factor	PNEC (μg/L)
4-NP	*Melosira varians*	Algae	10 d, Cell density, NOEC	4	(Julius et al. 2007)	3	0.480
	Chara sp.	Algae	122 d, Growth inhibition, NOEC	243	(Liber et al. 1999)		Gao et al., 2014
	Tapes philippinarum	Mollusca	96 h, Mortality, NOEC	190	(Matozzo et al. 2003)		
	Brachionus calyciflorus	Rotifers	96 h, Reproduction, NOEC	10	(Preston and Snell, 2001)		
	Crustacea sp.	Crustacean	122 d, population abundances, NOEC	76	(Liber et al. 1999)		
	Hyalella azteca	Crustacean	96 h, Mortality, LC50	20.7	(Brooke, 1993)		
	Oryzias latipes	Fish	104 d, Reproduction, NOEC	8.2	(Yokota et al. 2001)		
	Chironomus tentans	Insect	20 d, Survival, NOEC	42	(Kahl et al. 1997)		
4-*t*-OP	*Oryzias latipes*	Fish	10 d, Mortality, LC_{50}	0.45, 0.94	(Gray and Metcalfe, 1999)	1	0.122
	Cichlasoma dimerus	Fish	96 h, Mortality, LC_{50}	219.3	(Meijide et al. 2016)		EC, 2005
	Pomatoschistus minutus	Fish	56 d, Mortality, LC_{50}	23-35	(Robinson et al. 2004)		
BPA	*Physella acuta*	Mollusca	Hatchability	100	(Sánchez-Argüello et al. 2012)	2	0.860
	Oryzias latipes	Fish	90 d, Hatchability, Sex ratio, NOEC	120	(Metcalfe et al. 2001)		Guo et al., 2015
	Hydra oligactis	Cnidarian	35 d, 50 d, Reroduction, NOEC	170	(Fukuhori et al. 2005)		
	Daphnia magna	Crustacean	21 d, Reroduction, NOEC	800	(Brennan et al. 2006)		
TCC	*Pseudokirchneriella*	Algae	72 h, Growth inhibition, NOEC	< 10	(Yang et al. 2008)	1	0.661
	Pseudokirchneriella	Algae	72 h, Growth inhibition, LOEC	10	(Yang et al. 2008)		Chen et al., 2014a
	Pseudokirchneriella subcapitata	Algae	72 h, Growth inhibition, NOEC	5.7	(Tamura et al. 2013)		
	Ceriodaphnia Dubia	Crustacean	8 d, Reproduction NOEC	1.9	(Tamura et al. 2013)		
	Mysidopsis bahia	Crustacean	28 d, Reproduction, NOEC	0.062	(USEPA, 2009)		
	Danio rerio	Fish	9 d, Hatching, Survival, NOEC	24	(Tamura et al. 2013)		
	Pimephales promelas	Fish	35 d, Mortality, NOEC	5	(USEPA, 2009)		
TCS	*Scenedesmus subspicatus*	Algae	96 h, Biomass, NOEC	0.69	(Orvos et al. 2002)	1	0.026 ^{d}
	Pseudokirchneriella	Algae	72 h, Growth inhibition, NOEC	0.2	(Yang et al. 2008)		
	Pseudokirchneriella subcapitata	Algae	4 d, Growth, NOEC	8.3	(Harada et al. 2008)		
	Scenedesmus subspicatus	Algae	72 h, Growth rate, NOEC	0.5	(Orvos et al. 2002)		
	Dunaliella tertiolecta	Algae	96 h, Cell density, NOEC	3.55	(DeLorenzo and Fleming, 2008)		
	Pseudokirchneriella subcapitata	Algae	72 h, Growth inhibition, NOEC	0.53	(Tamura et al. 2013)		
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	40	(Orvos et al. 2002)		

Table S6. (Continued).

Compound	Species	Trophic group	Duration, Effect, Endpoint	Value (μg/L)	Reference	Assessment factor	PNEC (μg/L)
TCS	*Daphnia magna*	Crustacean	21 d, Survival, NOEC	200	(Orvos et al. 2002)		
	Ceriodaphnia dubia	Crustacean	7 d, Survival, NOEC	50	(Orvos et al. 2002)		
	Ceriodaphnia dubia	Crustacean	7 d, Survival, NOEC	339	(Orvos et al. 2002)		
	Ceriodaphnia dubia	Crustacean	8 d, Reproduction, NOEC	30	(Tamura et al. 2013)		
	Ceriodaphnia dubia	Crustacean	7 d, Reproduction, NOEC	6	(Orvos et al. 2002)		
	Ceriodaphnia dubia	Crustacean	7 d, Reproduction, NOEC	182	(Orvos et al. 2002)		
	Oncorhynchus mykiss	Fish	35 d, Survival, NOEC	34.1	(Orvos et al. 2002)		
	Danio rerio	Fish	9 d, Hatching, Survival, NOEC	26	(Tamura et al. 2013)		
	Oncorhynchus mykiss	Fish	35 d, Survival, LOEC	71.3	(Orvos et al. 2002)		
HHCB	*Pseudokirchneriella subcapitata*	Algae	72 h, Growth, Biomass, NOEC	201	(Balk and Ford, 1999b)	10	6.8
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	111	(Balk and Ford, 1999b)		
	Lepomis macrochirus	Fish	21 d, Growth, NOEC	93	(Balk and Ford, 1999b)		
	Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	1,000	(Carlsson and Norrgren, 2004)		
	Pimephales promelas	Fish	32 d, Larval growth, NOEC	68	(Balk and Ford, 1999b)		
	Folsomia candida	Insecta	28 d, Reproduction, NOEC	45,000	(Balk and Ford, 1999b)		
AHTN	*Pseudokirchneriella subcapitata*	Algae	72 h, Growth, Biomass, NOEC	374	(Balk and Ford, 1999b)	10	1
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	196	(Balk and Ford, 1999b)		
	Lepomis macrochirus	Fish	21 d, Growth, NOEC	89	(Balk and Ford, 1999b)		
	Pimephales promelas	Fish	36 d, Larval growth, NOEC	62.5	(Balk and Ford, 1999b)		
	Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	10	(Carlsson and Norrgren, 2004)		
	Brachydanio rerio	Fish	21 d, Growth, NOEC	35	(Balk and Ford, 1999b)		
	Folsomia candida	Insecta	28 d, Reproduction, NOEC	45,000	(Balk and Ford, 1999b)		
MX	*Scenedesmus subspicatus*	Algae	72 h, Biomass, NOEC	> sol. (150)	(Schramm et al. 1996)	10	1.0
	Selenastrum capricornutum	Algae	5 d, Fluorescence, NOEC	> 5,600	(Balk et al. 2004)		
	Daphnia magna	Crustacean	48 h, Swim activity, NOEC	> sol. (150)	(Schramm et al. 1996)		
	Daphnia magna	Crustacean	48 h, Swimming behaviour, NOEC	320	(Balk et al. 2004)		
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	56	(Balk et al. 2004)		
	Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	10	(Carlsson and Norrgren, 2004)		
	Brachydanio rerio	Fish	14 d, Growth, NOEC	< 100	(Balk et al. 2004)		

Table S6. (Continued).

Compound	Species	Trophic group	Duration, Effect, Endpoint	Value (µg/L)	Reference	Assessment factor	PNEC (µg/L)
MK	*Selenastrum capricornutum*	Algae	72 h, Growth, NOEC	88	(Balk et al. 2004)	10	0.33
	Scenedesmus subspicatus	Algae	72 h, Biomass, NOEC	>sol. (460)	(Schramm et al. 1996)		
	Acartia tonsa	Crustacean	5 d, Survival, NOEC	800	(Wollenberger et al. 2003		
	Daphnia magna	Crustacean	48 h, Swim activity, NOEC	>sol. (460)	(Schramm et al. 1996)		
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	169	(Balk et al. 2004)		
	Acartia tonsa	Crustacean	5 d, Survival, NOEC	800	(Wollenberger et al. 2003		
	Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	3.3	(Carlsson and Norrgren, 2004)		
	Onchorynchus mykiss	Fish	21 d, Growth, NOEC	125	(Balk et al. 2004)		
Clofibric acid	*Pseudokirchneriella subcapitata*	Algae	96 h, Growth, NOEC	75,000	(Ferrari et al. 2003)	10	24.6
	Ceriodaphnia dubia	Crustacean	7 d, Reproduction, NOEC	640	(Ferrari et al. 2003)		
	Brachionus calyciflorus	Rotifer	48 h, Reproduction, NOEC	246	(Ferrari et al. 2003)		
	Danio rerio	Fish	ELS 10 d, Survival, NOEC	70,000	(Ferrari et al. 2003)		
Naproxen	*Desmodesmus subspicatus*	Algae	96 h, Growth, NOEC	100000	(Cleuvers, 2004)	10	33.0
	Pseudokirchneriella subcapitata	Algae	96 h, Growth, NOEC	31820	(Isidori et al. 2005)		
	Daphnia magna	Crustacean	48 h, Mobility, NOEC	32,000	(Cleuvers, 2004)		
	Ceriodaphnids dubia	Crustacean	7 d, Reproduction, NOEC	330	(Isidori et al. 2005)		
	Brachionus calyciflorus	Rotifers	48 h, Reproduction, NOEC	560	(Isidori et al. 2005)		
	Hydra attenuata	Cnidarian	96 h, Morphology, NOEC	1000	(Quinn et al., 2008)		
Diclofenac	*Daphnia magna*	Crustacean	21 d, Mobility, NOEC	10,000	(Quinn et al. 2011)	3	49
	Daphnia magna	Crustacean	21 d, Survival, NOEC	25,000	(Lee et al. 2011)		
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	8,300	(Lee et al. 2011)		
	Moina macrocopa	Crustacean	7 d, Survival, NOEC	50,000	(Lee et al. 2011)		
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	10,000	(Han et al. 2006)		
	Moina macrocopa	Crustacean	7 d, Reproduction, NOEC	16,700	(Lee et al. 2011)		
	Danio rerio	Fish	72 h, Growth, NOEC	1,500	(van den Brandhof and Montforts, 2010)		
	Oryzias latipes	Fish	30 d, Survival, NOEC	> 10,000	(Lee et al. 2011)		
	Oryzias latipes	Fish	77 d, Survival, NOEC	> 10,000	(Lee et al. 2011)		
	Dunaliella tertiolecta	Algae	96 h, Cell density, NOEC	185,690	(DeLorenzo and Fleming, 2008)		
	Pseudokirchneriella subcapitata	Algae	96 h, Growth, NOEC	10,000	(Ferrari et al. 2003)		

Table S6. (Continued).

Compound	Species	Trophic group	Duration, Effect, Endpoint	Value (μg/L)	Reference	Assessment factor	PNEC (μg/L)
Diclofenac	*Brachionus calyciflorus*	Rotifers	48 h, Reproduction, NOEC	12,500	(Ferrari et al. 2003)		
	Ceriodaphnia dubia	Crustacean	7 d, Reproduction, NOEC	1,000	(Ferrari et al. 2003)		
	Salmo trutta f.fario	Fish	21 d, Histopathological alterations, NOEC	0.5	(Hoeger et al. 2005)		
	Oncorhynchus mykiss	Fish	21 d, Liver cytopathology, LOEC	1	(Triebskorn et al. 2007)		
	Oncorhynchus mykiss	Fish	28 d, Histopathological alterations, LOEC	5	(Schwaiger et al. 2004)		
	Danio rerio	Fish	ELS 10 d, Survival, NOEC	4,000	(Ferrari et al. 2003)		
Ibuprofen	*Desmodesmus subspicatus*	Algae	96 h, Growth, NOEC	32,000	(Cleuvers, 2004)	3	3.03
	Daphnia magna	Crustacean	48 h, Mobility, NOEC	75,000	(Cleuvers, 2004)		
	Daphnia magna	Crustacean	14 d, Survival, NOEC	20,000	(Heckmann et al. 2007)		
	Moina macrocopa	Crustacean	7 d, Reproduction, NOEC	25,000	(Han et al. 2010)		
	Daphnia magna	Crustacean	14 d, Population growth, LOEC	20,000	(Heckmann et al. 2007)		
	Daphnia magna	Crustacean	14 d, Survival, NOEC	20,000	(Heckmann et al. 2007)		
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	20,000	(Han et al. 2006)		
	Daphnia magna	Crustacean	21 d, Reproduction, NOEC	< 1,230	(Han et al. 2010)		
	Hydra attenuata	Cnidarian	96 h, Morphology, NOEC	100	(Quinn et al. 2008)		
	Oryzias latipes	Fish	120 d, Survival, NOEC	0.1	(Han et al. 2010)		
	Planorbis carinatus	mollusc	21 d, Growth, NOEC	1,020	(Pounds et al. 2008)		
	Planorbis carinatus	mollusc	21 d, Reproduction, NOEC	2,430	(Pounds et al. 2008)		
	Planorbis carinatus	mollusc	21 d, Survival, NOEC	5,360	(Pounds et al. 2008)		
	Lemna minor	duckweed	7 d, Growth, NOEC	310,000	(Fent et al. 2006)		
Indomethacin	*Thamnocephalus platyurus*	Crustacean	24 h, Mortality, LC_{50}	16,140	(Kim et al. 2009a)	1000	16.14
	Oryzias latipes	Fish	96 h, Mortality, LC_{50}	81,920	(Kim et al. 2009a)		
Gemfibrozil	*Pseudokirchneriella subcapitata*	Algae	72 h, Growth inhibition, NOEC	3,125	(Isidori et al. 2007)	10	7.8
	Daphnia magna	Crustacean	72h, Mobility, NOEC	30,000	(Zurita et al. 2007)		
	Daphnia magna	Crustacean	21 d, Mobility, NOEC	32,000	(Quinn et al. 2011)		
	Ceriodaphnids dubia	Crustacean	7 d, Population growth inhibition, NOEC	78	(Isidori et al. 2007)		
	Hydra attenuata	Cnidarian	96 h, Morphology, NOEC	100	(Quinn et al. 2008)		
	Brachionus calyciflorus	Rotifer	48 h, Population growth inhibition, NOEC	156	(Isidori et al. 2007)		
Mefenamic acid	*Danio rerio*	Fish	48 h, Mortality, NOEC	2,400	(Nadanaciva et al. 2013)	100	24.0

PNEC represents predicted no effect concentration; EC_{50} is an abbreviation of median effect concentration and LC_{50} means half lethal concentration. NOEC and LOEC represent no observed effect concentration and lowest observed effect concentration, respectively. For endpoints with no NOEC values, LOEC values were used in this study. PNECs values were derived using the statistical extrapolation methods where assessment factor is 1. Otherwise, PNECs were derived using assessment factor approach (Van Leeuwen, 2003). By this method, PNEC value was calculated by dividing the lowest acute EC_{50} or chronic NOEC value from the most sensitive species by an assessment factor, where the assessment factor of 1000, 100, 50 or 10 was used correspondingly for PNEC calculation when EC_{50} value for at least one trophic level or NOEC value for one, two or three trophic levels, with NOEC values prior to EC_{50} values to calculate the PNEC value.

Table S7. K_{OC} values of target compounds detected both in the water and sediment of urban rivers in Guangzhou.

Compound	n [a]	K_{OC}		
		Measured		Calculated [c]
		d_{10}-d_{90} [b]	Median	
4-NP	24	19400-105000	44500	38260
4-*t*-OP	19	12300-44700	16900	9979
BPA	24	2140-55000	10300	37670
TCC	24	33800-411000	141000	4070
TCS	24	5340-103000	22000	23400
HHCB	24	12000-117000	24200	19690
AHTN	19	18200-54400	32500	8678
MX	9	10100-82600	52700	33590
Clofibric acid	17	523-20700	5060	43.68
Naproxen	6	4380-11200	9960	335
Diclofenac	18	134-2150	744	458
Ibuprofen	19	42.9-1020	508	422.2

[a] measured sampling number with total sampling number n=24 in two seasons;

[b] d_{10}=first percentile, d_{90}=last percentile;

[c] Transformed from the LogK_{oc} values which were calculated by the EPI suite model based on the MCI method (USEPA, 2012).

Table S8. The percent of each distribution form for acid pharmaceuticals under the environmental pH values measured in the present study.

Season	Site	Naproxen		Ibuprofen		Ketoprofen		Diclofenac		Clofibric acid		Fenoprofen		Indomethacin		Meclofenamic		Mefenamic acid		Tolfenamic acid	
		Neutral	Ionized	Neutral	Ionized	Neutral	Ionized	Neutral	Ionized	Neutral	Ionized	Neutral	Ionized	Neutral	Ionized	Neutral	Ionized	Neutral	Ionized	Neutral	Ionized
Dry season	S0	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S1	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S2	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S3	0.00	1.00	0.01	0.99	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S4	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S5	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S6	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S7	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S8	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S9	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S10	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S11	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
Wet season	S0	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S1	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S2	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S3	0.00	1.00	0.01	0.99	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S4	0.00	1.00	0.01	0.99	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S5	0.00	1.00	0.01	0.99	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S6	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S7	0.00	1.00	0.01	0.99	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S8	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S9	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S10	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
	S11	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00

Table S9. The percent of each distribution form for 4-NP, 4-*t*-OP, BPA, TCS and TCC under the environmental pH values measured in the present study.

Season	Site	4-NP		4-*t*-OP		BPA		TCS		TCC	
		Neutral	Ionized	Neutral	Ionized	Neutral	Ionized	Neutral	Ionized	Neutral	Ionized
Dry season	S0	0.98	0.02	0.98	0.02	0.99	0.01	0.61	0.39	1	0
	S1	0.99	0.01	0.99	0.01	1	0	0.8	0.20	1	0
	S2	0.99	0.01	0.99	0.01	1	0	0.76	0.24	1	0
	S3	1	0	1	0	1	0	0.83	0.17	1	0
	S4	0.99	0.01	0.99	0.01	0.99	0.01	0.72	0.28	1	0
	S5	0.99	0.01	0.99	0.01	0.99	0.01	0.72	0.28	1	0
	S6	0.99	0.01	0.99	0.01	0.99	0.01	0.72	0.28	1	0
	S7	0.98	0.02	0.98	0.02	0.99	0.01	0.61	0.39	1	0
	S8	0.99	0.01	0.99	0.01	0.99	0.01	0.67	0.33	1	0
	S9	0.99	0.01	0.99	0.01	0.99	0.01	0.67	0.33	1	0
	S10	0.99	0.01	0.99	0.01	1	0	0.76	0.24	1	0
	S11	0.99	0.01	0.99	0.01	0.99	0.01	0.72	0.28	1	0
Wet season	S0	0.98	0.02	0.98	0.02	0.99	0.01	0.61	0.39	1	0
	S1	0.99	0.01	0.99	0.01	0.99	0.01	0.72	0.28	1	0
	S2	0.99	0.01	0.99	0.01	1	0	0.76	0.24	1	0
	S3	1	0	1	0	1	0	0.83	0.17	1	0
	S4	1	0	1	0	1	0	0.83	0.17	1	0
	S5	1	0	1	0	1	0	0.89	0.11	1	0
	S6	0.99	0.01	0.99	0.01	1	0	0.8	0.2	1	0
	S7	1	0	1	0	1	0	0.91	0.09	1	0
	S8	0.99	0.01	0.99	0.01	0.99	0.01	0.72	0.28	1	0
	S9	0.99	0.01	0.99	0.01	0.99	0.01	0.72	0.28	1	0
	S10	0.99	0.01	0.99	0.01	1	0	0.8	0.20	1	0
	S11	0.99	0.01	0.99	0.01	1	0	0.76	0.24	1	0

Table S10. Water quality assessment for urban rivers in Guangzhou in two seasons in 2015 by comprehensive water quality identification index.

Rivers	Site	Dry season	Wet season
Liuxi River	S0	III	III
	S1	IV	III
	S2	IV	IV
	S3	V	V
Zhujiang River	S4	V	V
	S5	V	V
	S7	V	V
	S10	V	V
Central urban rivers	S6	> V	> V
	S8	> V	> V
	S9	> V	> V
	S11	> V	> V

Table S11 Concentrations (ng/L) of EDCs and PPCPs detected in the surface water of urban rivers in Guangzhou in two seasons.

Season	Site	4-NP	4-*t*-OP	BPA	TCC	TCS	HHCB	AHTN	MX	MK	Clofibric acid	Naproxen	Diclofenac	Ibuprofen	Indomethacin	Gemfibrozil	Mefenamic acid
Dry season	S0	25.1±2.79	<LOD	2.30±0.43	2.54±0.15	1.81±0.33	2.70±0.72	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	S1	415±11.7	30.1±1.65	68.0±4.31	9.60±1.06	6.93±0.69	18.5±3.46	<LOD	8.86±0.76	<LOD	<LOD	<LOQ	4.87±0.07	2.97±0.14	<LOD	<LOD	<LOD
	S2	506±33.3	16.7±2.28	242±4.15	18.2±0.62	21.3±1.35	109±4.29	8.19±0.19	<LOD	7.94±0.60	0.26±0.03	0.94±0.03	10.53±0.52	20.2±1.51	2.10±0.35	<LOD	<LOD
	S3	2,140±99.8	55.3±5.78	461±10.7	66.0±0.95	73.5±0.64	273±8.79	22.0±0.79	<LOD	28.0±2.01	1.10±0.11	0.89±0.17	14.50±0.11	60.6±2.40	1.70±0.01	0.35±0.03	<LOQ
	S4	1,290±37.9	43.2±15.5	122±6.76	25.8±1.04	34.8±0.30	204±9.74	17.2±1.03	<LOD	13.5±1.13	1.38±0.11	0.95±0.24	20.59±0.64	48.9±2.08	2.30±0.18	0.57±0.01	<LOQ
	S5	2,010±58.4	74.2±5.82	397±7.22	183±0.44	50.8±1.73	281±14.1	33.7±1.37	<LOD	15.0±1.40	2.35±0.16	1.35±0.18	22.73±0.70	55.8±3.78	2.64±0.19	1.20±0.07	0.72±0.05
	S6	5,050±118	108±7.26	892±26.7	210±0.21	282±9.38	753±8.72	126±0.38	34.7±3.41	98.2±3.50	19.7±1.11	<LOD	645±28.3	542±7.97	69.6±2.21	9.52±1.84	<LOQ
	S7	1,100±68.9	29.3±3.36	83.8±9.45	43.5±0.99	28.0±0.14	125±1.93	17.6±1.00	<LOD	13.1±0.99	3.75±0.19	1.34±0.06	33.1±1.64	50.3±4.51	2.57±0.19	0.48±0.02	3.55±0.11
	S8	2,660±56.6	62.8±6.31	236±11.5	141±0.96	209±4.21	502±35.1	86.1±5.29	37.9±4.98	56.2±3.78	12.7±0.55	0.77±0.07	381.±25.9	475±23.36	40.2±1.12	9.83±1.01	2.65±0.23
	S9	980±39.1	21.9±1.67	382±7.32	88.4±0.63	142±6.33	306±7.82	41.0±2.78	28.3±1.84	25.2±1.71	0.72±0.03	0.96±0.09	51.2±7.24	121.4±1.75	8.05±0.58	3.12±0.31	2.75±0.19
	S10	1,300±132	85.8±13.54	310±16.8	42.4±1.67	32.1±1.05	159±2.99	22.9±0.29	<LOD	17.0±0.86	4.21±0.15	1.71±0.41	27.7±0.81	69.3±5.26	3.24±0.29	1.11±0.17	1.66±0.31
	S11	1,730±77.4	98.7±1.37	428±7.74	60.8±0.24	82.1±1.65	314±16.2	38.9±2.82	43.8±4.98	25.2±2.13	1.84±0.12	0.74±0.11	52.5±2.30	75.4±6.89	6.69±0.14	1.78±0.16	1.01±0.12
Wet season	S0	22.5±1.89	<LOD	2.69±0.52	2.37±0.06	5.06±0.17	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	S1	110±8.32	4.14±0.28	12.9±1.44	5.37±0.52	7.54±0.54	5.26±0.20	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.11±0.30	<LOQ	<LOD	<LOD
	S2	207±13.7	12.2±0.73	183±8.82	18.4±1.69	34.2±2.42	69.8±2.49	8.96±1.53	<LOD	2.96±0.68	0.29±0.03	0.46±0.03	6.06±0.35	6.63±0.40	0.89±1.22	0.35±0.05	0.99±0.31
	S3	835±72.6	41.4±2.01	92.0±2.68	17.9±0.49	39.5±2.86	128±1.68	13.1±1.54	<LOD	3.46±0.29	0.38±0.02	0.52±0.05	5.33±0.83	16.2±1.73	1.55±0.29	0.54±0.04	2.05±0.06
	S4	694±23.5	24.7±0.74	67.6±7.21	20.9±0.81	37.0±4.19	119±10.5	13.1±2.35	38.0±2.85	3.75±0.07	0.50±0.03	0.50±0.04	8.38±0.38	18.8±1.32	1.42±1.51	0.57±0.12	1.06±0.09
	S5	1,070±68.4	47.7±3.20	106±13.6	27.4±0.81	38.2±2.30	160±3.86	20.4±1.71	<LOD	4.40±0.26	0.73±0.03	0.73±0.08	8.07±1.05	20.4±1.80	1.49±1.14	0.71±0.07	<LOQ
	S6	3,310±209	95.9±5.11	544±16.5	88.0±0.80	220±2.17	685±46.3	64.9±4.16	19.4±1.52	15.8±0.55	1.45±0.10	1.81±0.30	20.84±1.77	31.8±2.40	1.36±0.26	<LOQ	<LOQ
	S7	823±19.6	86.4±3.75	215±6.95	40.8±1.35	39.8±2.50	166±6.82	20.9±1.24	<LOD	4.04±0.27	1.46±0.14	<LOQ	15.91±0.71	37.6±4.35	2.28±1.20	0.69±0.15	1.16±0.20
	S8	759±39.4	40.6±3.83	509±7.37	68.4±1.43	155±5.43	397±24.4	48.1±3.87	41.6±2.94	18.4±0.58	2.11±0.12	2.06±0.01	14.41±0.37	18.5±0.17	2.64±0.11	<LOQ	<LOD
	S9	1,420±114	24.4±1.91	744±44.9	135±2.19	253±6.91	540±10.9	74.0±2.56	15.7±1.47	13.0±1.60	2.53±0.12	4.28±0.34	7.90±1.61	14.0±1.57	1.54±0.14	<LOD	<LOQ
	S10	948±36.7	38.2±2.28	231±15.6	49.9±2.60	67.6±3.83	243±14.1	26.2±1.96	<LOD	5.91±0.51	1.43±0.22	1.28±0.24	9.75±0.55	24.4±2.32	1.69±0.36	0.75±0.07	<LOD
	S11	1,810.00±59.4	165±9.61	1,340±58.2	118±1.60	227±3.61	535±21.6	61.4±2.99	30.3±2.94	18.3±3.49	0.58±0.03	1.52±0.21	6.45±0.04	10.5±0.83	0.80±0.05	<LOD	<LOQ

[a] The data presented in this table were mean ± standard deviation (n=3); LOD, method limit of detection; LOQ, method limit of quantification.

Table S12. Comparison of the concentrations (ng/L) of EDCs and PPCPs in surface water in this study with other regions.

Compound	Asia			Europe									America
	China		Korea	Italy	Romania	Spain	Austria	Greece	Switzerland	Germany	the Netherlands	U.K.	USA
4-NP	22.5-5050	28.1-8890	360-3770	<0.5-1470		n.d-1030		558-2700	<29.0-195		n.d-4100		n.d-490
4-*t*-OP	n.d-165	1.0-2470	n.d-137			n.d-880			<1.20-6.90		n.d-6300	n.d-1290	n.d
BPA	2.30-1340	2.2-1030	n.d-637	<1.0-145		n.d-3700		55.0-162	2.00-46.0	42.0-417	n.d-1000	n.d-536	n.d-147
TCC	2.37-210	n.d-338				n.d-3.40							3.30-75.0
TCS	1.81-282	n.d-347	1.0-82		n.d-64.3	n.d-285		3.00-98.0		<3-10.0		n.d-95.0	0.5-28.3
HHCB	n.d-753	3.5-93.0	260-13920		153-340		n.d-32			n.d-678			3.95-794
AHTN	n.d-126	2.3-26.7	50.0-2800		76.0-120		n.d-<40			n.d-299			5.09-112
MX	n.d-43.8												
MK	n.d-98.2	n.d-34.64	n.d-420										
Clofibric acid	n.d-19.7	n.d-16.8	n.d-14.0							0.1-119		n.d-164	3.20-26.7
Naproxen	n.d-4.28	n.d-125	n.d-360					<3-322		0.3-92.9		n.d-146	n.d-135.2
Ibuprofen	n.d-645	n.d-685	n.d-414		n.d-136			n.d-67.0		0.1-33.6		n.d-100	n.d-37.9
Diclofenac	n.d-542	7.8-170	n.d-150					n.d-1040		0.4-245		n.d-261	

Table S12. (Continued).

Compound	Asia			Europe									America
	China		Korea	Italy	Romania	Spain	Austria	Greece	Switzerland	Germany	the Netherlands	U.K.	USA
Ketoprofen								n.d-395				n.d-14.0	
Gemfibrizi	n.d-9.83	n.d-63.4	n.d-46.0							0.2-34.5			
Indomethacin	n.d-69.6	6.8-77.9	n.d-33.5										
Mefenamic acid	n.d-3.55	n.d-24.6	n.d-326									n.d-169	
	This study	Chen et al., 2014a	Kim et al., 2014	Laganà et al., 2004	Moldovan, 2006	Brix et al., 2010	Clara et al., 2011	Stasinakis et al., 2012	Jonkers et al., 2009	Bester, 2005a	Vethaak et al., 2005	Kasprzyk-Hordern et al., 2008	Chase et al., 2012
		Dai et al., 2015	Kim et al., 2007	Pojana et al., 2007		Gorga et al., 2015				Dsikowitzky et al., 2002			Gautam et al., 2014
		Hu et al., 2011	Kim et al., 2009b			Kantiani et al., 2008				Möder et al., 2007			Katz et al., 2013
		Zhang et al., 2008	Lee et al., 2010			Salgueiro-González et al., 2015				Quednow and Püttmann, 2008			Klosterhaus et al., 2013
		Zhao et al., 2009	Sim et al., 2010			Villaverde-de-Sáa et al., 2010							Kumar et al., 2010
		Zhao et al., 2010 a	Yoon et al., 2010										Loyo-Rosales et al., 2007
		Zhao et al., 2010b											Reiner and Kannan, 2011
													Wilson et al., 2009
													Zhang et al., 2007

Table S13. Concentrations (ng/g) of EDCs and PPCPs detected in the sediment of urban rivers in Guangzhou in two seasons.

Season	Sites	4-NP	4-*t*-OP	BPA	TCC	TCS	HHCB	AHTN	MX	Clofibric acid	Naproxen	Diclofenac	Ibuprofen
Dry season	S0	10.9±1.01	<LOD	2.54±0.30	1.82±0.25	0.84±0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	S1	31.7±2.20	<LOD	2.72±0.77	2.45±0.43	1.49±0.17	10.9±3.15	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	S2	1,000±31.7	11.4±1.01	78.1±3.02	483±13.5	145±3.73	196±10.2	24.0±1.47	28.7±4.02	0.34±0.09	<LOD	<LOD	1.10±0.21
	S3	4,560±196	54.9±3.94	269±7.44	373±7.06	62.0±4.16	191.±4.91	29.2±1.79	37.3±0.89	0.79±0.21	0.38±0.08	0.72±0.16	2.47±0.14
	S4	3,100±172	33.8±3.50	120±7.67	197±3.30	134±4.32	189±15.1	30.2±3.14	<LOD	<LOQ	0.42±0.12	0.59±0.12	1.26±0.19
	S5	4,340±208	58.9±4.04	194±10.0	330±4.29	101±3.68	127±10.2	29.9±7.68	12.1±1.02	<LOD	0.29±0.05	0.64±0.09	1.03±0.23
	S6	14,400±221	191±0.30	145±2.20	3,290±137	534±20.2	1,280±15.5	235±6.59	376±5.52	0.25±0.04	<LOD	10.6±0.87	2.16±0.28
	S7	4,370±37.3	42.5±0.31	249±19.4	373±7.81	46.4±4.32	117±9.82	31.1±2.60	<LOD	<LOD	<LOD	0.67±0.11	2.59±0.19
	S8	5,840±687	88.7±12.3	89.2±8.76	2,300±140	689±7.18	1,480±21.4	210±8.15	70.8±3.52	0.64±0.18	<LOD	8.49±0.58	1.08±0.05
	S9	7,670±78.3	68.4±0.78	164±12.7	3,440±210	109±4.35	842±9.42	142±12.0	82.3±1.96	0.34±0.07	<LOD	0.65±0.13	0.91±0.18
	S10	2,780±61.8	27.1±1.52	79.2±6.72	359±10.0	94.6±2.87	216±6.62	47.6±2.0	<LOD	<LOQ	<LOD	0.42±0.06	3.19±0.28
	S11	5,100±410	260.9±36.9	56.9±3.57	581±27.4	215±8.93	545±4.91	86.9±3.28	145.6±6.51	0.56±0.11	<LOD	0.98±0.14	0.91±0.02
Wet season	S0	17.9±5.68	<LOD	6.47±1.94	1.95±0.36	0.87±0.08	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	S1	42.7±1.06	<LOD	6.67±1.17	2.68±0.44	1.70±0.50	7.59±1.10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	S2	115±5.87	<LOD	16.6±0.40	2.48±0.51	1.01±0.30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	S3	5,220±32.2	104±9.20	216±8.75	155±12.7	76.3±2.73	148±5.83	27.2±6.00	17.7±1.88	<LOD	0.23±0.03	89±0.15	0.38±0.07
	S4	1,700±32.8	21.7±0.71	154±1.60	148±8.56	67.3±3.95	106±3.92	21.4±4.08	55.4±1.65	0.24±0.04	0.24±0.05	0.42±0.07	0.44±0.09
	S5	1,730±40.9	24.6±1.45	158±8.53	184±6.62	93.0±5.08	111±4.16	23.3±4.08	54.8±4.01	0.27±0.05	0.36±0.09	0.35±0.05	0.29±0.05
	S6	11,700±570	143±3.03	433±7.79	1,210±42.5	199±7.35	1,430±45.8	205±6.70	205±0.79	<LOQ	<LOD	0.99±0.13	1.02±0.20
	S7	5,120±123	63.6±3.08	352±6.22	276±6.15	54.3±1.94	232±9.90	48.6±2.61	<LOD	0.27±0.01	<LOD	0.97±0.19	0.37±0.11
	S8	796±12.8	31.0±1.45	92.9±7.28	272±68.0	36.7±3.83	293±19.9	34.3±2.00	<LOD	0.67±0.72	<LOD	0.64±0.12	0.45±0.12
	S9	7,950±326	67.3±5.69	301±15.8	1,090±55.1	47.7±5.02	774±31.2	132±15.6	87.1±0.28	0.46±0.39	<LOD	0.43±0.08	0.51±0.10
	S10	2,140±57.9	31.6±6.65	130±6.21	443±32.7	32.1±5.24	268±4.49	43.4±4.11	<LOD	0.37±0.13	<LOD	0.39±0.05	0.29±0.06
	S11	8,591±156	219±9.02	211±3.14	899±20.4	134±7.48	1,179±5.41	179±3.07	124±1.22	0.34±0.10	<LOD	0.44±0.01	0.67±0.15

[a] The data presented in this table were mean ± standard deviation (n=3); LOD, method limit of detection; LOQ, method limit of quantification.

Table S14. Comparison of the concentrations (ng/g) of EDCs and PPCPs in sediment in this study with other regions

	Asia			Europe					America
Compound	China		Korea	Italy	Spain	Austria	Germany	the Netherlands	US
4-NP	10.9-14400	1.5-7808	4.9-14500	47-192	n.d-4460			ND-3800	21.5-7000
OP	n.d-261	1.0-93.0	n.d-1360		n.d-76			ND-26	
BPA	2.54-433	n.d-33.8	1.7-122	2-118	n.d-130			ND-43	
TCC	1.82-3440	1.79-5649			n.d-29				2.89-52.0
TCS	0.84-689	1.43-1329			n.d-388				<0.5-400
HHCB	n.d-1480	1.5-121.0	n.d-6.3			n.d-120	<2-191		3.2-388
AHTN	n.d-235	2.0-42.5	n.d2.3			n.d-20	<2-1399		0.96-544
	This study	Chen et al., 2006	Lee et al., 2014		Brix et al., 2010	Clara et al., 2011	Dsikowitzky et al., 2002	Vethaak et al., 2005	Cantwell et al., 2010
		Chen et al., 2014a	Kim et al., 2014		Gorga et al., 2015				Katz et al., 2013
		Hu et al., 2011		Pojana et al., 2007	Salgueiro-González et al., 2015				Klosterhaus et al., 2013
		Wang et al., 2011							Kumar et al., 2010
		Zeng et al., 2008							Loyo-Rosales et al., 2007
		Zhang et al., 2008							Reiner and Kannan, 2011
		Zhao et al., 2009							Peck et al., 2006
		Zhao et al., 2010a							Wilson et al., 2009
		Zhao et al., 2010b							

Table S15. RQ values of EDCs and PPCPs detected in the surface water of urban rivers in Guangzhou in two seasons.

Season	Site	4-NP	4-*t*-OP	BPA	TCC	TCS	HHCB	AHTN	MX	MK	Clofibric acid	Naproxen	Diclofenac	Ibuprofen	Indomethacin	Gemfibrozil	Mefenamic acid
Dry season	S0	0.05	0	0	0	0.07	0	0	0	0	0	0	0	0	0	0	0
	S1	0.86	0.25	0.08	0.01	0.26	0	0	0	0	0	0	0	0	0	0	0
	S2	1.05	0.14	0.28	0.03	0.81	0	0	0.01	0	0	0	0	0	0	0	0
	S3	4.46	0.45	0.54	0.10	2.81	0.01	0	0	0	0	0	0	0	0	0	0
	S4	2.69	0.35	0.14	0.04	1.33	0.01	0	0	0	0	0	0	0	0	0	0
	S5	4.19	0.61	0.46	0.28	1.94	0.01	0.01	0	0	0	0	0	0	0	0	0
	S6	10.5	0.89	1.04	0.32	10.8	0.02	0.02	0.03	0	0.04	0	0	0.04	0	0	0
	S7	2.29	0.24	0.10	0.07	1.07	0	0	0	0	0.01	0	0	0	0	0	0
	S8	5.54	0.51	0.27	0.21	7.98	0.01	0.01	0.04	0	0.03	0	0	0.04	0	0	0
	S9	2.04	0.18	0.44	0.13	5.42	0.01	0.01	0.03	0	0	0	0	0.01	0	0	0
	S10	2.71	0.70	0.36	0.06	1.23	0	0	0	0	0.01	0	0	0.01	0	0	0
	S11	3.60	0.81	0.50	0.09	3.13	0.01	0.01	0.04	0	0	0	0	0.01	0	0	0
Wet season	S0	0.05	0	0	0	0.19	0	0	0	0	0	0	0	0	0	0	0
	S1	0.23	0.03	0.02	0.01	0.29	0	0	0	0	0	0	0	0	0	0	0
	S2	0.43	0.10	0.21	0.03	1.31	0	0	0	0	0	0	0	0	0	0	0
	S3	1.74	0.34	0.11	0.03	1.51	0	0	0	0	0	0	0	0	0	0	0
	S4	1.45	0.20	0.08	0.03	1.41	0	0	0.04	0	0	0	0	0	0	0	0
	S5	2.23	0.39	0.12	0.04	1.46	0	0	0.00	0	0	0	0	0	0	0	0
	S6	6.90	0.79	0.63	0.13	8.40	0.02	0.01	0.02	0	0	0	0	0	0	0	0
	S7	1.71	0.71	0.25	0.06	1.52	0	0	0	0	0	0	0	0	0	0	0
	S8	1.58	0.33	0.59	0.10	5.92	0.01	0.01	0.04	0	0	0	0	0	0	0	0
	S9	2.96	0.20	0.87	0.20	9.66	0.01	0.01	0.02	0	0.01	0	0	0	0	0	0
	S10	1.98	0.31	0.27	0.08	2.58	0.01	0	0	0	0	0	0	0	0	0	0
	S11	3.77	1.35	1.56	0.18	8.66	0.01	0.01	0.03	0	0	0	0	0	0	0	0

Table S16. RQ values of EDCs and PPCPs detected in the sediment of urban rivers in Guangzhou in two seasons.

Season	Sites	4-NP	4-*t*-OP	BPA	TCC	TCS	HHCB	AHTN	MX	Clofibric acid	Naproxen	Diclofenac	Ibuprofen
Dry season	S0	0.02	0	0.01	0	0.06	0	0	0	0	0	0	0
	S1	0.37	0	0.08	0.01	0.64	0	0	0	0	0	0	0
	S2	0.89	0.10	0.16	0.10	4.67	0	0	0.02	0	0	0	0
	S3	3.91	0.49	0.56	0.07	1.97	0	0	0.02	0.01	0	0	0.01
	S4	3.04	0.34	0.28	0.04	4.86	0	0	0	0	0	0	0
	S5	4.15	0.58	0.45	0.07	3.58	0	0	0.01	0	0	0	0
	S6	4.70	0.65	0.11	0.25	6.46	0.01	0.01	0.08	0	0	0	0
	S7	3.36	0.34	0.46	0.07	1.32	0	0	0	0	0	0	0.01
	S8	3.28	0.52	0.12	0.30	14.3	0.02	0.01	0.03	0	0	0	0
	S9	3.86	0.36	0.20	0.40	2.03	0.01	0.01	0.03	0	0	0	0
	S10	6.20	0.63	0.43	0.18	7.82	0.01	0.01	0	0	0	0	0.02
	S11	4.04	2.14	0.11	0.11	6.31	0.01	0.01	0.07	0	0	0	0
Wet season	S0	0.05	0	0.05	0	0.10	0	0	0	0	0	0	0
	S1	0.18	0	0.07	0	0.27	0	0	0	0	0	0	0
	S2	0.26	0	0.09	0	0.08	0	0	0	0	0	0	0
	S3	6.30	1.30	0.63	0.04	3.41	0	0	0.01	0	0	0	0
	S4	1.73	0.23	0.38	0.03	2.53	0	0	0.04	0	0	0	0
	S5	1.84	0.27	0.40	0.04	3.66	0	0	0.04	0	0	0	0
	S6	5.63	0.71	0.50	0.13	3.55	0.02	0.01	0.06	0	0	0	0
	S7	4.16	0.54	0.69	0.05	1.63	0	0	0	0	0	0	0
	S8	1.60	0.64	0.45	0.13	2.73	0.01	0.01	0	0.01	0	0	0
	S9	6.02	0.53	0.55	0.19	1.34	0.01	0.01	0.04	0	0	0	0
	S10	1.90	0.29	0.28	0.09	1.06	0.01	0	0	0	0	0	0
	S11	5.20	1.37	0.31	0.12	3.01	0.02	0.01	0.05	0	0	0	0

Table S17. Risk assessment of 4-NP and TCS to each taxa in surface water and sediment in two seasons.

Compound	Threshold	Water		Sediment	
		Dry season	Wet season	Dry season	Wet season
4-NP	Algae	92%	75%	83%	75%
	PNEC	83%	75%	75%	75%
	Fish	75%	58%	75%	67%
	Rotifer	67%	33%	75%	42%
	Crustacean	17%	8%	17%	33%
	Insect	8%	0%	0%	0%
TCS	Algae	83%	83%	83%	75%
	PNEC	75%	83%	83%	75%

Thresholds were PNEC values as well as those derived from toxicity data for different taxa (Algae, fish, rotifer, crustacean and Insect). Toxicity thresholds, endpoints and PNECs are given in Table S6. The risk probability (*p*) was obtained by dividing the number of sampling site where a compound concentration exceeded the toxicity threshold of a particular species by the total number of sampling site where this compound was detected.

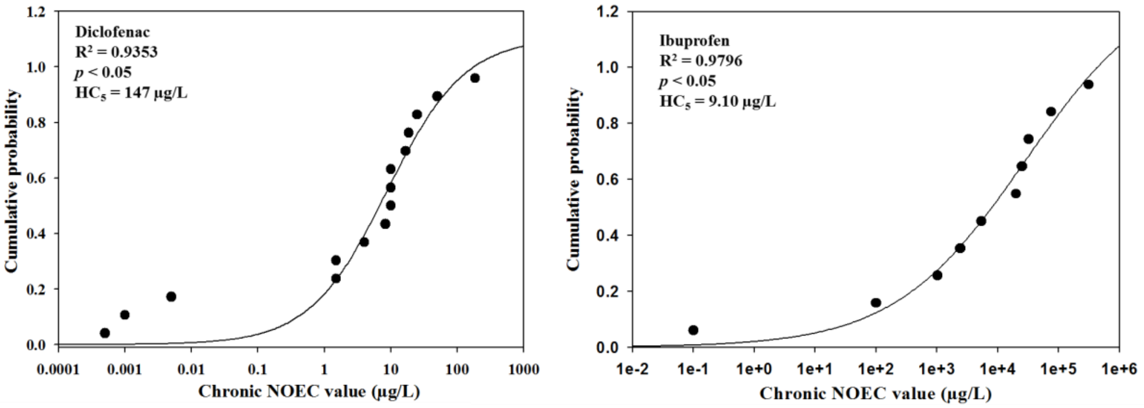

Figure S1. Derivation of the PNEC values for diclofenac and ibuprofen using species sensitivity distribution.

Chapter 3

Correlation of benthic invertebrate and microbial biodiversity in typical urban rivers with environmental and emerging chemicals

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Abstract

Urban rivers often function as harbors receiving various contaminants. Sediment in these rivers may suffer contamination and further influences the biological community in the river environments. We performed a comprehensive biological survey of the benthic macroinvertebrate and microbial community distribution in the sediments of six rivers from the suburb to the central urban area of Guangzhou city (South China), and evaluated their correlations with emerging organic contaminants, heavy metals and nutrients. The results showed that benthic macroinvertebrate community shifted from gastropods to oligochaeta along a contamination gradient, except for the site in the Sha River where chironomids were abundant. The most distinct trait was asexual reproduction that was significantly correlated with chromium and total phosphorus. There was no significant difference in benthic macroinvertebrate composition between the dry season and wet season. Likewise, the sediment microbial community composition also changed along a contamination gradient. In both seasons there was a significant difference in relative abundances of *Nitrospirae, Gemmatimonadetes*, *Chlorobi*, *Cloacimonetes* and *Synergistetes* among the Liuxi River, Zhujiang River and central urban rivers. These phyla were significantly correlated with organic contaminants, heavy metals and nutrients. However, the site in the Sha River had different benthic macroinvertebrate and microbial community composition from the other urban rivers. Our results suggest that input of sewage significantly altered the benthic macroinvertebrate and microbial community compositions in sediments.

1. Introduction

Urban rivers are often exposed to anthropogenic impacts such as wastewater discharge. In the aquatic environment, pathogens, nutrients, heavy metals and organic chemicals can bind to suspended particles that eventually settle to the bottom of rivers (Burton, 2002). Consequently, sediments may accumulate contaminants and serve as a reservoir of contaminants for bioaccumulation and trophic transfer, even causing significant contamination to the overlying water and loss of desirable species (Burton, 2002). In highly urbanized areas such as Guangzhou (South China), wastewater treatment plants (WWTPs) have been constructed with effluents routinely discharging into the freshwater ecosystems. Thus, nutrients, organic and inorganic constituents may be released to the receiving waters, which may directly influence the composition of benthic macroinvertebrate and microbial communities in the sediment (Fredrickson et al., 1989; Martino et al., 1998; Li et al., 2012). However, little is known about the effects of contaminants release on the biological communities in the sediments of these receiving rivers.

Biological assemblages have been used as an effective tool to evaluate contamination caused by sewage (Hilty and Merenlender, 2000; Moreno and Calisto, 2006). Benthic macroinvertebrates are the most preferred bio-indicators to evaluate ecological status in the freshwater environment (Moreno and Calisto, 2006), because of their several properties including sedentary lifestyle, large size, relatively long life span and variable tolerance to pressures (Dauer, 1983; Jordan and Smith, 2005). Recently, biological traits analysis (BTA), using a series of functional traits (e.g. feeding habitats, body size, reproduction strategy), have been recognized as an useful analytical approach to describe ecological functioning (Bremner et al., 2003; Van den Brink et al., 2011; Bolam and Eggleton, 2014), even better than traditional methods (Díaz and Cabido, 2001). For example, Munari et al. (2013) reported that biological traits composition of the benthic community was more stable than its taxonomic composition. In the freshwater ecosystems, the BTA can reliably distinguish the effects of disturbances (e.g. Dolédec et al., 1999; Statzner and Bêche, 2010), make community structure less confounded by naturally spatial gradients, and more reliably indicate human impacts in comparison with traditionally used approaches (Dolédec et al., 1999). However, there are a few weaknesses for BTA, such as traits autocorrelation, traits redundancy, data management and knowledge development (Van den Brink et al., 2011). Therefore, in this study, BTA and taxonomy-based approach were applied jointly to investigate and characterize correlations between benthic macroinvertebrate community and urban contamination that focused on emerging organic contaminants, heavy metal contaminants and nutrients.

As another widely used indicator of river environmental conditions (Atlas, 1984; Boothroyd and Stark, 2000), indigenous bacteria in the sediments can play a vital role in the transformation and mineralization of inorganic and organic contaminants in wastewater discharges (Fredrickson et al., 1989; Li et al., 2016). In turn, nutrients and contamination can significantly influence the microbial community composition (Staley et al., 2014; Ibekwe et al., 2016). In addition, bioturbation activities of benthic macroinvertebrates can also influence microbial community composition in sediments (Zeng et al., 2014). Although benthic macroinvertebrate and microbial community composition have been individually characterized spatially and temporally in various environments (e.g., Bolam and Eggleton, 2014; Souza et al., 2013; 2016; Li et al., 2016; Staley et al., 2014; 2015), they have rarely been assessed together in field research. However, understanding the correlations between contamination and biological community composition of invertebrates and microbes in sediments is highly important for sediment quality assessment.

The lower Zhujiang River and many of its tributaries cut through Guangzhou city (south China), a highly urbanized megacity with a population of 13.1 million (http://www. guangzhou. gov.cn/). Many of these tributaries function as channels receiving sewage effluent, as WWTP effluents are routinely released into these rivers (http://www.gzepb.gov.cn/). Currently, little is known about the ecological states in these rivers. However, such information can help identify whether restoration (remediation) activities such as dredging are needed to restore the ecosystem quality in these waterways.

Given the vital role of benthic macroinvertebrates and microbes in the ecological functioning of rivers, the objectives of this study were: (i) to investigate the benthic macroinvertebrate and microbial community distribution in the sediment of six rivers in Guangzhou City (South China) following a contamination gradient, (ii) to identify their differences across space and season, (iii) to reveal environmental variables that may correlate with the taxon composition among rivers, and (iv) to identify traits that modulate the species-environment relationship. Therefore, we measured concentrations of seven emerging organic compounds (i.e., 4-nonylphenol (4-NP), 4-tert-octylphenol (4-*t*-OP), bisphenol A (BPA), triclocarban (TCC), triclosan (TCS), galaxolide (HHCB) and tonalide (AHTN)), eight heavy metals (i.e., arsene (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn)), and four nutrients (i.e., total organic carbon (TOC), total phosphorus (TP), total nitrogen (TN) and ammonia-nitrogen (NH_3-N)) in sediments of 12 sampling sites in six rivers from rural area to urban area in the dry season (March) and wet season (July). Meanwhile, we explored the benthic macroinvertebrate community distribution in six rivers using the biological traits-based method in combination with the traditional taxonomy-based method, and determine sediment microbial community composition and diversity using the illumina Hiseq 2000 sequencing system.

2. Materials and methods

2.1. Study area and sample collection

The study area and sampling locations were the same as reported by Peng et al. (2017). Seasonal sampling was performed in the dry (March 2015) and wet season (July 2015) at 12 different sites (S0-S11) in six connected rivers. These sites were selected based on the extent of wastewater impact. Liuxi reservoir (S0) and the upstream part of the Liuxi River (S1) are located in the preserved headwater of Guangzhou city, and are minimally exposed to human stressors, whereas the remaining sampling sites are exposed to urbanization of its surrounding landscape and WWTP effluent discharges. The sampling map and detailed sites description are shown in Fig. 1 and Table S1. At each site, surface sediment samples were collected utilizing a van Veen grab (area: 0.0435 m^2; volume: 5 L). Sediment samples for benthic macroinvertebrates analysis were wet sieved (300 μm) on site. Benthic macroinvertebrates were then picked out and preserved in 70% ethanol. Additional sediment samples were taken for the analysis of microbial community, organic compounds, heavy metals and nutrients. In the laboratory, the organisms were further sorted and identified to genus level (where possible) under a microscope (Dataset S1).

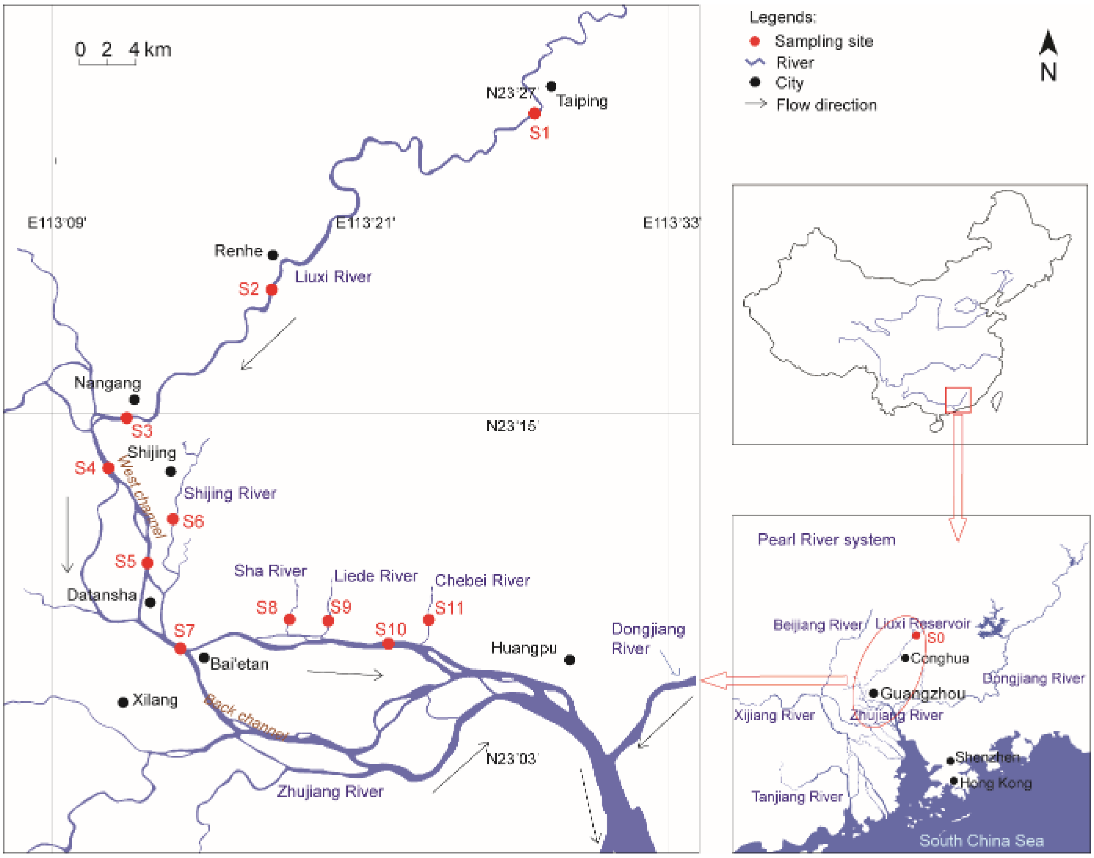

Figure 1. Map showing sampling locations in urban rivers of Guangzhou in 2015. Specifically, S0 is situated in Liuxi Reservoir that is a drinking water source in Guangzhou. S1, S2 and S3 are located in upstream, midstream and downstream of Liuxi River, respectively; S4, S5, S7 and S10 are in the main stem of Zhujiang River; S6, S8, S9 and S11 are located in Shijing River, Sha River, Liede River and Chebei River in urban region of Guangzhou, respectively.

2.2. Environmental variables analysis

Except for the seven emerging organic compounds and four nutrients that have been reported in Peng et al. (2017), eight common heavy metals including As, Cd, Cr, Cu, Pb, Hg, Ni and Zn

(Dataset S2) were also measured in the sediment samples because of their ubiquitous presence in urban runoff and their potential to impact the environment (Cheung et al., 2003; Liu et al., 2011) using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500, Agilent) (Yuan et al., 2014).

2.3. Traits information collection

The biological traits information of benthic macroinvertebrate community was obtained by matching the monitored benthic macroinvertebrate taxa in this study with the biological trait data available in the Tachet database (Tachet et al., 2000; Usseglio-Polatera et al., 1999, 2000). As we only focused on the correlations with contamination in this study, we excluded traits that are more related to the environmental conditions of the ecosystem, such as substrate and current velocity. The final trait dataset contained 56 trait states and is provided in the supporting information (Dataset S3), which corresponds to that reported by Rico et al. (2016).

2.4. DNA extraction and amplicon sequencing

Microbial DNA was extracted from 0.3 g of sediment using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). Universal primers 515F 5’-barcode-GTGCCAGCMGCCGCGG)-3’ and 907R 5’-CCGTCAATTCMTTTRAGTTT-3’ with a unique 8-nt barcode were used to amplify the V4-V5 region of the bacterial 16S ribosomal RNA gene by polymerase chain reaction (PCR). All samples were run in technical triplicate reactions to account for PCR variability. The amplification reactions contained 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. PCR conditions were 95 ˚C for 2 min for initial denaturation, followed by 25 cycles at 95 ˚C for 30 s, 55 ˚C for 30 s, and 72 ˚C for 30 s and a final extension at 72 ˚C for 5 min. 2% agarose gels was used to extract amplicons, followed by purification using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer’s instructions. Purified PCR products were further quantified by Qubit®3.0 (Life Invitrogen). The pooled DNA product was used to construct Illumina Pair-End library according to the Illumina’s genomic DNA library preparation procedure. After that, the amplicon library was paired-end sequenced on an Illumina HiSeq 2000 platform following the standard protocols.

2.5. Data analysis

To examine the benthic macroinvertebrate species-environment relationship and trait-environment relationship, multivariate analyses were performed using Canoco 5.1 software (Ter Braak and Šmilauer, 2018a). Partial redundancy analysis (RDA) with forward selection using season as covariate was performed to examine the species-environment relationship, as the expected relationship is monotonic. Monte Carlo permutation test under the RDA was performed using season as explanatory variable and rivers to test the significance of the difference in benthic macroinvertebrate community composition between the two seasons. Double constrained ordination is an ordination of a rectangular response data table in which the scores of both the rows (cases) and the columns (response variables) are constrained by linear combinations of predictor variables (Ter Braak et al., 2018b). In particular, double constrained principal component analysis (dc-PCA) constructs linear combinations of the traits and of the environmental variables that best explain the species data by a bilinear model and thereby summarizes the relationship between traits and environmental variables (Ter Braak et al., 2018b). The forward selection variant of dc-PCA (dc-PCA-FS) was performed (with season as covariate) so as to select the most important trait(s) and environmental variable(s). Species data (abundance value) were ln (x+1) transformed before analysis. The statistical significance of each variable was set at 5% under Monte Carlo simulation tests.

The sequence data were processed using quantitative insights into microbial ecology (QIIME) (version 1.17). Sequences were clustered into operational taxonomic units (OTUs) with 97% similarity cut-off using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed with Uchime algorithm (Edgar et al., 2011). The phylogenetic affiliation of each 16S rRNA gene sequence was analysed by Ribosomal Database Project classifier (http://rdp.cme.msu. edu/) against the silva (SSU123) 16S rRNA database using confidence threshold of 70% (Amato et al., 2013). The rarefaction analysis was conducted using Mothur v.1.21.1 to reveal the diversity indices including the Chao and Shannon diversity indices (Schloss PD et al., 2009). To detect the influence of wastewater discharge on benthic microbial community composition, the relative abundances of sewer infrastructure-associated bacterial genera (i.e., *Acinetobacter*, *Arcobacter* and *Trichococcus*) (VandeWalle et al., 2012; Newton et al., 2013), denitrifying genera (i.e., *Comamonas*, *Hyphomicrobium*, *Paracocccus*, *Thauera* and *Zoogloea*) that have been found in the activated sludge of WWTPs (Tsai et al., 2012; Wang et al., 2014), and potential bacterial pathogen sequences suggested by Ibekwe et al. (2016) were analysed in the present study. To examine the dissimilarity of microbial community between sampling sites or seasons, a PCA was performed based on the OTU table. Monte Carlo permutation test under the RDA was performed using rivers as explanatory variables and season as covariate, to test the significance of the difference in benthic microbial community composition between rivers. Similarly, Monte Carlo permutation test under the RDA using season as explanatory variable was performed to test the significance of the difference in benthic microbial community composition between season. The linear discriminant analysis effect size (LEfSe) method was used to characterize bacteria specific to different seasons (March and July) or rivers (Liuxi River, Zhujiang River and central urban rivers). Based on the results of LEfSe analysis, Pearson or Spearman correlation coefficients were calculated to identify the associations between environmental variables and relative abundances of the phyla that were significant different among rivers.

3. Results

3.1. Environmental variables

An overview of the environmental variables is given in the supporting information (Dataset S2). The concentrations of the selected organic compounds and nutrients concentrations have been reported in Peng et al. (2017). All the eight heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) were detected in the sediment samples at a wide range of levels. Higher concentrations of heavy metals were found at sites in the Zhujiang River and Shijing River than the remaining sites. There was no significant difference in individual heavy metals levels between the two season, except that As showed significantly higher levels in the dry season than the wet season ($p < 0.05$). The selected emerging organic compounds, nutrients and heavy metals had broadly similar occurrence patterns in the sediment among sampling sites (Dataset S2).

3.2. Benthic macroinvertebrates and correlation with environmental variables

A total of thirteen and twelve taxa were identified in the dry and wet season, respectively (Dataset S1). From the upstream part of the Liuxi River (minimally influenced) to the downstream part of the Liuxi River, Zhujiang River and the central urban rivers (contaminated), the prevalent benthic macroinvertebrates shifted from gastropoda and bivalvia to annelids *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi* (Dataset S1). However, *Orthocladiinae* was the predominant benthic macroinvertebrate at site S8 (Sha River) in both seasons. Interestingly, no benthic macroinvertebrate was found at site S9 (Liede River) in the dry season, and in any sampling sites in the central urban rivers except for site S8 in the wet season (Dataset S1). There was no significant difference in benthic macroinvertebrate community composition between the two seasons (Monte Carlo permutation tests under RDA; $p > 0.05$).

Among all the measured environmental variables, Cr, TP, HHCB and TCS (conditional effects) significantly explained a part of the variation in benthic macroinvertebrate community composition. Together they explained 60.6% (51.8% is the adjusted estimate) of the total variance with 52.3% and 7.0% on the first and second axis, respectively (Fig. 2). Results of Monte Carlo permutation test revealed that Cr, TP, HHCB and TCS each explained 23.8%, 14.0%, 12.1% and 10.6% of the total variance, respectively. Species on the left-hand side of the diagram, such as *L. hoffmeisteri* and *B. sowerbyi*, were positively correlated with Cr and TP concentrations, and occurred more in the samples from the downstream of the Liuxi River and Zhujiang River with higher Cr and TP concentrations. In contrast, those on the right side (e.g., *Bellamya*, *Cipangopaludina*, *Corbicula* and *Limnoperna*) were negatively correlated with the Cr, TP, HHCB and TCS concentrations, and occurred more in the samples from the up- and midstream part of the Liuxi River. Emerging chemicals such as HHCB and TCS showed higher levels in the samples from the central urban rivers (except for Sha River) where less benthic macroinvertebrates were found compared to those from the Liuxi River and Zhujiang River.

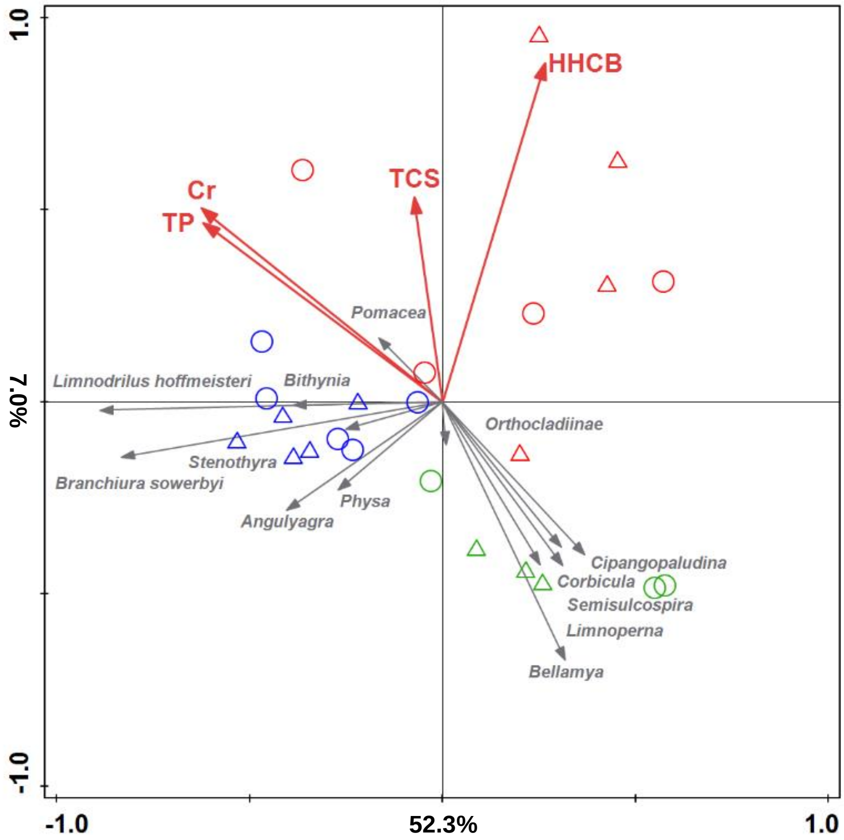

Figure 2. Partial redundancy Analysis (partial RDA) ordination diagram based on the environmental variables that significantly explained the variation of the benthic macroinvertebrate assemblage using Monte Carlo permutation tests. Of all the variance observed in the dataset, approximately 60.6% (adjusted: ~52.8%) can be explained by the included environmental variables. Circle and up triangle symbols represent samples from the dry and wet season, respectively. Green, blue and red symbols represent samples from the Liuxi River (S0-S2), downstream of the Liuxi River and Zhujiang River (S3-S5, S7 and S10), and central urban rivers (S6, S8, S9 and S11), respectively. Red arrows represent environmental variables significantly explaining the taxa variation across samples, whereas grey arrows represent benthic macroinvertebrate taxa. Notably, no benthic macroinvertebrate was found in S9 in both seasons, S6 and S11 in the wet season. For interpretation see text.

3.3. Correlation between macroinvertebrates traits and environmental variables

Only one trait (Reproduction: asexual reproduction) significantly modulated the species-environment relationship ($p < 0.05$), where the environment was represented by Cr and TP. Accordingly, only the first axis was significant. Cr and TP were positively correlated with the above trait (Fig. 3). When constrained by both the functional traits and environmental variables, 44.7% (44.2% is the adjusted estimate) of the total variation in species data was explained. Furthermore, 52.7% (46.7% is the adjusted estimate) of the total variation in species composition data was explained when constrained only by environmental variables (i.e., Cr and TP), whereas 54.9% (50.8% is the adjusted estimate) of the total variation in transposed species composition data was explained when constrained only by trait (i.e., Reproduction: asexual reproduction for dc-PCA-FS).

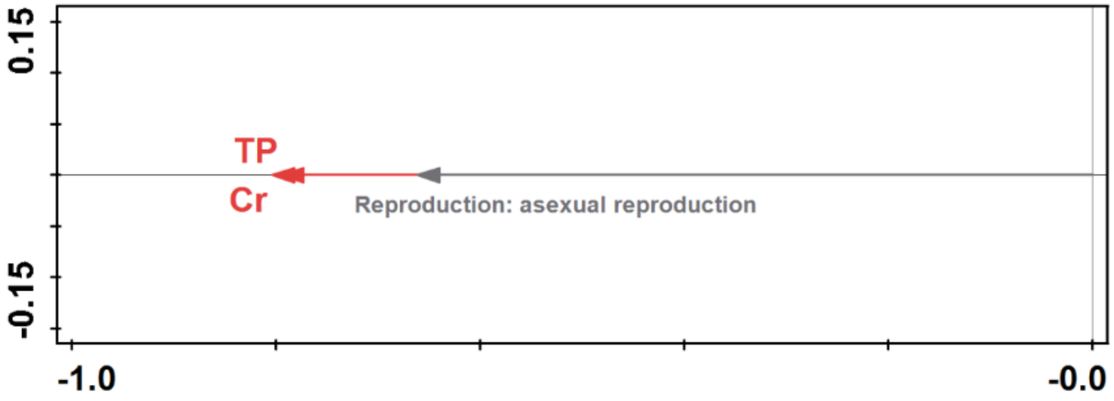

Fig.3 Bi-plots of dc-PCA-FS relating one selected trait (grey) and two selected environmental variables (red) in the benthic macroinvertebrates dataset with 24 sites and 13 macroinvertebrates species. Only the first axis was significant. For interpretation see text.

3.4. Microbial community diversity and composition

The relative abundances of the bacterial 16S rRNA gene at the phylum and genus level were examined to determine the microbial community composition (Fig. 4a; b). A total of 52 bacterial phyla and 1028 genus were identified in the sediment samples. The five most dominant phyla were *Firmicutes* (37%), *Proteobacteria* (24%), *Chloroflexi* (9%), *Actinobacteria* (9%) and *Bacteroidetes* (6%) (Fig. 4a). In general, *Firmicutes* was the most dominant phylum in most of the samples, whereas *Proteobacteria* was the most abundant phylum at sites S4 and S8 in the dry season and sites S0, S5 and S8 in the wet season. However, *Actinobacteria* and *Chloroflexi* occupied the largest portion of the bacterial sequences at S9 in the dry season and S6 in the wet season, respectively. At the genus level, *Bacillus*, *Clostridium sensu stricto 1*, *Lactococcus*, *Peptostreptococcaceae* and *Anaerolineaceae* were the five most abundant genera (Fig. 4b). *Bacillus* was prevalent at site S3 (downstream part of the Liuxi River) in the wet season, accounting for 49.7% of the bacterial sequences. *Acinetobacter*, *Arcobacter* and *Trichococcus* showed much greater abundance at site S8 than the remaining sites, with *Acinetobacter* as the predominant genus at site S8 in the wet season by occupying 45.3% of the bacterial sequences (Table S2). Although sites S6, S9 and S11 are also located in the central urban area, the abundances of *Acinetobacter*, *Arcobacter* and *Trichococcus* were far lower than site S8. Denitrifying genera showed higher relative abundances in the samples from the central urban rivers compared with the Liuxi River and Zhujiang River (Table S3). All analysed potential genera showed rather low relative abundance across all the samples, except that *Nocardia* showed a proportion of 11.5% at site S9 in the dry season (Table S4).

3.5. Comparison of microbial community composition across space and time

The results of PCA showed a clear separation among the rivers in terms of microbial community composition (Fig. 5). The samples from the up- and midstream part of the Liuxi River (S0-S2) clustered to the upper right quadrant of the PCA ordination diagram, while samples from the central urban rivers (S6, S8, S9 and S11) clustered to the upper left one.

Furthermore, most samples from the downstream part of the Liuxi River (S3) and Zhujiang River (S4, S5, S7 and S10) clustered to the bottom part of the diagram. Results of the Monte Carlo permutation tests showed that there was a significant difference in microbial community composition between the above three groups ($p = 0.002$), and between the dry and wet season ($p = 0.042$).

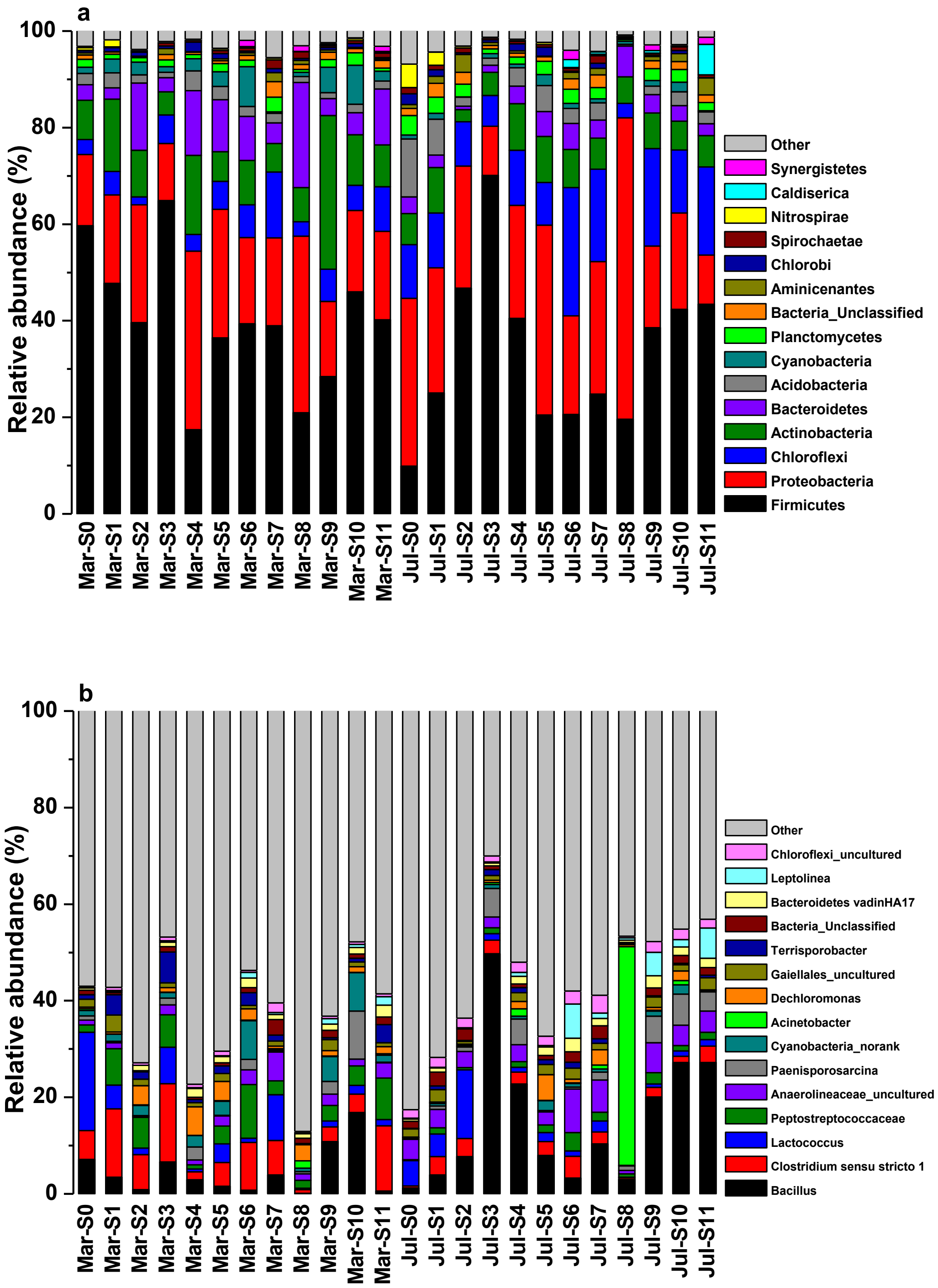

Figure 4. Relative abundance (%) of the top 15 phyla (a) and genera (b) in the sediments from the six rivers of Guangzhou in the two seasons.

The results of LEfSe indicate that 9 and 10 phyla were significantly different between these rivers in the dry season and the wet season, respectively (Fig. S1 and Fig. S2). In the dry season, the relative abundance of *Nitrospirae*, *Gemmatimonadetes* and *Deinococcus-Thermus* were significantly higher in the Liuxi River than those in the Zhujiang River and central urban rivers. Also, while *Chlamydiae* and *Chlorobi* showed higher relative abundance in the Zhujiang River, *Cloacimonetes*, *Hydrogenedentes*, *Lentisphaerae* and *Synergistetes* showed greater relative abundance in the central urban rivers (Fig. S1). In the wet season, the relative abundance of *Nitrospirae* and *Gemmatimonadetes* were again higher in the Liuxi River compared to the other two rivers. Also, while higher relative abundances of *Gracilibacteria*, *Chlorobi*, *RsaHF231*, *Saccharibacteria* and *WCHB1-60* appeared in the Zhujiang River, higher relative abundances of *Cloacimonetes*, *Caldiserica* and *Synergistetes* occurred in the central urban rivers (Fig. S2). Season-associated phyla were also observed using LEfSe. At the phylum level, *Fibrobacteres*, *Cyanobacteria*, *Bacteroidetes* and *Tenericutes* were more abundant in the dry season, whereas *Chloroflexi*, *Aminicenantes*, *SHA-109*, *Planctomycetes* and *PAUC34* showed the opposite trend (Fig. S3).

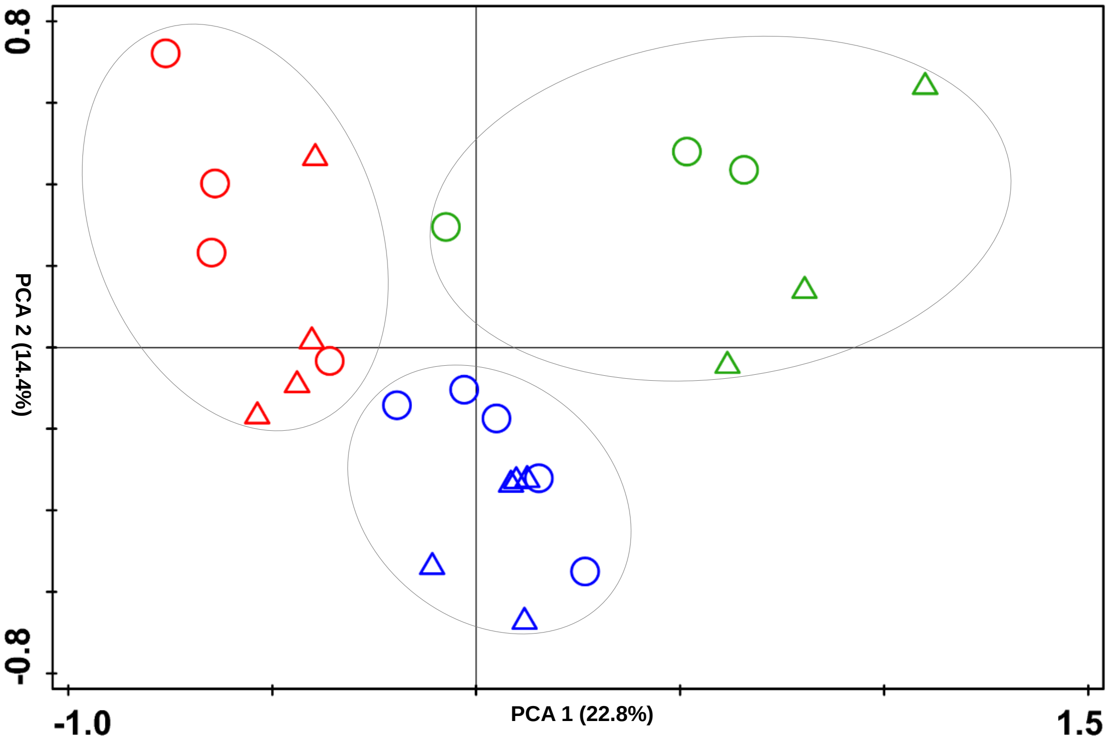

Figure 5. Principal component analysis (PCA) ordination diagram performed on the OTU table for sediment samples from the six rivers of Guangzhou in the two seasons. Circle and up triangle symbols represent samples from the dry and wet season, respectively. Green, blue and red symbols represent samples from the Liuxi River (S0-S2), downstream of the Liuxi River and Zhujiang River (S3-S5, S7 and S10), and central urban rivers (S6, S8, S9 and S11), respectively.

Table 1. Correlation coefficients between the environmental variables and relative abundance of bacterial phyla that showed significant differences among rivers.

Season	Phyla	4-NP	4-t-OP	BPA	TCC ^{s}	TCS	HHCB	AHTN	Cu	Zn	Ni	Pb ^{s}	Cd	Cr	Hg	As	NH_3-N	TN	TP	TOC
Dry season	*Nitrospirae*	-0.72**	-0.53	-0.38	-0.65*	-0.81**	-0.89**	-0.71*	-0.42	-0.33	-0.31	-0.36	-0.32	-0.20	-0.47	-0.15	-0.92**	-0.87**	-0.60*	-0.83**
	Gemmatimonadetes	-0.71**	-0.50	-0.32	-0.81**	-0.92**	-0.97**	-0.86**	-0.50	-0.42	-0.39	-0.53	-0.39	-0.28	-0.51	-0.24	-0.95**	-0.89**	-0.50	-0.80**
	Deinococcus-Thermus	-0.60*	-0.36	-0.28	-0.55	-0.72**	-0.62*	-0.53	-0.52	-0.47	-0.45	-0.60*	-0.36	-0.33	-0.30	-0.48	-0.54	-0.53	-0.37	-0.55
	Chlamydiae	-0.04	-0.35	0.40	-0.25	0.10	0.04	-0.55	0.29	0.32	0.30	0.05	0.26	0.27	0.58*	0.35	0.16	-0.25	0.42	-0.05
	Chlorobi	-0.47	-0.77**	-0.17	-0.41	-0.08	-0.58	-0.61	0.10	0.04	0.12	-0.15	0.20	0.15	-0.42	0.17	-0.40	-0.48	-0.28	-0.50
	Cloacimonetes	0.47	0.49	-0.24	0.75**	0.63*	0.73*	0.78*	0.20	0.21	0.16	0.59	0.16	0.00	0.15	0.12	0.61*	0.71*	0.01	0.50
	Hydrogenedentes	0.70*	0.79**	0.00	0.71*	0.58*	0.86**	0.87**	0.27	0.41	0.31	0.64*	0.24	0.15	0.61*	0.17	0.76**	0.75**	0.25	0.68*
	Lentisphaerae	0.50	0.67*	-0.11	0.80**	0.67*	0.71*	0.64*	0.21	0.11	0.10	0.41	0.16	-0.03	0.28	0.09	0.66*	0.64*	0.33	0.57
	Synergistetes	0.63*	0.74*	-0.15	0.61*	0.57	0.92**	0.91**	0.21	0.33	0.24	0.51	0.23	0.06	0.47	0.09	0.78**	0.79**	0.15	0.67*
Wet season	*Nitrospirae*	-0.43	-0.22	-0.39	-0.62*	-0.67*	-0.70*	-0.24	-0.71**	-0.41	-0.32	-0.41	-0.15	-0.39	-0.27	-0.32	-0.55	-0.46	-0.25	-0.43
	Gemmatimonadetes	-0.54	-0.14	-0.62*	-0.80**	-0.80**	-0.88**	-0.50	-0.82**	-0.62*	-0.60	-0.69*	-0.43	-0.61*	-0.35	-0.41	-0.71*	-0.58	-0.43	-0.61*
	Gracilibacteria	0.30	-0.23	0.29	0.63	0.54	0.48	0.30	0.66	0.80*	0.47	0.69	0.39	0.40	0.39	0.50	0.82*	0.86*	0.53	0.68
	Chlorobi	-0.51	-0.64	-0.31	-0.59*	-0.29	-0.55	-0.66	-0.35	-0.26	-0.13	-0.22	-0.14	-0.12	-0.16	0.10	-0.35	-0.36	0.04	-0.37
	Saccharibacteria	0.01	-0.68*	0.33	0.26	0.62*	0.11	-0.74*	0.70*	0.37	0.42	0.50	0.14	0.50	0.18	0.82**	0.43	0.28	0.73**	0.27
	WCHB1-60	-0.25	-0.79*	0.11	-0.06	0.27	0.11	-0.74*	0.40	0.42	0.33	0.36	0.18	0.35	-0.20	0.44	0.27	0.09	0.60	0.06
	Cloacimonetes	0.58	0.40	0.63	0.85**	0.36	0.67*	0.57	0.28	0.70*	0.42	0.61	0.47	0.20	0.36	-0.37	0.58	0.50	0.33	0.58
	Caldiserica	0.86**	0.75*	0.77**	0.87**	0.75**	0.75*	0.87**	0.56	0.57	0.53	0.73**	0.42	0.46	0.87**	0.47	0.83**	0.91**	0.61*	0.91**
	Synergistetes	0.81**	0.65	0.73*	0.96**	0.76**	0.96**	0.96**	0.54	0.64*	0.52	0.67*	0.45	0.41	0.81**	0.07	0.81**	0.82**	0.38	0.85**

^{s} For TCC and Pb, the Spearman correlation was use, whereas Pearson correlation was used for the rest environmental variables.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

3.6. Correlation between microbial community and environmental variables

Significant correlations were observed between environmental factors and bacterial phyla whose levels were significant different between rivers (Table 1). Specifically, in the dry season, the relative abundance of bacterial phyla *Nitrospirae*, *Gemmatimonadetes*, *Deinococcus-Thermus* and *Chlorobi* were significantly negatively correlated with the concentrations of organic compounds (e.g., 4-NP, TCC, TCS, HHCB and AHTN) and nutrients (e.g., TN and TOC), whereas the relative abundances of *Cloacimonetes, Hydrogenedentes, Lentisphaerae* and S*ynergistetes* were significantly positively correlated with the concentrations of organic compounds and nutrients. However, no significant correlations were observed between the concentrations of heavy metals and the relative abundances of bacterial phyla in the dry season, except for Pb and Hg. Similar to the dry season, the relative abundances of *Nitrospirae*, *Gemmatimonadetes* and *Chlorobi* were significantly negatively correlated with the concentrations of organic compounds (e.g., 4-NP, TCC, TCS, HHCB and AHTN) and nutrients (e.g., TN and TOC), whereas the relative abundances of *Cloacimonetes*, *Caldiserica* and *Synergistetes* were positively correlated with the concentrations of organic compounds, heavy metals and nutrients.

4. Discussion

4.1. Environmental variables

In comparison with the sediment quality guidelines for freshwater ecosystems (Table S5, MacDonald et al., 2000), the concentrations of heavy metals in several sites exceeded the toxic effect threshold (TET) values as set by the guideline. For example, arsenic contamination was ubiquitous in the investigated rivers, with concentrations higher than the recommended TET value (17 mg/kg) in 19 samples out of 24 samples. In addition, Cr, Cu, Hg and Ni were the other four metal contaminants with levels higher than the recommended TETs in more than half of samples. Nevertheless, all the heavy metals concentrations were below the TETs at site S0 and S1, suggesting that the heavy metal contamination of the headwater of Liuxi River will not pose ecological risks. However, no guideline was available for the nutrients or the selected organic contaminants, except for 4-NP. Compared to the Canadian Sediment Quality Guideline (1400 ng/g dw), most of the measured 4-NP concentrations exceeded this guideline value (Canada, 2002) in both seasons.

4.2. Benthic macroinvertebrates distribution and correlations with environmental variables

Among all the identified benthic macroinvertebrates, *L. hofmeister* was the predominant macroinvertebrates in all sampling sites except for S0, S1 and S8 (Dataset S1, Fig. 2), indicating that organically enriched habitat can be a favourable conditions to recruiting deposit feeding organisms, as demonstrated by Goodnight and Whitney (1960). Similar results have been reported by Sang (1987) that *L. hofmeister* was the most abundant macroinvertebrates in the Guangzhou Reach of the Zhujiang River. In contrast, gastropods mainly occurred at the sites S0 and S1 that were subjected to negligible environmental stress (Dataset S1, Fig. 2). This is likely related to the different tolerance of various benthic macroinvertebrates to the variation of certain abiotic variables, such as availability of oxygen and the substrate (Paraskevopoulou et al., 2015). A decreasing trend in species diversity was observed from the Liuxi River to Zhujiang River and the central urban rivers (Dataset S1), which is in agreement with the human-impact gradient in these rivers. In contrast, an increasing trend in density of annelid (i.e., *L. hofmeister* and *B. sowerbyi*) was found from the Liuxi River to the Zhujiang River. This is likely due to the high organic matter and hypoxic conditions in the Zhujiang River and central urban rivers (Souza et al., 2013; Omena et al., 2012). The drastic reductions in benthic macroinvertebrates diversity accompanying the presence of a few opportunistic species (e.g., Tubificidae) with a high density suggests that rivers in the urban area of Guangzhou city were subjected to excessive organic contamination (Bigot et al., 2008; Omena et al., 2012). This is also supported by the high levels of emerging chemicals measured in these rivers.

The results of RDA further demonstrated that Cr, TP, HHCB and TCS together explained 60.6% of the total variation in benthic macroinvertebrates composition among sites (Fig. 2). This is in agreement with findings of Souza et al. (2013), who demonstrated that the effects of the urban sewage contamination was the primary forces in determining benthic macroinvertebrates distribution. The absence of benthic macroinvertebrates in samples from the central urban rivers, thus, could be attributed to the discharge of urban effluents (Dataset S1).

4.3. Correlation between benthic macroinvertebrates traits and environmental variables

The most distinctive trait was asexual reproduction (Fig. 3), suggesting that species changing along the environmental gradients also caused changes in the ecological functioning of the species assemblages (Oug et al., 2012). This is in line with the findings of a previous study investigating the benthic macroinvertebrates in the Danube River, which found that traits including reproduction types explained the variability of benthic macroinvertebrates between sampling sites (Rico et al., 2016). Environmental variables (i.e., Cr and TP) and trait (asexual reproduction) showed equal influence on taxa composition. In the sites with higher levels of TP and Cr, the dominant trait were feeding habits (e.g., absorber and deposit feeder) and reproduction (e.g., asexual reproduction) (Datasets S1, S2 and S3, Fig. 3), supporting the predictions that opportunistic traits are beneficial in disturbed areas (Bremner et al., 2006). Although there are a few limitations for biological traits analysis (Van den Brink et al., 2011; Munari et al., 2013), this approach was useful to evaluate the effects of contamination on the benthic macroinvertebrate community in urban rivers, as traits can reflect the function of every species in a community (Bremner, 2008).

4.4. Microbial community composition and correlations with environmental variables

The microbial community composition shifted along the rural-urban gradient, which is in line with the findings observed in the urban and suburban rivers of Chicago (Drury et al., 2013a). This indicates that human activities (e.g., WWTP effluent discharges) strongly altered the microbial community composition directly or indirectly in the contaminated sites. For example, *Paracocccus*, *Zoogloea* and *Thauera* were prominent denitrifying genera in the activated sludge of WWTPs (Tsai et al., 2012; Wang et al., 2014), but they were observed at sites S6, S8 and S11 (Table S3). This is likely related to the WWTPs effluents and/or illegal wastewater discharging by the surrounding area. As wastewater-associated bacteria *Acinetobacter*, *Arcobacter* and *Trichococcus* (VandeWalle et al., 2012; Newton et al., 2013) reached much greater abundance at site S8 (Table S2), this site was likely more affected by WWTP effluents than the other sites. Interestingly, chironomids was also more abundant at site S8 in the wet season. It has reported that the bioturbation of benthic macroinvertebrates can affect the relative abundances of specific bacterial groups in sediments (Zeng et al., 2014). Therefore, there seems to be interactions among contaminants, benthic macroinvertebrates and microbial community at site S8. However, Laboratory work will be required to elucidate such interactions, as this could not be determined here.

Unlike the other sites from the Liuxi River, site S3 had rather high relative abundance of *Bacillus* (49.7%) in the dry season (Fig. 4), which could be attributed to fact that the downstream part of the Liuxi River is located in the urban area of Guangzhou and suffered more contamination relative to the up- and midstream part. Despite the totally different environmental conditions (e.g., hydrological conditions, chemical and biological characteristics), the microbial community in the downstream part of the Liuxi River was similar to those in the Zhujiang River. This suggests that WWTP effluents entering the environment can increase biotic homogenization between natural ecosystems (Drury et al., 2013a). Likewise, McKinney (2006) and Epelde et al. (2015) also found that the evenness of the microbial communities increased with contamination in sediment.

Compared to the Liuxi River, the relative abundances of *Nitrospirae* and *Gemmatimonadetes* were significantly lower in the Zhujiang River and the central urban rivers receiving WWTP effluents in both seasons, which indicates that these two phyla may be sensitive to some components of WWTP effluents. In contrast, the relative abundance of *Cloacimonetes* and *Synergistetes* were significantly higher in the sampling sites in urbanized rivers receiving the WWTP effluents, which is likely due to the increased nitrogen and organic matters concentrations at these sites. For example, *Synergistetes* showed substantial existence in the anaerobic sludge (Wang et al., 2013) and can supply short-chain fatty acids and sulfate for terminal degraders such as methanogens and sulfate reducing bacteria (Vartoukian et al., 2007). Because there was an obvious seasonal variation in microbial community composition between the two seasons (Fig. S3), temperature was likely to be an important fact influencing microbial community composition. Indeed, temperature has been previously reported as an important factor in shaping microbial community composition (Staley et al., 2015).

Bacterial phyla showing significant differences among rivers were significantly and strongly correlated with emerging chemicals (e.g., 4-NP, TCC, TCS, HHCB and AHTN), nutrients (e.g., TN and TOC) and metal contaminants (e.g., Pb and Hg) (Table 1), which is in agreement with the findings reported from other large/urban rivers in California (Ibekwe et al., 2016) and Upper Mississippi River in Minnesota (Staley et al., 2014, 2015). Therefore, it is required to improve removal efficiency of organic and inorganic compounds in WWTPs to reduce they entering urban rivers.

5. Conclusions

Our data showed that there were significant correlations between environmental variables (i.e., most analysed emerging chemicals, heavy metals and nutrients) and benthic biological communities in urban rivers. The input of domestic and/or industrial sewage effluents influenced the sediment quality and distribution of local benthic macroinvertebrates, their functional attributes and sediment microbial communities in the rivers of Guangzhou (south China). Absence of benthic macroinvertebrates was recorded in a few samples collected from the central urban rivers. Restoration activities, thus, are recommended to restore the ecosystem quality in these waterways.

Acknowledgments

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Supporting information

Table S1. Basic information for each sampling site in urban rivers of Guangzhou in 2015.

Site	Geographic location (N, E)		Site location	District	Site characteristics
S0	113º28'47"	23º56'45"	Liuxi Reservoir	Conghua district	Source of Liuxi River
S1	113°28'10"	23°26'05"	Taiping	Conghua district	Upstream of Liuxi River
S2	113°17'28"	23°19'38"	Renhe	Baiyun district	Midstream of Liuxi River
S3	113°12'26"	23°14'47"	Nangang	Huangpu district	Downstream of Liuxi River
S4	113°11'07"	23°13'15"	Yagang	Baiyun district	Zhujiang River
S5	113°12'22"	23°08'15"	Shabeilijiao	Baiyun district	Zhujiang River
S6	113°13'50"	23°11'39"	Shijing River	Baiyun district	Tributary of Zhujiang River, receiving wastewater
S7	113°13'58"	23°06'22"	Baietan	Liwan district	Zhujiang River
S8	113°12'08"	23°07'10"	Sha River	Yuexiu district	Tributary of Zhujiang River, receiving wastewater
S9	113°19'43"	23°07'08"	Liede River	Tianhe district	Tributary of Zhujiang River, receiving wastewater
S10	113°21'52"	23°06'44"	Pazhou	Tianhe district	Zhujiang River, near the discharge port of a WWTP
S11	113°24'11"	23°07'12"	Chebei River	Tianhe district	Tributary of Zhujiang River, receiving wastewater

Table S2. Sewer-affiliated genera in the sediments from urban rivers in Guangzhou.

Season	Site	*Acinetobacter* (%)	*Arcobacter* (%)	*Trichococcus* (%)
Dry season	S0	0.30	0	0.24
	S1	0.09	0	0.06
	S2	0.10	0	0.01
	S3	0.07	0.05	0.12
	S4	0.01	0.01	0.07
	S5	0.11	0.05	0.08
	S6	0.11	0.01	0.24
	S7	0.18	0.06	0.13
	S8	1.49	0.81	3.46
	S9	0.12	0.00	0.03
	S10	0.03	0.01	0.04
	S11	0.24	0.04	0.25
Wet season	S0	0.07	0	0.06
	S1	0.16	0	0.06
	S2	0.21	0.03	0.21
	S3	0.41	0.02	0.04
	S4	1.52	0	0.03
	S5	0.06	0	0.03
	S6	0.12	0.01	0.02
	S7	0.86	0.01	0.07
	S8	45.30	0.02	0.12
	S9	0.19	0	0.06
	S10	0.88	0	0.02
	S11	0.13	0	0.03

All these genera are cited from Newton et al. (2013).

Table S3. Denitrifying genera in the sediments from urban rivers in Guangzhou.

Season	Site	*Comamonas* (%)	*Hyphomicrobium* (%)	*Paracocccus* (%)	*Thauera* (%)	*Zoogloea* (%)
Dry season	S0	0.028	0.004	0.036	0.157	0.088
	S1	0.139	0.093	0.013	0.010	0.043
	S2	0.040	0.003	0.023	0.063	0.080
	S3	0.016	0.016	0.016	0.008	0.008
	S4	0	0	0.006	0.006	0.061
	S5	0	0.011	0.017	0.011	0.044
	S6	0.028	0.016	0.313	0.442	0.135
	S7	0.009	0.014	0.005	0.005	0.009
	S8	0.314	0.003	0.782	2.424	6.962
	S9	0	0.004	0.069	0.141	0.027
	S10	0.009	0.029	0.038	0.072	0.040
	S11	0.030	0.020	0.268	1.384	0.817
Wet season	S0	0.171	0.050	0.008	0	0.025
	S1	0.146	0.076	0.012	0.047	0.053
	S2	0.097	0.004	0.036	0.004	0.069
	S3	0.004	0.009	0	0	0
	S4	0.022	0.035	0.009	0.035	0.035
	S5	0.016	0.031	0.012	0.035	0.027
	S6	0.004	0.044	0.205	0.282	0.077
	S7	0.031	0.021	0.017	0.024	0.069
	S8	0.050	0.014	0.622	0.490	1.021
	S9	0.010	0.079	0.074	0.098	0.010
	S10	0.004	0.050	0.014	0.032	0.028
	S11	0.009	0.025	0.084	0.028	0.012

All these genera are have been reported in the active sludge (Juretschko et al., 2002; Tsai et al., 2012; Wang et al., 2014).

Table S4. Percent of potential bacterial pathogen sequences at the genus level in the sediments from urban rivers in Guangzhou.

Season	Site	*Actinobacteria*			*Bacteroidetes*	*Proteobacteria*					*Spirochaetes*	*Firmicutes*	
		Nocardia	*Corynebacterium*	*Mycobacterium*	*Bacteroides*	*Arcobacter*	*Aeromonas*	*Legionella*	*Citrobacter*	*Shigella*	*Leptospira*	*Bacillus*	*Staphylococcus*
Dry	S0	0.01	0.02	0.20	0	0	0	0	0	0	0.01	0	0
	S1	0.02	0.02	0.29	0	0	0.01	0	0	0	0	0	0
	S2	0.01	0.01	0.42	0.88	0	0.11	0.01	0	0	0	0	0
	S3	0.02	0.01	0.33	0.10	0.05	0.07	0	0	0	0	0	0
	S4	0.78	0	0.57	0.15	0.01	0.10	0.01	0	0	0	0	0
	S5	0.01	0.01	0.43	0.76	0.05	0.22	0	0	0	0	0	0
	S6	0.04	0.15	0.83	0.07	0.01	0.03	0	0	0	0	0	0
	S7	0.28	0.01	0.39	0	0.06	0.53	0	0	0	0	0	0
	S8	0.02	0.18	0.23	1.20	0.81	0.68	0	0	0	0	0	0
	S9	11.53	0	0.57	0.01	0	0	0	0	0	0	0	0
	S10	0.25	0.02	1.01	0.01	0.01	0.01	0.01	0	0	0	0	0
	S11	0.01	0.14	0.40	0.10	0.04	0.07	0	0	0	0	0	0
Wet	S0	0	0	0.05	0	0	0	0.02	0	0	0	0	0
	S1	0.01	0	0.46	0	0	0.01	0	0	0	0.01	0	0
	S2	0	0	0.01	0	0.03	0	0.01	0	0	0	0	0
	S3	0.03	0	0.21	0	0.02	0	0.01	0	0	0	0	0
	S4	0.08	0.03	0.45	0	0	0	0.01	0	0	0	0	0
	S5	0.03	0.02	0.64	0	0	0.01	0.03	0	0	0	0	0
	S6	0.01	0.05	0.28	0.03	0.01	0.01	0	0	0	0	0	0
	S7	0.02	0.02	0.30	0.01	0.01	0.07	0.01	0	0	0	0	0
	S8	0.01	0.12	0.11	0.18	0.02	0.15	0	0	0	0	0	0
	S9	0.05	0.01	0.39	0	0	0	0	0	0	0	0	0
	S10	0.01	0	0.67	0.01	0	0	0.01	0	0	0	0	0
	S11	0.03	0.02	0.14	0.01	0	0.01	0.02	0	0	0	0	0

All these genera are cited from Ibekwe et al. (2016).

Table S5. Sediment quality guidelines for metals (mg/kg) under different effect levels.

Effect level	Sediment quality guideline	Arsenic	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Zinc
Threshold effect	Threshold effect level	5.9	0.6	37.3	35.7	35	0.17	18	123
Midrange effect	Probable effect levels	17	3.5	90	197	91.3	0.486	36	315
Extreme effect	Toxic effect threshold	17	3	100	86	170	1	64	540

The values belonging to threshold effect level are same as Canadian Freshwater Sediment Guidelines (NOAA 1999)
Reference: MacDonald et al., 2000.

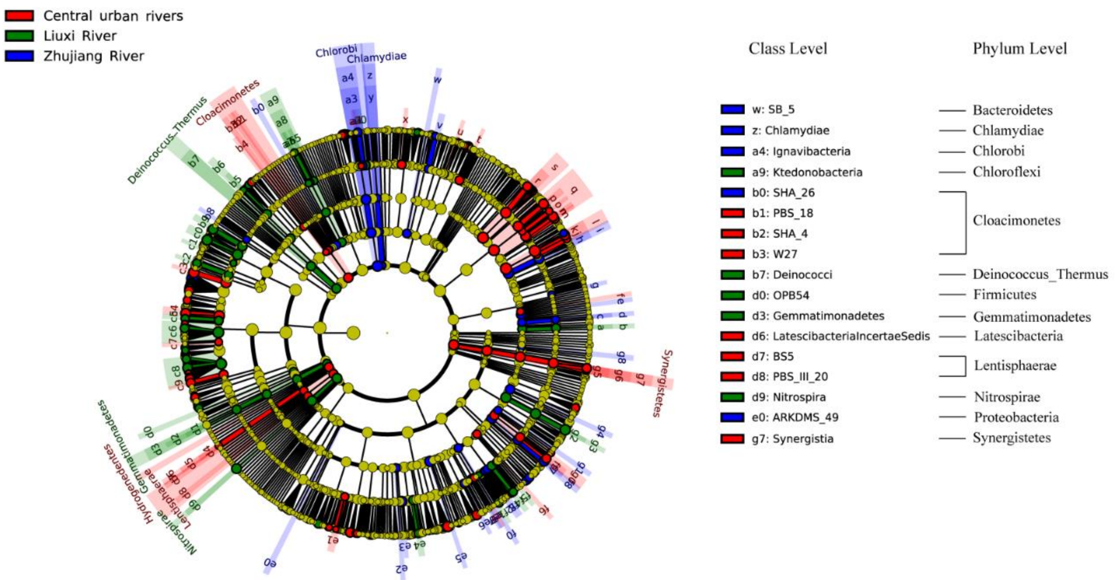

Figure S1. Linear discriminant analysis effect size (LEfse) analysis on bacterial assemblage among Liuxi River, Zhujiang River and the central urban rivers in the dry season.

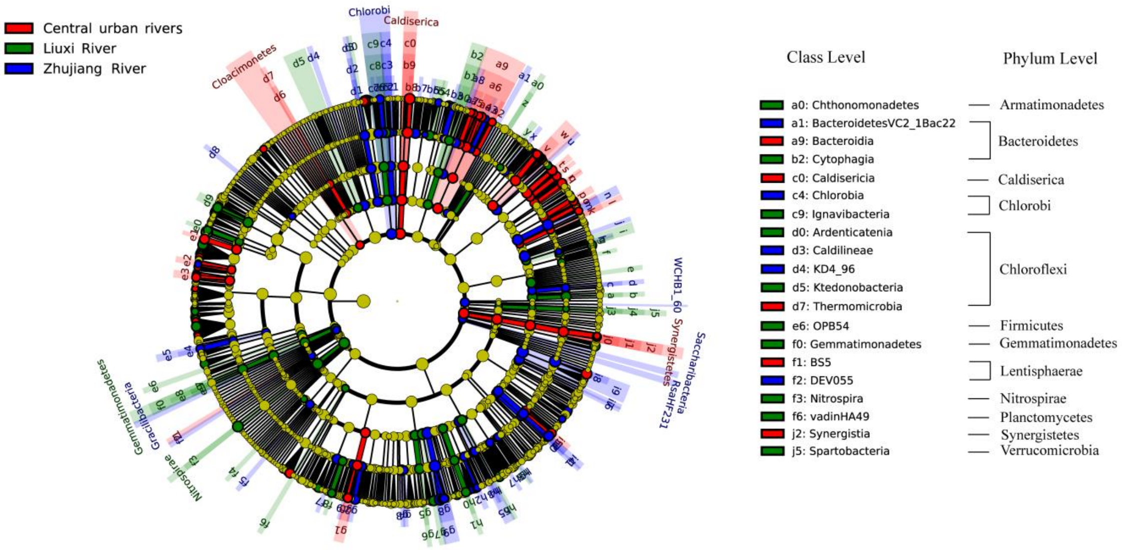

Figure S2. Linear discriminant analysis effect size (LEfse) analysis on bacterial assemblage among Liuxi River, Zhujiang River and the central urban rivers in the wet season.

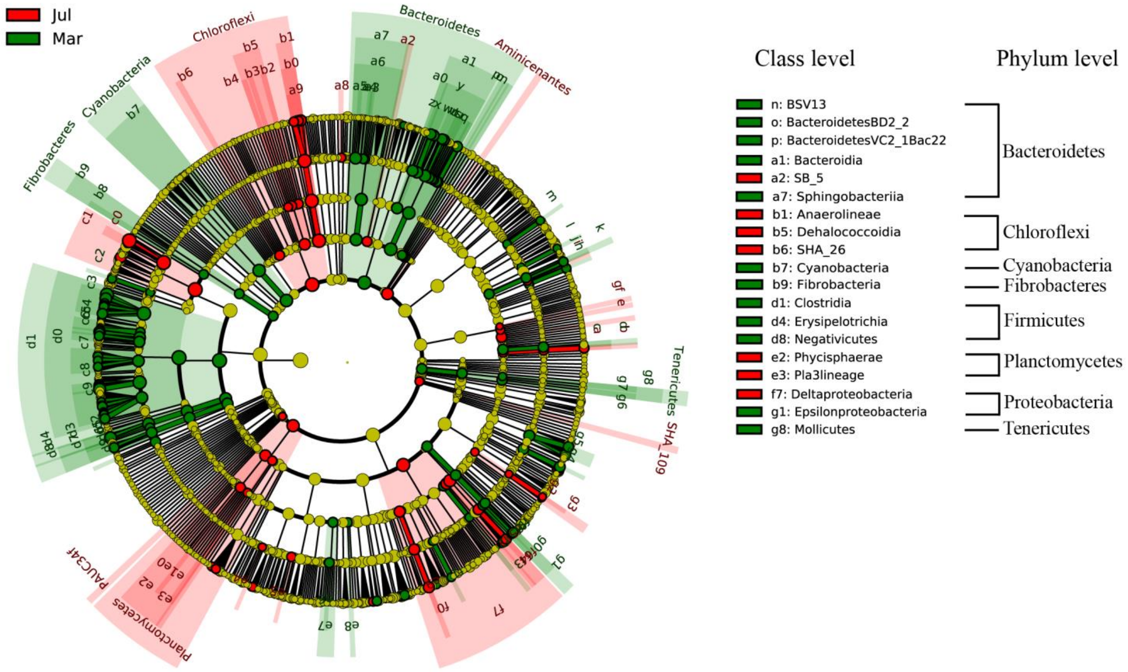

Figure S3. Linear discriminant analysis effect size (LEfse) analysis on bacterial assemblage between March and July.

Chapter 4

Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms

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Abstract

Triclosan (TCS) is an antibacterial agent that is commonly used in personal care products. Because of the sediment-binding properties, TCS exposure presents a potential threat to sediment-dwelling aquatic organisms. Currently our knowledge of the fate and effects of sediment-associated TCS in aquatic systems is limited. To understand the impact of sediment-associated TCS, we used microcosms to assess TCS exposure on a diverse range of organisms selected to mimic a subtropical community, with an exposure period of 28 days. We included the oligochaete freshwater worm *Limnodrilus hoffmeisteri* to evaluate the interaction between sediment-associated TCS and sediment-dwelling organisms, including potential loss of TCS from the sediment due to biological activity and bioaccumulation. Benthic macroinvertebrate presence significantly increased the TCS levels from 0.013 ± 0.007 µg/L to 0.613±0.030 µg/L in the overlying water through biological activity, posing a potential additional risk to pelagic species, but this did not result in a significant reduction of the sediment concentration. Furthermore, worms accumulated TCS with estimated Biota-Sediment-Accumulation-Factors (BSAFs) ranging between 0.38-3.55. Other than for algae, TCS at environmental concentrations did not affect the survival of the introduced organisms, including the *L hoffmeisteri*. Our results demonstrate that, although TCS at currently detected maximum concentration may not have observable toxic effects on the benthic macroinvertebrates in the short term, it can lead to bioaccumulation in worms.

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1. Introduction

Chemicals used in personal care products are frequently discharged into the aquatic environment, due to high consumer use throughout the world, and have a range of environmental impacts (Bedoux et al., 2012). Some substances used in personal care products are environmentally persistent and can bioaccumulate in the tissues of aquatic organisms (Brausch and Rand, 2011; Liu and Wong., 2013). Triclosan (TCS; 5-chloro-(2,4-dichlorophenoxy) phenol) is a synthetic and broad-spectrum antibacterial agent that is widely used in personal care products, textiles, plastics, and food contact materials (SCCS 2010). In the aquatic environment, TCS is one of the most commonly detected organic contaminants (Halden and Paull, 2005; Brausch and Rand, 2011) with concentrations of 1.02 µg/L detected in some regions (Bedoux et al., 2012). TCS exposure can affect a variety of aquatic organisms (e.g., Orvos et al., 2002; Yang et al., 2008; Khatikarn et al., 2016; Zhang et al., 2016a), with algae being particularly sensitive at environmentally relevant concentrations (EC_{50} of 0.7 µg/L) (Orvos et al., 2002). Currently, our knowledge of the toxic effects of TCS exposure is limited to laboratory-based single-species studies performed in the water phase. However, because of the hydrophobic property of TCS (Reiss et al., 2002), it is likely that sediment-dwelling aquatic organisms may be affected by TCS exposure. Nevertheless, there is limited knowledge of the effects of TCS on sediment-dwellers in a multi-species community.

TCS can adsorb to particles in the water column as well as accumulate in the sediment due to high hydrophobicity (log Kow of 4.8, log Koc 3.11 to 4.48) (Reiss et al., 2002). In the water phase, TCS can readily degrade under controlled laboratory conditions via various mechanisms (e.g. photolysis, chlorination, ozonolysis, and sonochemical treatment) and has short half-lives in the range of less than 3 ms-around 1 day (Bedous et al., 2012). In soil and sediment, however, TCS is more resistant to biodegradation with reported half-lives of up to 239 days (Ying et al., 2007; Wu et al., 2009; Huang et al., 2015). TCS is frequently identified in the sediments of water bodies (e.g., Cantwell et al., 2010; Katz et al., 2013; Gorga et al., 2015), with the highest reported level of 1.33 µg TCS/g dry weight (dw) sediment (Zhao et al., 2010a). However, there are no sediment quality guidelines and there is limited information in the literature on sediment toxicity of TCS on sediment-dwelling invertebrates. This limits the prediction of the TCS effect of maximum environmental concentrations on aquatic organisms, especially benthic species.

In addition to a sink of hydrophobic contaminants, sediments can serve as a source of contaminants when sediment is re-suspended during physical disturbance or biological activity (Burton, 2002). Through their feeding habits and bioturbation behaviour (e.g., sediment irrigation and particle mixing), sediment-dwelling benthic macroinvertebrates, such as oligochaete worms, can alter the physicochemical properties of sediment and sequentially the fate and effects of sediment-bound contaminants. For example, bioturbation can provide elevated oxygenation that can promote microbial degradation processes (Banta and Andersen, 2003; Eggleton and Thomas, 2004). Bioturbation may also increase the transfer of contaminants from the sediment to the overlying water (Eggleton and Thomas, 2004). For example, bioturbation by benthic macroinvertebrates, such as polychaetes and oligochaetes, enhanced the transport of hydrophobic organic contaminants from sediment to the overlying water (Thibodeaux et al., 2001; Josefsson et al., 2010). Thus, bioturbation may influence the fate of sediment-bound contaminants (Schaffner et al., 1997) and potentially increase contaminant-associated risks to pelagic species (Pang et al., 2012). Indirect removal of contaminants from the sediment may occur via ingestion of sediment-associated contaminants, followed by bioaccumulation in the tissues of sediment-dwellers and potentially biotransformation (i.e., degradation). For example, TCS accumulated in the freshwater sediment-dwelling worm *Lumbriculus variegatus* with a 28-d Biota-Sediment-Accumulation-Factor (BSAF) of 1.4 (Dang et al., 2016). Despite this interaction between sediment-bound contaminants and benthic macroinvertebrates, the influence of these organisms on sediment-associated TCS has not been well explored.

We explored the effects of TCS in an environmentally realistic manner using a microcosm setup. The microcosms comprised of TCS contaminated sediment and representative aquatic communities mimicking a subtropical freshwater ecosystem. Additionally, to investigate the effects of benthic macroinvertebrates for the fate of TCS in the microcosms, we included a sediment-dwelling worm, *Limnodrilus hoffmeisteri*, from the Naididae family. The Naididae family (formerly known as Tubificidae) are highly abundant in contaminated sediments due to their tolerance to a range of contaminants (Vivien et al., 2015). As such, Naididae are commonly used to assess the quality of aquatic ecosystems in field and laboratory studies, such as in ecotoxicological tests and bioaccumulation studies (Milbrink, 1973; Rodriguez and Reynoldson, 2011). Specifically, we aimed to (i) determine the impact of L hoffmeisteri for the fate of sediment-associated TCS in the microcosm, and (ii) get a better understanding of the direct and indirect toxic effects of sediment-associated TCS on the biological community of a subtropical aquatic environment. To do this, we quantified the fate of sediment-associated TCS in indoor benthic microcosms with and without the presence of introduced organisms, and examined the effects of sediment-associated TCS on algae, Daphnia, snails, midges and sediment-dwelling worms.

2. Material and methods

2.1. Chemicals

The TCS standard was obtained from J&K Scientific Ltd (Beijing, China) with a purity of 97%, and $^{13}C_{12}$-triclosan was purchased from Cambridge Isotope Laboratories (Andover, USA). The HPLC grade reagents including methanol, acetonitrile, ethyl acetate, hexane, dichloromethane, formic acid and acetic acid were supplied by Merck (Germany), CNW Technologies (China), and Tedia (USA). Glass fiber filters (GF/F, pore size 0.7 μm) were obtained from Whatman (Maidstone, UK). Oasis HLB cartridges (200 mg, 6 mL) were from Waters (Milford, USA). HPLC-grade water was from a Milli-Q water purification system (Millipore, Watford).

2.2. Test organisms

Following extensive biomonitoring in six urban rivers of Guangzhou City, South China in 2015, several key common species were identified and selected as representative species. To create a representative subtropical community, we used: five species of algae (*Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Selenastrum capricornutum*, *Scenedesmus acuminatus*, and *Chlamydomonas reinhardtii*); a filter-feeding cladoceran (*Daphnia magna*); a filter-feeding detritivorous snail (*Viviparidae bellamya*); an insect midge larvae (*Orthocladiinae*) at fourth instar stage; and a sediment-dwelling worm (*Limnodrilus hoffmeisteri*).

The algae were laboratory cultured in BG11 medium at 25 °C and a light intensity of 4000 lux with a dark/light cycle of 14 h/10 h. The preparation of BG11 medium can be found in a previous study (Rippka et al., 1979). *D. magna* was laboratory reared in an incubator at 20 °C under a cool white fluorescent lamp on a photoperiod of 16 h light: 8 h dark. *D. magna* was fed with yeast and the algae *Chlorella pyrenoidosa*. Snails, midge larvae, and juvenile worms were purchased from an aquatic market (Guangzhou, South China), as they could not be collected in the field, and placed into separate 18-L glass holding tanks with aerated tap water and unbleached tissue paper as substrate. Snails and worms with similar size and mass (representing similar age) were selected and cultured for 2 weeks in the laboratory at the same light and temperature conditions as the microcosm experiment (light/dark: 12/12 h, temperature: 27 ± 1 °C). Midge larvae were only cultured for 2 days as they were near emergent stage. During the culturing period (2 weeks), snails were fed with lettuce in small slices every day, while worms were fed with ground fish food (Tetramin; Tetra Werke) twice a week. The culturing systems for snails, midge larvae and worms were cleaned every day by taking out all animals and washing the glass tanks with aerated tap water.

One day before being added to the microcosms, midges, worms and snails were transferred to glass beakers separately and allowed to clear their guts by defecating in aerated tap water overnight (Dai et al., 2012). In this study, the endpoint was based on survival and reproduction for *D. magna*, and on growth inhibition assessed as changes in wet weight, survival and reproduction for midges, worms, and snails. The wet weights (ww, mg) of midges, worms, and snails were estimated by measuring 50 randomly selected individuals at the start of the experiment (Sartorius balance, Germany) (see Table S1 for all body lengths and wet weights).

2.3. Test sediment and TCS spiking

Sediment was collected in September 2015 from an uncontaminated site (Liuxi Reservoir, Guangzhou, South China) (Zhao et al., 2010a; Peng et al., 2017). The sediment was wet sieved through a 300 μm sieve using deionized water to remove potential benthic macroinvertebrates. No organisms were observed in the collected sediment, probably due to the depth of the water column (40-50 meters) in the reservoir. After settling for a week, the overlying water was removed, and the wet sediment was first well mixed using a stainless steel mixer, followed by a manual mixture using a metal spade to achieve sediment homogenization. The sieved sediment contained 62% water. The sediment was composed primarily of clay (56%), but also silt (43%), and sand (0.65%). It contained 1.9% organic matter (OM), 0.04% total phosphorus, 0.16% total nitrogen, and 0.01% ammonia nitrogen (NH_3) quantified using standard methods (Clesceri et al., 1998). The TCS concentration was found to be less than 0.002 µg/g dw in the sediment and considered negligible for the purposes of this study.

The sediment was spiked with TCS dissolved in acetone to reach actual TCS concentrations of 0.8 (treatment 1, T1), 8 (treatment 2, T2), 80 (treatment 3, T3) and 240 µg/g dw (treatment 4, T4). The same volume of acetone (6.45 mL) was added to all treatments. Both solvent control and water control treatments were included by replacing TCS stock solution with the same volume of acetone and aerated Milli-Q water, respectively. The lowest TCS concentration used here is at environmentally relevant levels (1.33 µg/g dw, Zhao et al., 2010a). However, the remaining TCS levels were used to evaluate the effects of sediment-associated TCS on the test organisms. After spiking, the sediment was thoroughly mixed for 30 min in a stainless steel mixer. Further pre-equilibration was performed by manually mixing the spiked sediment for 5 minutes using a spade during the following four days. Before introduction to the microcosms, each spiked sediment was further mixed for 20 min using the stainless steel mixer.

2.4. Microcosm set-up

We used 52 indoor glass microcosms (30 x 30 x 20 cm) in a temperature-controlled room (27 ± 1 °C), to mimic static subtropical aquatic sediment systems. For each TCS treatment level (T1, T2, T3 and T4), 9 microcosms were filled with 4 cm of corresponding TCS spiked sediment (3.6 L) and 14 cm of aerated tap water (12.6 L; 0.056% total organic carbon (TOC), 0.001% total phosphorus, 0.164% nitrate (NO_3^-), 0.0002% nitrite (NO_2^-) and 0.030% NH_3). Eight microcosms each were used for water and acetone controls. After introduction of spiked sediment and aerated tap water, the particles in the overlying water were allowed to settle for 3 days before addition of the test organisms. For each TCS treatment, 1 replicate of the 9 microcosms was sacrificed to measure TCS concentrations in the overlying water and sediment phase at the start of the exposure (day 0). Therefore, there were 8 microcosms for each treatment level (water control, acetone control, T1, T2, T3 and T4): 4 with sediment and water only (i.e., without test organisms) and 4 with sediment, water, and test organisms (40 midges, 240 worms, 6 snails, 30 *D. magna*, and algae at a density of approximately 104 cells/Ml per microcosm). Test organisms were added on day 0 and the experiment ran for 28 days. Because periphyton can develop in the microcosms during the experiment, the effect of sediment-associated TCS on periphyton community development was investigated by introducing five microscopic glass slides (7.5 x 2.5 cm) into each microcosm at a water depth of about 10 cm.

Microcosms were illuminated with a cool white fluorescent light (2200 lux) and a daily photoperiod of 12 h. Each microcosm was aerated using a glass pipette connected to an aeration system. During the experiment, evaporated water was replenished with aerated tap water weekly to maintain the original water level. Nitrogen (0.7 mg/L as urea) and phosphorus (0.09

mg/L as triple super phosphate) were added biweekly to the systems to provide nutrients for algal growth (Rico et al., 2014).

To determine the effects of abiotic degradation of TCS during the exposure, we performed two parallel fate experiments using similar conditions as the microcosm experiment but covered in aluminium foil to prevent light exposure (see supporting information for test details).

2.5. Sampling, water and sediment quality parameter analysis

Dissolved oxygen (DO: mg/L), temperature (°C), pH and conductivity (µS/cm) of the microcosms were monitored in the afternoon (15:00 hrs) on days -1, 1, 2, 4, 7, 11, 14, 18, 21 and 28 using a multi-meter placed at a water depth of 7 cm. Turbidity (NTU) was measured (15:00 hrs) using a turbidimeter on days 0, 1, 7, 14, 21 and 28.

At the end of the experiment (day 28), two water samples (0.5 L) were taken using a Teflon siphon from each microcosm. One sample was used to determine TCS concentration, while the other was used to determine water parameters including NH_3, NO_3^-, NO_2^-, total phosphorus and TOC. To measure TCS concentration and sediment quality parameters including total nitrogen, NH_3, total phosphorus and OM, a sediment column (depth 4 cm, diameter 3 cm) was collected from each microcosm using a thin-walled metal corer (inner diameter 3 cm, height 8 cm). Sediment samples were amended with sodium azide (1 g/L) to suppress any potential microbial activity, frozen at -20 °C, freeze-dried, homogenized, and passed through a 0.83 mm mesh. The sediment was stored at 4 °C until extraction.

2.6. TCS analysis

TCS concentrations in the overlying water and sediment were determined at the start (day 0) and end (day 28) of the experiment, while TCS concentrations in the worms were assessed prior to addition to the microcosms and on day 28. Additionally, TCS concentrations were also determined in the initial aerated tap water and spiked sediment that were added to the microcosms. TCS in water samples was extracted using solid-phase extraction (SPE), while TCS in sediment samples was extracted by ultrasonic extraction combined with SPE purification (Chen et al., 2012). TCS in worm tissue was extracted by QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction combined with dispersive solid-phase extraction (d-SPE) clean-up method (Yao et al., 2016). Detailed extraction methods are given in the supporting information . TCS in extracts were analysed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) with electrospray ionization (ESI) in the negative mode according to Chen et al. (2010) (see supporting information).

2.7. Organism sampling and analysis

At the end of the experiment, algae, D. magna, midges, worms and snails were sampled from the 24 microcosms where they had been introduced. To assess the algal biomass in the overlying water of these 24 microcosms, the chlorophyll-a content was measured by removing a 1 L water sample from each microcosm and filtering it through a Whatman GF/C glass-fibre filter (mesh size: 0.45 µm). Chlorophyll-a samples were extracted following the methods outlined in Zhou et al. (2012). The chlorophyll-a extracts were analysed spectrophotometrically following the methods of Li et al. (2000). Using a Teflon siphon, a further 10 L of water was collected from each microcosm and passed through a plankton net (mesh size: 55 µm) to sample D. magna abundance. Lugol's iodine solution was added to the sieved samples to fixate any organisms and samples were stored at 4 °C until further analysis. D. magna individuals were counted using a binocular microscope at a magnification of 15-25×.

After removing the overlying water, the sediment was gently sieved using a 300 µm mesh to collect midges, worms and snails. The collected benthic macroinvertebrates were then placed overnight into bottles with aerated tap water to empty their guts and remove any adhering TCS. The wet weight of each snail was measured after 28-d exposure and preserved in 70% ethanol. The wet weight of worms was estimated by randomly taking out a sub-sample of 20 worms from each microcosm with introduced organisms, worms were then released back to the container where they purged. Subsequently, worms were frozen at -20 °C until further TCS analysis. No midges were found in any of the systems at the end of the experiment, so no measurements were performed.

2.8. Data analysis

To assess body burden of TCS in the worms tissue, Biota-Sediment-Accumulation-Factor (BSAF) was calculated at the end of the exposure (day 28) using the following formula (Ankley et al., 1992): $(C_{org,WW}/f_{lip})/(C_{sed,DW}/f_{OM})$, where C_{org} is the chemical concentration in the organism (µg/g wet weight (ww)), C_{sed} is the chemical concentration in sediment (µg/g dw), f_{lip} is the lipid percentage of the organism (ww) and f_{OM} is the percentage of organic matter (dw). The bioaccumulation model proposed by Diepens et al., (2015) was used to explain differences between measured BSAF values among treatments, to identify the most significant uptake pathways and to check if a steady state at the end of the experiment was reached (see supporting information for model parameterization). The model equation is:

$$BSAF_t = \frac{C_{L,t=0}}{C^{SED}_{OM,\,t=0}} \times e^{-(k_e+k_g)t} + \frac{k_e K_{lip}/K^{SED}_{OM} + \alpha I[\beta+(1-\beta)\gamma]}{(k_e+k_g)} \times (1\text{-}e^{-(k_e+k_g)t}) \tag{1}$$

where $C_{L,t=0}$ is the measured background concentration in the macroinvertebrate lipid at time zero, $C^{SED}_{OM,\,t=0}$ the measured concentration in the sediment at time zero, normalized to organic matter content, k_e (d^{-1}) the rate constant for overall elimination, k_g (d^{-1}) the growth dilution, t (d) the exposure time, K_{lip} the lipid/water partition coefficient, K^{SED}_{OM} the sediment OM-water partition coefficient, α (-) the chemical assimilation efficiency, and I (≥0, $k_{gOM} \times k^{-1}_{gLIP} \times d^{-1}$) the mass of OM ingested per unit of time and organism lipid weight, β (0<β < 1) the fraction of ingested OM originating from the sediment, γ the constant ratio to estimate the relation between K^{SS}_{OM} (suspended solid OM-water partition coefficient) and K^{SED}_{OM}. In the model, we assumed that *L. hoffmeisteri*, as a bulk feeder, feed only sediment (SED) (β = 1), which includes sediment particles and recently settled particles from the water column, corresponding to earlier model results for a similar freshwater worm *Lumbricules variegatus* (Sidney et al., 2016). The percentage uptake through water is calculated based on equation as:

$$\%WaterUptake = \frac{k_e}{k_e + \alpha I[\beta + (1-\beta)\gamma]K_{OM}^{SED}/K_{lip}} \quad (2)$$

The fraction of steady state reached (F_{SS}, $0 < F_{SS} < 1$) in the 28-days bioaccumulation test (t = 28 d) was calculated as:

$$F_{SS} = 1 - e^{-(k_e + k_g)t} \quad (3)$$

No observed effect concentrations (NOECs) were calculated for water parameters, sediment parameters, phytoplankton chlorophyll a, periphyton chlorophyll a and survival of benthic macroinvertebrates using the Williams test (Williamss, 1972) which assumes a concentration-effect relationship and is, therefore, used extensively for the analysis of the results of eco-toxicity tests. The Williams tests were performed using the Community Analysis computer program, version 4.3.05 (Hommen et al., 1994). In order to down-weigh high abundance values and obtain approximately a normal distribution of the data, the abundance values of species were ln (Ax + 1) transformed, where x represents the abundance data and A was set as 2 (Van den Brink et al., 2000; Rico et al., 2014).

Differences in water parameters, sediment parameters, TCS concentrations, loss of TCS in the microcosms, phytoplankton chlorophyll-a, and periphyton chlorophyll-a concentrations were examined between microcosms with and without macroinvertebrates, using SPSS version 23.0. Prior to analyses, data were examined for normality using Shapiro-Wilk test, and homogeneity of variance between systems was detected by Levene's test. Accordingly, a two-way analysis of variance (ANOVA), one-way ANOVA, Kruskal-Wallis test, two independent samples t-test, Mann-Whitney test or Wilcoxon matched-pairs test was conducted. When significant ANOVA main effect was detected, Tukey’s test was used for post hoc comparisons. A significance level of $p = 0.05$ was set for comparison between means or rank-sum values.

3. Results

3.1. Water and sediment quality parameters

Dissolved oxygen concentration and temperature remained between 6-7 mg/L and 25-26 °C in the microcosms during the exposure period, respectively. During the first week of exposure pH was stable between 7.2-7.5 in all of the microcosms (Table S2). By the end of the experiment, a slight drop in pH was measured (from 7.45±0.05 to 6.29±0.11) in all microcosms. While conductivity values in most of the microcosms remained around 120-150 µS/cm, conductivity was higher in T4 in the microcosms containing organisms than those from all the remaining treatments (140-180 µS/cm) (Table S3). Turbidity decreased from day 0 (~ 200 NTU) to day 28 (< 15 NTU) in all microcosms with and without introduced organisms (Table S4).

In comparison to the initial added aerated tap water, NO_2^- and total phosphorus in the water phase remained at similar low levels after 28 days exposure in all treatments, whereas the TOC concentration increased approximately 6-fold to 9-fold after 28 days exposure in all treatments (Wilcoxon matched-pairs test, $p < 0.05$) (Table S5). In the microcosms containing the introduced organisms, T4 showed significantly higher levels of NH_3, NO_2^- and TOC than controls; NO_3^- concentration was significantly higher in all TCS treatments than controls (Williams test, $p < 0.05$) (Table S5). In the microcosms without introduced organisms, T4 showed significantly higher levels of NO_2^- and TOC than controls; NO_3^- concentration was significantly higher in T2, T3 and T4 than controls (Williams test, $p < 0.05$) (Table S5). There was no significant difference in total phosphorous (two independent samples t-test), NO_2^- (two independent samples t-test) and TOC (Mann-Whitney test) between systems with and without introduced organisms. NH_3 and NO_3^- concentrations were significantly higher in the system with introduced organisms than without (Mann-Whitney test, $p < 0.05$).

By the end of the experiment, the total nitrogen and NH_3 concentration in the sediment were significantly lower in both systems compared to those in the original sediment (Wilcoxon matched-pairs test, $p < 0.05$). OM and total phosphorous concentrations did not change significantly over time in any of the microcosms (Wilcoxon matched-pairs test) (Table S6). In the microcosms containing the introduced organisms, T4 showed significantly higher level of NH_3 than the controls (Williams test, $p < 0.05$). In the microcosms without introduced organisms, T3 and T4 showed significantly higher levels of NH_3 than the controls (Williams test, $p < 0.05$). There was no significant difference in OM, total nitrogen, total phosphorous and NH_3 between systems with and without introduced organisms (two independent samples t-test).

3.2. TCS concentrations in water, sediment and worms

The TCS concentrations in the overlying water, sediment and worms were analysed at the start and end of exposure (day 0, 28) (Table 1). The concentrations of TCS in both the water and sediment phase of the control groups were negligible (<0.002 µg/L or 0.002 µg/g dw) at both time points. After 28 days exposure, TCS concentrations decreased (> 90%) significantly in the overlying water both in systems with and without introduced organisms (Wilcoxon matched-pairs test, $p < 0.05$) (Table 1). In the microcosms containing introduced organisms, TCS concentration was significantly higher at T2 and T3 than T1 and T4 (one-way ANOVA, $p < 0.05$), with the highest level (0.613±0.030 µg/L) measured at T2 (Table 1 and Fig. 1). In the microcosms without introduced organisms, TCS concentration was significantly higher (~38-fold) at T4 than T1 (Kruskal-Wallis test, $p < 0.05$). There was a significant difference in TCS concentration in the overlying water between systems with and without introduced organisms (Mann-Whitney test, $p < 0.05$), such that the overlying water concentration was ~1.1-fold to 56-fold higher in systems with than without organisms.

By the end of experiment, in both systems TCS concentrations in the sediment were significantly lower than the initial spiked TCS concentrations by 3.43%±0.56%-11.76%±0.64% in system with introduced organisms and 3.46%±0.43%-10.91%±0.50% in system without introduced organisms (Wilcoxon matched-pairs test, $p < 0.05$) (Table 1). There was no significant difference in TCS concentration in the sediment between systems with and without introduced organisms at experimental end (Mann-Whitney test).

Mass-balance calculations that measure TCS dissipation in the microcosms showed significant loss of TCS in both systems relative to the initial spiked TCS amount (Wilcoxon matched-pairs test, $p < 0.05$) (Table S7 and Fig. 2). In microcosms containing the introduced organisms, the decreased TCS amount in the microcosms of T1 (11.4%±0.63%) was significantly higher than that of T4 (3.43%±0.56%) (Kruskal-Wallis test, $p < 0.05$) (Table S7 and Fig. 2). In the microcosms without introduced organisms, the decreased TCS amount in the microcosms of T1 (10.9±0.50%) was significantly higher than that of T4 (3.46±0.43%) (Kruskal-Wallis test, $p < 0.05$). Although TCS dissipation in the sediment was greater (0.12-0.50% in different treatments) in the microcosms with introduced organisms compared to those without, there was no significant difference in decreased TCS amount between systems with and without introduced organisms (Mann-Whitney test).

In the synchronized photolysis and hydrolysis experiments that were used to determine the abiotic degradation of TCS, under light conditions, TCS remained at the initial spiked concentration of 2 mg/L in the aerated tap water until day 14 and decreased to approximately 1 mg/L by day 28 (Table S8). In contrast, under the dark conditions, TCS remained at around 2 mg/L in the aerated tap water throughout the 28 days. In the water collected from the sacrificed microcosms of T1, there was no significant difference in TCS concentration between light conditions (5.24±0.56 µg/L) and dark conditions (5.27±0.34 µg/L) on day 28 (Table S8).

Table 1. TCS concentration in overlying water, sediment and worm at the start and end of exposure.

Treatment	Actual spiked concentration (µg/g dw)	Overlying water			Sediment (day 28) (µg/g dw)		Worm (day 28)	
		Day 0 (µg/L)	Day 28 (µg/L)		System with introduced organisms	System without introduced organisms	Concentrations (µg/g lw)	BSAF
			System with introduced organisms	System without introduced organisms				
CK1	0	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< LOQ	-
CK2	0	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< LOQ	-
T1	0.8	5.29±0.16	0.395±0.022	0.007±0.003	0.706±0.01	0.713±0.00	154±4.96	3.55±0.16
T2	8	39.6±1.51	0.613±0.030	0.013±0.007	7.11±0.05	7.17±0.04	552±12.3	1.28±0.07
T3	80	292±6.05	0.554±0.053	0.123±0.005	72.6±0.53	72.7±0.27	1650±63.8	0.38±0.01
T4	240	1190±14.3	0.237±0.029	0.216±0.017	232±1.34	232±1.02	*	*

CK1 and CK2 represent water control and acetone control, respectively.

T1, 0.8 µg/g dw treatment; T2, 8 µg/g dw treatment; T3, 80 µg/g dw treatment; 240 µg/g dw treatment.

dw, dry weight.

ww, wet weight.

BSAF = $(C_{org,WW}/f_{lip,\ WW})/(C_{sed,DW}/f_{OM,\ DW})$.

LOQ means method limit of quantitation (= 8 ng/g lw).

* No worms were present in T4 at the end of the experiment.

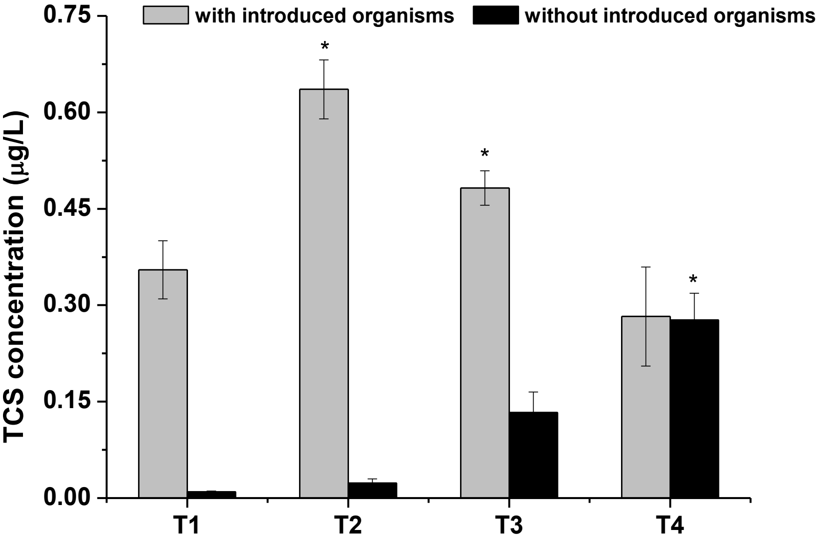

Figure 1. TCS concentrations (µg/L) in the overlying water at the end of exposure (day 28). Error bars represent standard error of the mean (n = 4). T1, T2, T3 and T4 represent treatments with actual TCS concentrations of 0.8, 8, 80 and 240 µg/g dry weight (dw), respectively. Gray and black bars represent treatment from microcosms with and without introduced organisms, respectively.

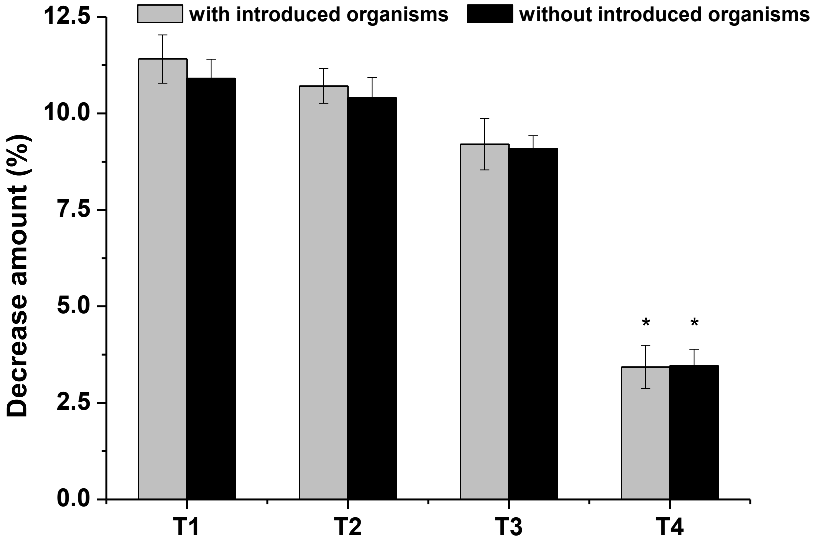

Figure 2. Changes of total TCS amount (%) in the water/sediment systems at the end of exposure (day 28). Error bars represent standard error of the mean (n = 4). T1, T2, T3 and T4 represent treatments with spiked TCS concentrations of 0.8, 8, 80 and 240 µg/g dry weight (dw), respectively. Gray and black bars represent treatment from microcosms with and without introduced organisms, respectively.

At experimental end, TCS was detected in the worm, with concentrations of 154, 552, 1650 µg/g tissue lipid weight (lw) in T1, T2 and T3, respectively (Table 1). Since all worms died in T4 (see section 3.3 below), no measurement was performed here. The TCS concentration was significantly higher in T3 than T1 (Kruskal-Wallis test, $p < 0.05$). Although the tissue concentration increases with increasing chemical concentration, the rate of increase decreases. Thus, the tissue concentrations are relatively higher in T2 compared to T1 (3.6x increase) and in T3 compared to T2 (3x increase) while the increase in sediment concentration stays equal among treatments (i.e., 10x increase). To gain a 10x increase in tissue concentration, the sediment concentration needs to increase 100x (from T1 to T3). Correspondingly, BSAF values decreased with increasing exposure concentration, decreasing from 3.55 (±0.16) in T1 to 0.38 (±0.01) in T3 (Table 1). The bioaccumulation model was used as an aid to further explain this observation. The model provided a good fit: BSAFs matched well with the measured BSAFs for all treatments (Fig. S1, Table S9). However, parameter estimates need to be interpreted and used carefully. To obtain this good fit, the ingestion rate (I) needed to be estimated for each treatment separately, though sediment and species do not differ between treatments. The bioaccumulation model provided satisfactorily narrow confidence intervals for the fitted parameters in all treatments (Table S9). Similar to BSAF values, the fitted ingestion rates decreased with increasing exposure concentration (T1 > T2 > T3, Table S9). The model shows that systems reached steady state at the end of experiment (Table S10). Sediment was the main uptake route (100%) for L. hoffmeisteri across all treatments (Table S10).

3.3. Effects of TCS on algae, benthic macroinvertebrates, and *D. magna*

After 28 days, the phytoplankton chlorophyll-a concentrations, used to assess algal density, were low in all treatments (< 0.25 µg/L) and were significantly lower at higher treatment levels (T2, T3 and T4, 0.13-0.20 µg/L) compared to T1 and controls (~0.24 µg/L) (Williams test, $p < 0.05$) (Table S11, Fig. 3). Periphyton chlorophyll-a levels varied between treatments and systems. As with phytoplankton chlorophyll-a, periphyton chlorophyll-a was higher in controls and low TCS treatments than higher TCS treatments in both systems (Table S11, Fig. 3). In the microcosms containing the introduced organisms, periphyton chlorophyll-a concentration was significantly lower in T2, T3, and T4 (0.27-0.4 µg/dm^2) than controls and T1 (0.64-0.65 µg/dm^2) (Williams test, $p < 0.05$). In the microcosms without introduced organisms, periphyton chlorophyll-a concentration was significantly lower in T3 and T4 (0.37-0.4 µg/dm^2) compared to controls, T1, and T2 (6.25-6.57 µg/dm^2) (Williams test, $p < 0.05$). Between the two systems, periphyton chlorophyll-a levels were significantly higher in the controls, T1, and T2 in the system without introduced organisms (6.25-6.57 µg/dm^2 compared to 0.27-0.65 µg/dm^2); levels in T3 and T4 were similar in both systems (~0.4 µg/dm^2).

Differences in survival of snails and worms were observed between treatments with higher TCS concentrations (T3 and T4) and lower concentrations (controls, T1 and T2) (Williams test, $p < 0.05$). A reduction in snail count (16.67% ± 0.3%) and worm count (85.2% ± 0.02%) was observed in T3 by the end of the experiment (Table S12). Complete mortality of snails and worms was observed in T4 after 28 days exposure (Table S12). There was no evidence of reproductive activity in either the snails or worms in any of the treatments during the study.

Based on these observed effects, the 28-d survival NOEC values for snails and worms were determined to be 8 μg/g dw TCS. There was no significant difference in wet weight of the surviving snails or worms between treatments at the end of the experiment (Kruskal-Wallis test).

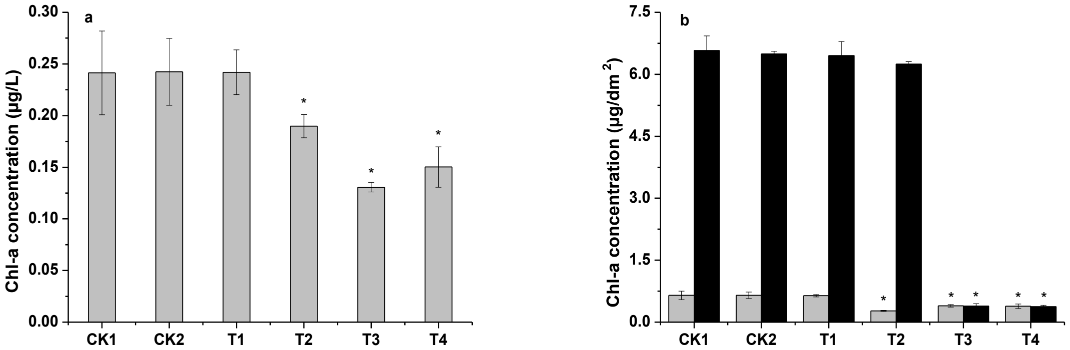

Figure 3. Chlorophyll a content in the water samples (a) and on the glass slides (b) at the end of exposure (day 28). Phytoplankton chl-a (μg/L) (a) was analysed only in microcosms with introduced organisms, since no algae was introduced into the microcosms without introduced organisms. Error bars represent standard error of the mean (n = 4). CK1 and CK2 represent water control and acetone control, respectively. T1, T2, T3 and T4 represent treatments with TCS concentrations of 0.8, 8, 80 and 240 μg/g dry weight (dw), respectively. Gray and black bars represent treatment from microcosms with and without macroinvertebrates, respectively.

There were no D. magna left in any of the treatments by the end of the exposure period, except for T3 where 2 ind./L were recorded. Additionally, no midge larvae or adults were found in any of the microcosms at the end of the experiment. Although midge emergence was observed after day 2, it was difficult to record their emergence number and date, since the microcosms were open systems.

4. Discussion

Overall, there was a significant dissipation of TCS in the microcosms after 28 days exposure in this study (3.43 (±0.56)-11.4 (±0.63)%), which is in agreement with data reported from other studies of TCS degradation by microorganisms (e.g., Ying et al., 2007 (half-life of 18 days under aerobic conditions and little degradation within 70 days under anaerobic conditions); Huang et al., 2015 (half-lives of 55-239 days)). TCS accumulated in the worm with BSAFs in the range of 0.38-3.55: BSAF decreased with increasing concentration. Although the presence of introduced organisms increased TCS concentrations in the overlying water compared to systems without introduced organisms, it did not significantly influence TCS dissipation in the microcosms. TCS at 8 μg/g dw, 5-fold higher than the currently detected maximum (1.33 μg/g dw, Zhao et al., 2010a), did not influence the survival and growth of snails and worms under the conditions in this study.

4.1 Fate of TCS in the microcosms

Deposition of suspended particles from the overlying water to the sediment phase and the partitioning of TCS between water and sediment likely contributed to the TCS decrease in the overlying water during the exposure period. This was confirmed by the measured turbidity values of larger than 100 NTU at the start of exposure given the 3 days deposition of systems. Because the aqueous TCS concentration was significantly higher in the microcosms with introduced organisms than without, it suggests that worms via bioturbation flushed TCS from the sediment phase to the overlying water. The contribution of abiotic degradation (i.e., photolysis and hydrolysis) to the loss of TCS in the microcosms was negligible during the experiment period, therefore the dissipation of TCS observed in the microcosms can mainly be attributed to biotic degradation and bioaccumulation. However, the presence of the introduced organisms did not significantly increase the dissipation of TCS in microcosms in this study. Unlike this study, Dai et al (2012) examined the fate of a sediment-associated fragrance material, acetyl cedrene (AC), in sediments inhabited by the polychaete, *Capitella teleta*, and found that more than 88% of the AC disappeared during 14 days of exposure when *C. teleta* was present (both at medium and high densities) compared to only about 25-30% in the absence of this worm. This difference can be attributed to many factors including a difference in invertebrate density, species differences in e.g., biotransformation capacity and properties of chemicals (e.g., Log Kow).

The amount of TCS that accumulated in the worm tissue varied between treatments, with BSAFs in the range of 0.38-3.55. This is likely related to differences in ingestion rate among treatments, as demonstrated by the bioaccumulation model: higher TCS levels in sediment lead to lower ingestion rates (Table S10). This suggests that worms actively avoided ingesting TCS contaminated sediments. This leads to lower uptake of TCS from the ingested sediment, which is also reflected in the BSAF values. Similar results have been reported for adult gastropod *Potamopyrgus antipodarum* exposure to galaxolide (HHCB, a polycyclic musk), whose feeding rate declined with increasing HHCB concentration (Pedersen et al., 2009). In a similar species of sediment dwelling worms (*Lumbriculus variegatus*), studies report both similar 28-day BSAF for TCS (1.4) as the current study (Dang et al., 2016), but also higher BSAF values (9.04) (Karlsson et al., 2016). For other species, BSAFs for TCS were lower. For example, in the freshwater clam, *Corbicula fluminea*, BSAF values of less than 1 have been reported (Edziyie et al. 2011). Such differences may be related to differences in sediment concentration that can impact the ingestion rate of organism (Pedersen et al., 2009), and sediment characteristics, such as organic matter content and pH value (Diepens et al., 2015; Karlsson et al., 2016), which would affect the retention of TCS in sediment and uptake of TCS into the worms. Likewise, differences in species traits (e.g., body size, diet preference and ingestion rate) can also be influencing factors (Diepens et al., 2015). Additionally, the possible biodegradation of TCS in the worms and/or by sediment microbial community could have lowered the tissue levels of TCS. For example, TCS is biodegraded into methyl-triclosan by nematodes, causing an increase in concentrations of methyl-triclosan but a lowering of TCS in the nematodes tissue (Macherius et al., 2014). However, the methyl-TCS is more persistent than TCS and is more prone to bioaccumulate in aquatic organisms, such as fish (James, et al., 2012; Pycke et al., 2014). Except for methylation, dihydroxylation is another degradation pathway occurring on the aromatic rings of TCS, which has been reported for microorganisms with 2,4-dichlorophenol as the major intermediate (Kim et al., 2011; Lee et al., 2012). However, little (eco)toxicological information is known about these degradation products of TCS. Further work, therefore, will be needed to assess their risks on aquatic organisms.

4.2. Effects of TCS on introduced organisms in the microcosms

The effects of TCS differed amongst the organisms that were introduced to the microcosms. In these microcosms, both phytoplanktonic and periphytonic chlorophyll-a content decreased as the concentrations of TCS in the sediment increased. Algae are known to be particularly sensitive to TCS, with growth inhibition typically being recorded at EC_{50} values of 0.53 µg/L to 430 µg/L (Table S13), depending on the test conditions and species (e.g., Orvos et al., 2002; Yang et al., 2008). Algal growth was likely inhibited in higher TCS treatments due to elevated water concentrations of TCS and increased turbidity during the first week, both a result of sediment resuspension at the start of the experiment. Initial high turbidity (30-131 NTU) and grazing pressure from *D. magna* during the first week likely contributed to low chlorophyll-a contents in T1 and controls. In microcosms without introduced organisms, the periphyton chlorophyll-a levels were also reduced in the higher TCS concentration treatments, demonstrating that TCS was an important factor influencing the growth of periphyton given the similar turbidity values among treatments during the exposure period.

Almost complete loss of *D. magna* from all treatments, including controls, indicates that shortage of food was probably the main cause of death, rather than TCS stress. However, because multiple measurements of survival were not taken during the experiment, the effects of TCS on *D. magna* cannot be discounted. NOEC for *D. magna* survival was determined at 200 µg/L (21-d TCS exposure, Orvos et al., 2002), and a 96 h LC_{50} at 338 µg/L TCS (Wang et al., 2013). As these concentrations are in line with, or less than, some of the TCS concentrations used here, it is likely that TCS would have influenced D. magna survival in the highest TCS treatment.

Here, the survival of snails and worms was reduced once TCS concentrations reached 80 µg/g dw, with a complete loss of individuals at 240 µg/g dw TCS. Based on these values, we determined the 28-d survival NOEC values for these organisms as 8 µg/g dw TCS. On the same species of worm as used here, the toxic effects of TCS have been observed to begin at much higher concentrations. For example, an LC_{50} value of 2046 µg/L has been reported for *L. hoffmeisteri* in a 96-h acute toxicity test (Wang et al., 2013), which is higher than the TCS initial level (1190 µg/L) in the overlying water of the highest TCS treatment used here. This may be related to the difference in pH between the two studies (8.0 in that study compared to below 7.5 in this study), which can affect the toxicity of TCS as TCS in neutral form is more toxic than in ionic form (Orvos et al., 2002; Roberts et al., 2014). Additionally, the presence of sediment in the microcosms used here increases the possible modes of TCS uptake by *L. hoffmeisteri* (pore water, sediment and overlying water), and also that TCS concentration in the overlying water could include TCS sorbed to colloids in the presence of sediment, which is not the case for water-only setups (as in Wang et al., 2013).

4.3. Implications for environmental risk assessment

Although previous research has reported bioaccumulation of various organic contaminants in aquatic organisms, there are no data on the fate of sediment-associated TCS in microcosms consisting of water, sediment and a diverse community. TCS concentrations up to a few µg/g dw have been monitored in subtropical freshwater surface sediments impacted by direct and indirect discharge of wastewater (Table S14). Our results suggest that sediment-associated TCS at such environmental concentrations is unlikely to impose a direct toxicological risk, at least in the short term, to snails and worms present in the water-sediment systems (NOEC = 8 µg/g dw TCS). We also measured the bioaccumulation of TCS in the oligochaete worm, and the results suggest that sediment-associated TCS is available for uptake in *L. hoffmeisteri* and may be biotransformed. However, further studies are required to investigate whether there is metabolism of TCS in worms and their related ecotoxicity to aquatic organisms.

In conclusion, this study showed that TCS accumulated in the worm used in this study. Although benthic invertebrates may have contributed to the mobilization of TCS from the sediment to the overlying water, they did not make a significant contribution to the removal of TCS from the microcosms under the experimental conditions used here. Partitioning of TCS into sediment can decrease aquatic concentrations and reduce toxicity of TCS to pelagic species, but could pose an increased risk to benthic species. However, the results shown here indicate that current maximal measured environmental concentrations in the subtropics probably do not affect the survival and growth of snails and worms in contaminated sediments, at least in the short term. For ERA, long term effects, indirect effects and bio-magnification of TCS in the food web should be further investigated.

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Supporting information

Materials and Methods

Photolysis and hydrolysis experiment

We conducted 2 experiments in Erlenmeyer flasks to determine TCS photolysis and hydrolysis under conditions in the present study. Experiment 1: 50 µL of TCS dissolved in acetone (20 g/L) was added into a 1 L Erlenmeyer flask and let evaporated. 500 mL of de-chlorinated tap water was then added into the flask to achieve final TCS concentration of 2 mg/L. Subsequently, flasks were placed in a shaker to equilibrate for 2 h in the dark. Then three Erlenmeyer flasks with TCS solution were applied to measure TCS photolysis and hydrolysis under the same conditions as microcosm experiment, while the other three flasks with same TCS solution were covered with aluminum foil to prevent possible photolysis of TCS. 1 mL of TCS solution was sampled from each flask on day 0, 3, 7, 14, 21 and 28. For sample extraction, 50 ng of internal standard was added into each sample and mixed well. Then, 2 mL of n-hexane was added, manually shaken for 1 min and put at room temperature in the dark for 5 min. The supernatant of n-hexane phase was transferred carefully to a 5 mL glass centrifugal tube using a glass pipette. The extraction procedures of the second round were same as the first time. Then hexane mixture was dried under a gentle nitrogen stream. The final extract was re-dissolved in 1 mL of methanol, transferred to a 2 mL amber glass and stored at -20 °C until analysis.

Experiment 2: Same as in the experiment 1, six replicates were set in this experiment with three of them covered with aluminum foil to keep solutions away from light. Specifically, each Erlenmeyer flask (volume: 500 mL) was added with 250 mL of overlying water collected from the microcosm with spiked TCS concentration of 0.8 µg/g dw for determination of TCS concentration at the start of exposure. Then, Erlenmeyer flask with overlying water were placed in the same conditions as microcosm experiment. On day 28, the whole Erlenmeyer flask was sacrificed to determine TCS concentrations. The extraction method was same as water samples from the microcosm.

Extraction of TCS in water samples

The water samples (500 L each) were extracted by solid phase extraction (SPE) with Oasis HLB SPE cartridges (200 mg, 6 mL), which is similar to a previous reported method (Chen et al., 2012). The collected water samples were filtered using 0.7-µm glass fiber filters, spiked with the internal standards (100 ng/L each), loaded onto cartridges preconditioned with 10 mL of methanol and 10 mL of Milli-Q water. The filtered water samples were passed through cartridges at a flow rate of 5-10 mL/min. Each sample bottle was rinsed twice with 50 mL of Milli-Q water containing 5 % methanol (v/v) and passed through the SPE cartridge. The cartridges were then dried under vacuum for 3 h. The cartridges were eluted with 3×2 mL of methanol followed by 3×2 mL of ethyl acetate. The eluates were dried under a gentle nitrogen gas, re-dissolved in 1 mL of MeOH, transferred to a 2 mL amber glass vial with filtering through a 0.22 μm nylon membrane filter, and finally stored at -18 °C until analysis.

Extraction of TCS in sediment samples and suspended particles

Sediment samples or suspended particles were extracted three times by ultrasonic extraction, followed by SPE purification (Chen et al., 2012). Briefly, 1 g of freeze-dried solid samples were spiked with 100 ng of each internal standard in a 30-mL glass centrifuge tubes, manually mixed, left in a fume hood to volatilise organic solvent, and stored in a 4 °C cold room overnight. Each replicate was then mixed with 9 mL of methanol containing 0.1% formic acid (v/v), sonicated for 15 min at room temperature, and then centrifuged at 1370 *g* for 10 min. The supernatants of the three times of extraction were combined, diluted to about 300 mL with Mill-Q water and purified using SPE with same method as above water samples.

Extraction of TCS in worm tissue

The quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction combined with dispersive solid-phase extraction (d-SPE) clean-up method reported by Yao et al. (2016) was applied to extract TCS in the tubifex. Briefly, 0.5 grams of tubifex tissue sample was weighted into a 50-mL polypropylene centrifuge tube, spiked with internal standard (50 ng) into each tube and vortexed for 30 s. Then samples were stored at 4 °C for 30 min for equilibration. After that, five milliliters of Milli-Q water and two ceramic homogenizers were added into each tube and vortexed for 1 min to homogenize the sample sufficiently. Subsequently, 10 mL of acetonitrile containing 1 % acetic acid, a salt set containing 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous sodium acetate were added and hand shaken for 1 min immediately. Samples were then ultrasonic extracted for 15 min and centrifuged at 2364 *g* for 10 min. Supernatants were transferred to 15 mL d-SPE tube containing 900 mg anhydrous $MgSO_4$, 150 mg PSA, and 150 mg C18 and vortexed for 1 min, followed by centrifugation at 2364 *g* for 10 min. Then supernatants were dried under a gentle stream of nitrogen and re-dissolved by 0.5 mL methanol, transferred to a 2 mL amber glass vial with filtering through a 0.22 μm nylon membrane filter, and finally stored at -18 °C until instrumental analysis.

LC-MS/MS analysis

TCS was analyzed by an Agilent 1200 rapid resolution liquid chromatograph coupled to Agilent G6460A triple quadrupole mass spectrometer under electrospray negative ionization (ESI) mode (Chen et al., 2010). A 10 μL aliquot of each extract was injected into an Agilent SB-C18 column (3.0 mm × 100 mm ID, 1.8 μm particle size) at temperature of 40 °C with an RRLC in-line pre-column filter (4.6 mm, 0.2 μm filter), with Milli-Q water containing 0.01% acetic acid (v/v) (solvent A) and acetonitrile : methanol (1:1, v/v) (solvent B) as the mobile phase at a flow rate of 0.3 mL/min. The gradient program was given as follows: 60% B at 0 min, then increased to 90% B at 3 min and kept at 90% B for 4 min, then returned to the initial 60% B at 9 min and let column re-equilibrate for 6 min. The capillary was maintained at 3500

V. Dry and sheath gas flows were kept at 8 and 12 mL/min, respectively. Both dry and sheath temperatures were kept at 350 °C.

Model parameterization

The model was implemented in Microsoft Excel 2010 and fitted to the experimental BSAF data using the Excel Solver tool with scaling of parameters and a relative least-squares criterion. The parameter k_g was obtained from weight measurements and the parameters k_e, I and the affinity of chemical partitioning to organic matter in the relation (b) were estimated using a two-stage iterative approach. Confidence intervals (90% CI) were calculated according to Draper and Smith (1981). *L. hoffmeisteri* is a bulk sediment feeder therefor β was set to 1, as was previously also done for a similar worm *Lumbricules variegatus* (Sidney et al., 2016). The constant ratio γ was set to 0 as the model was insensitive to changes in this parameter. This reduces the number of parameters that need to be estimated with 2. Additionally, it was previously shown that reducing the number of parameters did not significantly affected the model performance (Diepens et al., 2015; Sidney et al., 2016).

Note that this model exercise was not intended to provide a full parameter set for *L. hoffmeisteri* but merely as a way to explain observed BSAF values in the experiment and calculate the steady state body burden. During the parameterization, the global minimum may not have been reached for this specific parameter set, as the number of observations (n=12) was small compared to the number of parameters (p=5) to be estimated. However, with other parameters sets with less residual sum of squares the same conclusions were reached. Moreover, the model was able to give satisfactory small confidence intervals. Despite this, parameter estimates need to be interpreted and used carefully.

Table S1. Traits of *Limnodrilus hoffmeisteri*, *Viviparidae Bellamya* and *Orthocladiinae*.

Traits	*Limnodrilus hoffmeisteri*	*Viviparidae Bellamya*	*Orthocladiinae*
			
Food type	microorganisms, living microphytes, mud, sand and detritus	living microphytes, living macrophytes, dead animal, living microinvertebrates, dead plant and detritus	living microphytes, living macrophytes, living microinvertebrates and detritus
Body length (mm) [a]	15-20	23-25	7-9
Weight (mg) [a]	1-1.6	2290-3250	1.9-2.6
Average lipid content (SD) (%) [a]	1.50 ± 0.1	not determined	not determined
Respiration type	tegument	tegument, gill	tegument,gill
Life cycle duration	> 1 year	> 1 year	≤ 1 year
Generation time (years)	0.5-1	1-2	<1
Life span (years)	> 1	> 5	0.6-1.9
Dispersal mode	passive	passive, active	passive, active
Dispersal ability	low	low	high
Aquatic stages	egg, larva, adult	egg, adult	egg, larva, nymph
Reproduction type	asexual reproduction	sexual reproduction	sexual reproduction
Feeding habits	absorber, deposit feeder	shredder, scraper, filter-feeder	deposit feeder, scraper, filter-feeder, parasite
Reproduction	clutches, cemented or fixed	ovoviviparity	clutches, cemented or fixed, clutches free, clutches
Current velocity	null-fast (> 50 cm/s)	null-medium (25-50 cm</s)	null-fast (> 50 cm/s)
Trophic status	mesotrophic, eutrophic	oligotrophic, mesotrophic, eutrophic	oligotrophic, mesotrophic, eutrophic
Temperature	eurythermic	eurythermic	eurythermic
Water depth	water surface, shallows, middle depths,	shallows, middle depths, bottom	shallows, middle depths, bottom

Table S1. (Continued).

Traits	*Limnodrilus hoffmeisteri*	*Viviparidae Bellamya*	*Orthocladiinae*
Locomotion and substrate relation	burrower, interstitial	crawler, burrower	full water swimmer, crawler, burrower, interstitial, temporarily attached
Transversal distribution	river channel, banks, connected side-arms, ponds, pools, disconnected side-arms, lakes	river channel, banks, connected side-arms, ponds, pools, disconnected side-arms, marshes, peat bogs, lakes	banks, connected side-arms, ponds, pools, disconnected side-arms, marshes, peat bogs, temporary waters, lakes, crenon
Substrate type	gravel, sand, silt, macrophytes, microphytes, twigs/roots, organic detritus/litter, mud	flags/boulders/cobbles/pebbles, gravel, macrophytes, mud	flags/boulders/cobbles/pebbles, gravel, sand, silt, macrophytes, microphytes, organic detritus/litter, mud

[a] Traits determined in the present study at the start of the experiment. The remainder traits information was retrieved from the PondFX (Heneghan et al., 1999) and Tachet database (Tachet et al., 2000; Usseglio-Polatera et al., 1999; 2000).

Table S2. pH values during the experiment period.

Group	Treatment	day-1	day1	day2	day4	day7	day11	day14	day18	day21	day28
System with introduced organisms	CK1	7.45±0.05	7.54±0.10	7.36±0.06	7.18±0.05	7.19±0.07	6.55±0.12	6.39±0.08	6.26±0.12	6.24±0.08	6.29±0.11
	CK2	7.45±0.03	7.50±0.01	7.41±0.03	7.31±0.04	7.19±0.05	6.54±0.06	6.50±0.02	6.48±0.02	6.52±0.09	6.49±0.02
	T1	7.45±0.05	7.44±0.06	7.41±0.02	7.32±0.03	7.29±0.03	6.58±0.07	6.55±0.03	6.56±0.03	6.43±0.05	6.46±0.06
	T2	7.39±0.02	7.42±0.01	7.31±0.13	7.27±0.08	7.34±0.01	7.00±0.04	6.75±0.10	6.41±0.09	6.42±0.14	6.34±0.10
	T3	7.39±0.02	7.41±0.03	7.44±0.04	7.28±0.02	7.43±0.04	7.21±0.07	7.03±0.15	6.43±0.07	6.33±0.31	6.30±0.15
	T4	7.43±0.03	7.44±0.07	7.50±0.10	7.35±0.02	7.62±0.03	7.40±0.06	7.52±0.17	7.28±0.07	7.19±0.12	6.61±0.06
System without introduced organisms	CK1	7.45±0.04	7.48±0.01	7.33±0.07	7.25±0.04	7.16±0.02	6.66±0.02	6.62±0.12	6.85±0.16	6.90±0.07	7.09±0.03
	CK2	7.42±0.08	7.51±0.03	7.49±0.02	7.33±0.04	7.31±0.05	6.84±0.06	6.94±0.02	7.04±0.08	7.23±0.04	7.22±0.01
	T1	7.42±0.03	7.44±0.05	7.47±0.03	7.30±0.04	7.33±0.03	6.84±0.09	6.82±0.08	6.87±0.10	6.97±0.09	7.22±0.07
	T2	7.36±0.02	7.38±0.04	7.40±0.03	7.19±0.05	7.45±0.02	7.06±0.02	6.86±0.07	6.89±0.09	7.01±0.02	7.25±0.01
	T3	7.38±0.03	7.43±0.02	7.41±0.03	7.28±0.02	7.41±0.01	7.11±0.05	6.99±0.10	6.90±0.07	6.90±0.07	6.62±0.05
	T4	7.44±0.01	7.54±0.04	7.51±0.03	7.38±0.06	7.47±0.02	7.17±0.01	7.13±0.04	7.11±0.04	7.04±0.20	6.81±0.04

CK1 represents water control while CK2 means acetone control.

T1, 0.8 μg/g dw treatment; T2, 8 μg/g dw treatment; T3, 80 μg/g dw treatment; 240 μg/g dw treatment.

Table S3. Conductivity values (μS/cm) during the experiment period.

Group	Treatment	day-1	day1	day2	day4	day7	day11	day14	day18	day21	day28
System with introduced organisms	CK1	134.13±1.19	135.35±2.00	136.33±3.23	134.13±7.11	132.60±4.24	126.38±1.35	125.05±1.67	129.85±2.00	131.18±4.48	135.60±7.05
	CK2	134.65±0.67	134.48±1.11	135.43±2.13	132.25±4.80	131.03±4.44	121.13±2.95	117.75±1.98	122.45±1.96	124.20±1.89	129.53±1.77
	T1	135.85±1.73	134.25±2.04	135.10±2.72	134.13±4.16	136.90±5.93	126.58±6.00	122.95±4.43	128.35±5.10	130.98±6.18	136.70±7.90
	T2	137.45±0.75	136.43±1.59	136.40±3.09	135.00±4.23	137.05±3.34	133.60±4.36	127.15±3.90	127.60±4.45	128.73±4.78	131.68±5.66
	T3	141.58±2.39	138.45±2.22	139.78±2.50	146.43±7.66	153.08±7.84	149.73±7.79	143.83±8.05	139.73±7.47	143.58±4.06	147.03±3.00
	T4	140.28±0.42	148.13±1.28	153.48±2.57	165.98±8.19	179.20±6.83	180.45±8.34	175.28±7.27	173.60±7.47	170.63±8.89	161.20±7.74
System without introduced organisms	CK1	133.38±3.23	132.53±4.62	133.05±3.80	129.38±4.19	129.73±2.29	124.73±1.61	123.60±1.37	128.63±2.96	128.73±3.81	134.53±5.32
	CK2	135.95±2.17	134.88±3.26	135.73±2.52	133.13±2.46	132.40±2.48	125.98±2.03	123.53±1.87	127.33±2.47	126.85±3.49	132.38±5.65
	T1	135.80±0.58	133.80±1.59	135.03±2.24	134.25±2.76	135.43±3.67	128.10±2.07	124.70±1.93	128.08±2.40	127.03±2.94	131.58±3.04
	T2	136.98±0.67	135.53±0.67	137.15±0.95	137.40±0.88	141.30±1.64	135.78±3.34	128.75±2.80	130.78±3.48	129.83±4.17	132.93±4.58
	T3	139.98±1.53	137.00±0.91	136.33±0.81	134.33±1.00	137.60±1.37	134.05±1.60	131.48±2.39	132.83±3.11	134.88±3.46	140.00±3.35
	T4	140.48±0.49	146.63±1.48	147.83±0.55	145.08±3.45	146.55±4.96	142.23±3.90	141.70±3.26	146.33±4.13	145.13±6.62	147.08±6.00

CK1 represents water control while CK2 means acetone control.

T1, 0.8 μg/g dw treatment; T2, 8 μg/g dw treatment; T3, 80 μg/g dw treatment; 240 μg/g dw treatment.

Table S4. Turbidity values (NTU) during the experiment period.

Group	Treatment	day0	day1	day7	day14	day21	day28
System with introduced organisms	CK1	93.63±17.37	54.43±13.55	28.55±9.02	14.25±3.91	9.15±2.63	11.47±6.62
	CK2	101.77±31.70	50.55±23.43	41.10±4.01	21.83±5.39	12.48±3.52	10.54±3.57
	T1	131.57±43.67	72.45±27.73	66.95±12.99	23.50±3.85	11.41±1.89	10.61±3.97
	T2	199.33±32.52	126.68±21.22	106.43±20.45	56.35±20.63	21.51±10.61	12.14±5.03
	T3	212.00±27.33	135.98±23.76	80.48±33.88	64.93±12.32	16.53±6.62	8.33±3.79
	T4	116.33±17.56	86.93±14.80	13.39±5.57	18.69±10.05	8.67±5.70	7.02±6.37
System without introduced organisms	CK1	151.17±55.34	125±41.07	35.72±33.45	16.79±15.57	4.63±1.54	3.00±0.62
	CK2	129.67±62.33	81.70±17.60	9.99±1.36	4.39±1.64	1.70±0.95	1.36±0.95
	T1	100.27±34.59	48.15±16.54	11.88±6.14	3.18±0.96	1.98±1.39	1.95±1.09
	T2	172.67±36.85	104.50±20.42	42.80±8.04	15.66±3.41	9.44±1.56	6.70±5.62
	T3	196.00±34.00	125.95±25.62	62.02±24.91	35.20±4.15	6.36±3.04	6.63±1.17
	T4	151.67±43.80	88.98±11.59	24.33±10.31	18.69±6.40	10.30±1.12	6.25±0.96

CK1 represents water control while CK2 means acetone control.

T1, 0.8 μg/g dw treatment; T2, 8 μg/g dw treatment; T3, 80 μg/g dw treatment; 240 μg/g dw treatment.

Table S5. Nutrients concentrations in the water phase (mg/L) at the end of exposure.

Group	Treatment	NH_3-N	NO_3-N	NO_2-N	TP	TOC
System with introduced organisms	CK1	0.17±0.04	1.01±0.24	0.00±0.00	0.01±0.00	3.97±0.14
	CK2	0.18±0.04	1.07±0.19	0.00±0.00	0.01±0.00	3.92±0.19
	T1	0.20±0.04	1.25±0.14 *	0.00±0.00	0.01±0.00	4.10±0.30
	T2	0.22±0.03	1.36±0.10 *	0.00±0.00	0.02±0.00	3.98±0.39
	T3	0.20±0.04	2.95±0.21 *	0.00±0.00	0.02±0.00	3.87±0.31
	T4	0.48±0.04 *	3.75±0.37 *	0.01±0.00 *	0.02±0.00	5.42±0.46 *
System without introduced organisms	CK1	0.15±0.01	0.07±0.00	0.00±0.00	0.02±0.01	4.17±0.11
	CK2	0.19±0.04	0.07±0.00	0.00±0.00	0.01±0.00	4.12±0.20
	T1	0.17±0.04	0.09±0.03	0.00±0.00	0.01±0.01	3.72±0.38
	T2	0.18±0.04	0.14±0.04 *	0.00±0.00	0.02±0.01	3.89±0.31
	T3	0.18±0.04	2.37±0.23 *	0.00±0.00	0.02±0.00	3.82±0.33
	T4	0.15±0.03	2.94±0.19 *	0.01±0.01 *	0.02±0.01	6.05±0.50 *
initial added aerated tap	-	0.30±0.01	1.64±0.01	0.00±0.00	0.01±0.00	0.56±0.02

* represents treatment showed significant difference using Williams test ($p < 0.05$). These significances were relative to the controls.

CK1 represents water control while CK2 means acetone control.

T1, 0.8 μg/g dw treatment; T2, 8 μg/g dw treatment; T3, 80 μg/g dw treatment; 240 μg/g dw treatment.

Table S6. Nutrients concentrations in the sediment (g/kg) at the end of exposure.

Group	Treatment	OM	TN	TP	NH_3-N
System with introduced organisms	CK1	18.52±0.06	1.15±0.08	0.40±0.01	0.04±0.00
	CK2	18.54±0.15	1.34±0.06	0.39±0.01	0.05±0.00
	T1	18.53±0.22	1.20±0.07	0.40±0.01	0.05±0.00
	T2	18.51±0.16	1.25±0.04	0.41±0.01	0.05±0.00
	T3	18.49±0.13	1.24±0.15	0.39±0.01	0.06±0.00
	T4	18.56±0.19	1.37±0.08	0.40±0.01	0.07±0.01 *
System without introduced organisms	CK1	18.56±0.20	1.22±0.12	0.40±0.01	0.05±0.00
	CK2	18.57±0.23	1.25±0.13	0.40±0.01	0.06±0.01
	T1	18.57±0.10	1.27±0.05	0.40±0.01	0.05±0.01
	T2	18.53±0.16	1.17±0.13	0.39±0.01	0.05±0.01
	T3	18.52±0.09	1.23±0.02	0.40±0.01	0.08±0.00 *
	T4	18.58±0.15	1.22±0.05	0.39±0.01	0.08±0.01 *
Original sediment	-	18.53±0.15	1.62±0.03	0.40±0.01	0.12±0.00

* represents treatment showed significant difference using Williams test ($p < 0.05$). These significances were relative to the controls.

CK1 represents water control while CK2 means acetone control.

T1, 0.8 μg/g dw treatment; T2, 8 μg/g dw treatment; T3, 80 μg/g dw treatment; 240 μg/g dw treatment.

Table S7. Changes of TCS amount (%) in the microcosms at the end of exposure.

Treatment	Decreased TCS amount		Contribution of introduced organisms
	System with introduced organisms	System without introduced organisms	
CK1	-	-	-
CK2	-	-	-
T1	11.4±0.63	10.9±0.50	0.50±0.66
T2	10.7±0.45	10.40±0.52	0.31±0.96
T3	9.20±0.66	9.09±0.33	0.12±0.99
T4	3.43±0.56	3.46±0.43	-

CK1 represents water control while CK2 means acetone control.

T1, 0.8 μg/g dw treatment; T2, 8 μg/g dw treatment; T3, 80 μg/g dw treatment; 240 μg/g dw treatment.

Table S8. TCS concentrations in the hydrolysis and photolysis experiment.

Sampling points	Aerated tap water (mg/L)		Overlying water (μg/L)	
	Light	Dark	Light	Dark
day 0	2.01±0.01	2.01±0.01	5.29±0.21	5.29±0.21
day 3	2.00±0.01	2.00±0.02	-	-
day 7	2.01±0.03	2.01±0.03	-	-
day 14	2.00±0.01	2.00±0.02	-	-
day 21	1.18±0.04	2.01±0.01	-	-
day 28	0.96±0.05	1.99±0.03	5.24±0.56	5.27±0.34

Table S9. Parameters and their 90% confidence intervals obtained from fitting with data for *L. hoffmeisteri* using the bioaccumulation model. Ingestion 'I' was fitted separately for treatments: T1, T2 and T3.

Parameters		CI	Value
a (-)		L90	5.338
			5.361
		H90	5.385
b (-)*		L90	1.445
			3.995
		H90	-
I (kg OM/kg lipids/d)	T1	L90	14.705
			16.160
		H90	17.758
	T2	L90	5.295
			5.819
		H90	6.394
	T3	L90	1.582
			1.738
		H90	1.910
$SS_{min} = 3.259*10^{-3}$	n = 12	p = 5	F-ratio = 2.883

T1, 0.8 µg/g dw treatment; T2, 8 µg/g dw treatment; T3, 80 µg/g dw treatment; 240 µg/g dw treatment.

L90, lower boundary of the 90% Confidence Interval; H90, higher boundary of the 90% Confidence Interval.

'-' represents confidence limit not within two orders of magnitude above or below estimated value.

*LogKOM,sed=LogKOW+b

Table S10. Fraction of steady state reached (FSS) * and uptake from sediment in the 28 d bioaccumulation test with *L. hoffmeisteri* obtained from the bioaccumulation model.

Treatment	Fraction of steady state reached	Fraction of uptake from sediment
T1	1.0	1.0
T2	1.0	1.0
T3	1.0	1.0

* Fraction of Steady State reached (FSS) with 0 < FSS <1. A value of 0 means that bioaccumulation is zero (t=0) and a value of 1 means that bioaccumulation is at steady state.

T1, 0.8 µg/g dw treatment; T2, 8 µg/g dw treatment; T3, 80 µg/g dw treatment; 240 µg/g dw treatment.

Table S11. Chlorophyll-a contents of phytoplankton and periphyton at the end of exposure.

Treatment	Phytoplanktonic chl-a (μg/L)	Periphytonic chl-a (μg/dm^2)	
	System with invert	System with introduced organisms	System without introduced organisms
CK1	0.241±0.040	0.647±0.105	6.574±0.355
CK2	0.242±0.032	0.650±0.079	6.491±0.064
T1	0.242±0.022	0.639±0.030	6.451±0.341
T2	0.190±0.011 *	0.273±0.014 *	6.246±0.059
T3	0.131±0.005 *	0.396±0.031 *	0.390±0.058 *
T4	0.150±0.020 *	0.384±0.055 *	0.374±0.034 *

chl-a represents chlorophyll-a; chlorophyll-a concentration of phytoplankton was analysed only in microcosms with introduced organisms, because they were added with phytoplankton at the beginning of experiment.

[*] represents treatment showed significant difference using Williams test ($p < 0.05$). These significances were relative to the controls.

CK1 represents water control while CK2 means acetone control.

T1, 0.8 μg/g dw treatment; T2, 8 μg/g dw treatment; T3, 80 μg/g dw treatment; 240 μg/g dw treatment.

Table S12. Counts of organisms survived at the end of exposure (day 28).

Treatment	Snail	Daphnia	Worm	Midge
CK1	6	0	233±7	0
CK2	6	0	234±6	0
T1	6	0	235±4	0
T2	6	0	232±7	0
T3	5 *	23±3	35±5 *	0
T4	0 *	0	0 *	0

CK1 and CK2 represent water control and acetone control, respectively.

T1, T2, T3 and T4 represent treatments with TCS spiked concentrations of 0.8, 8, 80 and 240 μg/g dry weight (dw), respectively.

[*] represents treatment showed significant difference using Williams test ($p < 0.05$). These significances were relative to the controls.

Table S13. Summary of the aquatic eco-toxicity data for TCS.

Species	Trophic group	Duration, Effect, Endpoint	Value (μg/L)	Reference
Anabaena flos-aqua	Algae	96 h, Biomass, EC_{50}	0.97	(Orvos et al., 2002)
Dunaliella tertiolecta	Algae	96 h, Cell density, NOEC	3.55	(DeLorenzo and Fleming, 2008)
Navicula pelliculosa	Algae	96 h, Biomass, EC_{50}	19.1	(Orvos et al., 2002)
Pseudokirchneriella subcapitata	Algae	72 h, Growth inhibition, IC_{50}	0.53	(Yang et al., 2008)
Pseudokirchneriella subcapitata	Algae	72 h, Growth inhibition, NOEC	0.2	(Yang et al., 2008)
Pseudokirchneriella subcapitata	Algae	72 h, Growth inhibition, LOEC	0.4	(Yang et al., 2008)
Pseudokirchneriella subcapitata	Algae	96 h, Growth, NOEC	8.3	(Harada et al., 2008)
Pseudokirchneriella subcapitata	Algae	72 h, Growth inhibition, EC_{50}	5.1	(Tamura et al., 2013)
Pseudokirchneriella subcapitata	Algae	72 h, Growth inhibition, NOEC	0.53	(Tamura et al., 2013)
Scenedesmus subspicatus	Algae	72 h, Growth rate, EC_{50}	2.8	(Orvos et al., 2002)
Scenedesmus subspicatus	Algae	72 h, Growth rate, NOEC	0.5	(Orvos et al., 2002)
Scenedesmus subspicatus	Algae	96 h, Biomass, EC_{50}	1.4	(Orvos et al., 2002)
Scenedesmus subspicatus	Algae	96 h, Biomass, NOEC	0.69	(Orvos et al., 2002)
Selenastrum capricornutum	Algae	96 h, Biomass, EC_{50}	4.46	(Orvos et al., 2002)
Skeletonema costatum	Algae	96 h, Biomass, EC_{50}	>66.0	(Orvos et al., 2002)
Lemna gibba	Duckweed	7 d, Biomass, EC_{50}	>62.5	(Orvos et al., 2002)
Ceriodaphnia dubia	Crustacean	7 d, Survival, NOEC	50	(Orvos et al., 2002)
Ceriodaphnia dubia	Crustacean	7 d, Survival, LOEC	339	(Orvos et al., 2002)
Ceriodaphnia dubia	Crustacean	7 d, Reproduction, NOEC	6	(Orvos et al., 2002)
Ceriodaphnia dubia	Crustacean	7 d, Reproduction, NOEC	182	(Orvos et al., 2002)
Ceriodaphnia dubia	Crustacean	8 d, Reproduction, NOEC	30	(Tamura et al., 2013)
Daphnia magna	Crustacean	21 d, Reproduction, NOEC	40	(Orvos et al., 2002)
Daphnia magna	Crustacean	21 d, Reproduction, LOEC	200	(Orvos et al., 2002)
Daphnia magna	Crustacean	21 d, Survival, NOEC	200	(Orvos et al., 2002)
Daphnia magna	Crustacean	48 h, Mobility, EC_{50}	390	(Orvos et al., 2002)
Daphnia magna	Crustacean	48 h, Immobilization, EC_{50}	180	(Tamura et al., 2013)
Daphnia magna	Crustacean	48 h, Mobility, EC_{50}	338	(Wang et al., 2013)

Table S13. (Continued).

Species	Trophic group	Duration, Effect, Endpoint	Value (µg/L)	Reference
Daphnia magna	Crustacean	21 d, Reproduction, EC_{10}	45	(Wang et al., 2013)
Hyalella azteca	Crustacean	10 d, Survival, LC_{50}	200	(Dussault et al., 2008)
Hyalella azteca	Crustacean	10 d, Growth, EC_{50}	250	(Dussault et al., 2008)
Neocaridina denticulata sinensis	Crustacean	96 h, Mortality, LC_{50}	772	(Wang et al., 2013)
Thamnocephalus platyurus	Crustacean	24 h, Mortality, LC_{50}	470	(Kim et al., 2009a)
Chironomus riparius	Insect	10 d, Survival, LC_{50}	400	(Dussault et al., 2008)
Chironomus riparius	Insect	10 d, Growth, EC_{50}	280	(Dussault et al., 2008)
Chironomus plumosus	Insect	96 h, Mortality, LC_{50}	2890	(Wang et al., 2013)
Limnodrilus hoffmeisteri	Oligochaeta	96 h, Mortality, LC_{50}	2046	(Wang et al., 2013)
Tubifex tubifex	Oligochaeta	96 h, Mortality, LC_{50}	259	(Khatikarn et al., 2016)
Danio rerio	Fish	9 d, hatching, Survival, NOEC	26	(Tamura et al., 2013)
Lepomis macrochirus	Fish	24 h, Mortality, LC_{50}	440	(Orvos et al., 2002)
Lepomis macrochirus	Fish	48 h, Mortality, LC_{50}	410	(Orvos et al., 2002)
Lepomis macrochirus	Fish	96 h, Mortality, LC_{50}	370	(Orvos et al., 2002)
Oncorhynchus mykiss	Fish	35 d, Survival, NOEC	34.1	(Orvos et al., 2002)
Oncorhynchus mykiss	Fish	35 d, Survival, LOEC	71.3	(Orvos et al., 2002)
Oryzias latipes	Fish	96 h, Mortality, LC_{50}	600	(Kim et al., 2009)
Oryzias latipes	Fish	96 h, Larvae mortality, LC_{50}	602	(Ishibashi et al., 2004)
Oryzias latipes	Fish	96 h, Embryos mortality, LC_{50}	399	(Ishibashi et al., 2004)
Oryzias latipes	Fish	96 h, Mortality, LC_{50}	210	(Tamura et al., 2013)
Pimephales promelas	Fish	24 h, Mortality, LC_{50}	360	(Orvos et al., 2002)
Pimephales promelas	Fish	48 h, Mortality, LC_{50}	270	(Orvos et al., 2002)
Pimephales promelas	Fish	72 h, Mortality, LC_{50}	270	(Orvos et al., 2002)
Pimephales promelas	Fish	96 h, Mortality, LC_{50}	260	(Orvos et al., 2002)

Table S14. TCS concentrations in surface water and sediment.

Region	Surface waters (ng/L)	Sediment (µg/kg dw)	Reference
China	n.d-478	< LOQ-1329	1,2,3
Korea	n.d-82		4,5,6
Australia	14-75		7
Greece	3.00-98.0		8
Germany	<3-10.0		9
Romania	n.d-64.3		10
U.K.	n.d-95.0		11
Span	n.d-285	n.d-388	12,13,14
USA	0.5-28.3	<0.5-400	15,16,17,18

[1] Zhao et al., 2009; [2] Zhao et al., 2010a; [3] Chen et al., 2014a; [4] Kim et al., 2007; [5] Kim et al., 2009b; [6] Yoon et al., 2010; [7] Kookana et al., 2011; [8] Stasinakis et al., 2012; [9] Bester et al., 2005a; [10] Moldovan, 2006; [11] Kasprzyk-Hordern et al., 2008; [12] Kantiani et al., 2008; [13] Villaverde-de-Sáa et al., 2010; [14] Gorga etal., 2015; [15] Wilson et al., 2009; [16] Kumar et al., 2010; [17] Katz et al., 2013; [18] Gautam et al., 2014;

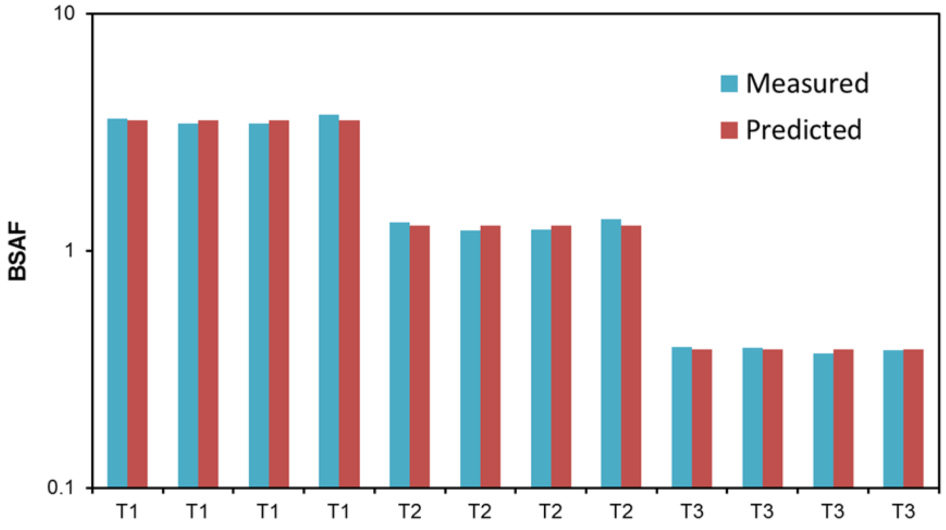

Figure S1. Measured and predicted BSAF values for each treatment in the 28 d bioaccumulation test with *L. hoffmeisteri*. T1, 0.8 µg/g dw treatment; T2, 8 µg/g dw treatment; T3, 80 µg/g dw treatment; 240 µg/g dw treatment.

Chapter 5

Response of sediment bacterial community to triclosan in subtropical freshwater benthic microcosms

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Abstract

The response of sediment bacterial communities to triclosan (TCS) exposure for 28 days in subtropical freshwater benthic microcosms was analyzed using Illumina high-throughput sequencing. This study highlights the interactive effect of sediment-associated TCS and the presence of benthic macroinvertebrates on the sediment bacterial communities. The results showed that TCS at concentrations ≥ 80 µg/g dry weight (dw) significantly altered the taxonomic composition and decreased richness and diversity of sediment bacterial communities in the absence of benthic macroinvertebrates while at concentrations ≥ 8 µg/g dw affected the bacterial community in the presence of benthic macroinvertebrates. TCS under these concentrations significantly reduced the relative abundance of *Bacteroidetes* and *Firmicutes* whereas increased the relative abundances of *Chloroflexi* and *Cyanobacteria*. However, the abundance of algae was dramatically reduced at TCS concentrations ≥ 80 µg/g dw, indicating that *cyanobacteria* were more resilient to TCS over other algae. Our data indicate that TCS alone would not significantly alter sediment bacterial community at environmentally relevant concentrations (up till 8 µg/g dw), but will have an effect in combination in the presence of benthic macroinvertebrates. Therefore, it is suggested to include the benthic macroinvertebrates when assessing the response of sediment bacterial communities to exposure to environmental stress such as organic contaminants.

1. Introduction

Triclosan (2,4,4’-tricloro-2’-hydroxydiphenyl ether, TCS) is an antimicrobial active ingredient used in more than 2000 products, such as soaps, toothpastes, detergents, clothing, toys, carpets, plastics, and paints (FDA, 2016; Halden et al., 2017). Recently, Europe has banned the use of TCS in human hygiene products in 2015 (ECHA, 2015). Also, the U.S. Food and Drug Administration (FDA) has banned the use of TCS in over-the-counter consumer antiseptic wash products (FDA, 2016). However, TCS is still in use in other personal care products and in other parts of the world. As a ‘down-the-drain’ chemical, TCS has been widely detected in aquatic environments (e.g., Katz et al., 2013; Peng et al., 2017). TCS has become one of the most frequently detected contaminants in the freshwater environment (Yueh and Tukey 2016). Toxicological studies suggest that TCS is toxic to bacteria, algae, crustaceans, fish (especially in early developmental stages), oligochaetes, insects, molluscs and amphibians at environmentally elevated concentration, with algae as the most sensitive group (Table S1). For example, the lowest toxicity value found for TCS is 0.2 µg/L based on the growth inhibition in a 72-h test for green alga *Pseudokirchneriella subcapitata* (Yang et al., 2008).

In aquatic environments, TCS is expected to adsorb onto the surface of suspended solids and sediments due to its lipophilic property (log Kow = 4.8) and low aqueous solubility (USEPA, 2010). However, sediment resuspension could occur due to disturbance at water-sediment interface, e.g. due to the presence of benthic invertebrates (Zhang et al., 2014), which may turn the sediment to become a source of contamination to the overlying water. Indeed, a microcosm experiment evaluating the fate and effects of TCS on benthic macroinvertebrates demonstrated that the presence of benthic macroinvertebrates in the microcosms caused significantly higher TCS concentration in the overlying water compared to microcosms without (Chapter 4).

As a crucial component of aquatic ecosystems, bacterial communities play important roles in nutrient re-mineralizing and organic matter decomposition (Burkhardt et al., 2014; Zeng et al., 2014). It is well known that TCS is toxic to bacteria through inhibiting the enzyme enoyl ACP reductase, an essential component of the bacterial fatty acid biosynthetic pathway (Heath et al., 1998). Since TCS is a broad spectrum antimicrobial agent and expected to be retained in the sediment, TCS may negatively affect the sediment bacterial community. Drury et al. (2013b) reported that 8 mg/L TCS in the artificial stream sediments led to reductions in diversity and shifts in taxonomic composition of sediment bacterial communities. However, little is known about the effect of sediment-associated TCS on the sediment bacterial community using more realistic concentrations and including communities like benthic macroinvertebrate. Benthic macroinvertebrates such as Naidid worm (e.g., *Limnodrilus hoffmeisteri*) are broadly distributed in freshwater ecosystems and represent essential links in the aquatic food web (Liu et al., 2014). Due to the biogeochemical processes in sediment, bioturbation from benthic macroinvertebrates could influence metabolism of bacteria and further alter the bacterial community (Zeng et al., 2014; Robinson et al., 2016). However, little is known about the interactive effect of TCS and the presence of benthic macroinvertebrates on the bacterial community structure in the sediment.

Therefore, this study was designed to assess the influence of TCS and the presence of benthic macroinvertebrates on bacterial community structure in the sediment of subtropical freshwater benthic microcosms. This study is part of a larger experiment assessing also the fate and effects of TCS on benthic macroinvertebrates (Chapter 4). The objectives of this study were (1) to examine the response of the sediment bacterial community after exposure to TCS for 28 days, and (2) to determine whether there was an interactive effect of TCS and the presence of benthic macroinvertebrates on the sediment bacterial community structure.

2. Material and methods

2.1. Microcosm operation

Experimental exposures were conducted in indoor glass rectangular microcosms (length and width 30 cm; depth 20 cm) in a temperature and light controlled room (27 ± 1 °C; light intensity: approximately 2200 lux; photoperiod: 12 h/12 h). Wet sieved (300 μm), homogenized, fresh sediment collected from the Liuxi Reservoir, a drinking water source of Guangzhou (South China), was spiked with TCS at concentrations of 0.8 (Treatment 1, T1), 8 (Treatment 2, T2), 80 (Treatment 3, T3) and 240 mg/kg dw (Treatment 4, T4) with acetone as carrier (0.625‰) in a metal mixer. Moreover, water control and acetone control were prepared by replacing TCS stock solution with the same volume of Mill-Q water and acetone, respectively. Each microcosm was filled with 4-cm spiked sediment (3.6 L) and 14-cm aerated tap water (12.6 L). For each treatment, 4 replicates of two types of systems were constructed, namely, (i) system A: with introduced organisms (i.e., algae, Daphnia, snail, midge and worm), and (ii) system B: without introduced organisms (i.e., only water and sediment). Hence, the effect of TCS on the sediment bacterial community can be examined through exposure in system B microcosms. Meanwhile, the effect of the presence of benthic macroinvertebrates and its interaction with TCS exposure on the sediment bacterial community can be further assessed by comparison of system A microcosms with system B microcosms. The exact setup of experiment, introduced organisms sampling, TCS extraction and analysis, sediment parameters (i.e., organic matter (OM), total phosphorus (TP), total nitrogen (TN), and ammonia nitrogen (NH_4-N)) analyses can be referred to Chapter 4.

2.2. DNA extraction, high-throughput sequencing and sequence analysis

DNA was isolated from sediment samples using PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) by following the manufacturer protocol. The concentration and purity of DNA extractions were monitored by gel electrophoresis in 2% agarose gels. The isolated DNA was stored at -80 °C until use. DNA was accordingly diluted to 10 ng/µL before further analysis using sterile water. To compensate for heterogeneity, DNA extraction was performed from three replicates of each system-treatment combination.

The bacterial 16S rDNA genes were amplified at V4 and V5 regions with the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3'). The PCR mixture consisted of 15 µL Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 µM of each primer, 10 ng template DNA and 2 µL H_2O. PCR conditions were 98 ˚C for 1 min for initial denaturation, followed by 30 cycles of 10 seconds at 98 ˚C, 30 seconds at 50 ˚C, 30 seconds at 72 ˚C and a final extension at 72 ˚C for 5 min. The 400-450 bp PCR products were selected by gel electrophoresis and further purified with GeneJET Gel Extraction Kit (Thermo Scientific). Sequencing libraries were constructed using TruSeq® DNA PCR-Free Sample Preparation Kit according to manufacturer's recommendations and added with index codes. The library quality was then checked using the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. After that, the library was sequenced using an Illumina HiSeq 2500 and 250 bp paired-end reads were then generated.

The produced paired-end reads were merged using Flash (Magoč and Salzberg 2011), and assigned to each sample according to the unique barcodes. Sequences were then analysed using QIIME software package (V1.7.0, Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2010). Reads were first filtered by QIIME quality filters to remove low quality reads. The chimera sequences were further removed from reads using UCHIME and Gold database (http://www.drive5.com/usearch/manual/chimera_formation.html). The resultant high-quality sequences with ≥ 97% similarity were then clustered into operational taxonomic units (OTUs) using Uparse software (Edgar, 2013). The most abundant reads from each cluster were assigned as the representative sequences and annotated with taxonomic information by RDP classifier (Wang et al., 2007).

2.4. Data processing and statistical analysis

Bacterial community composition: alpha diversity parameters (i.e., observed OTU number, Chao1, Shannon index and Good's coverage estimator) were analysed using in-house Perl scripts in QIIME software package. The Good's coverage is an estimator of sampling completeness and calculates the probability that a randomly selected amplicon sequence from a sequenced sample (Rea et al., 2011). Differences in alpha diversity indices and relative abundance of the six most abundant phyla/genera between system A and B microcosms or among TCS treatments were tested using Social Sciences v23.0 software (SPSS Inc., IL, USA). Before analysis, the normality of data was tested with Shapiro-Wilk test, and the variance homogeneity of data was detected using Levene's test. If both are satisfied, the two-way ANOVA (factors: TCS treatment and the presence of benthic macroinvertebrates) or one-way ANOVA (factor: TCS treatment) was conducted, otherwise the generalized linear model (GLM) with identity link (factors: TCS treatment and the presence of benthic macroinvertebrates) was conducted. When significant main effect was detected by the ANOVA, Tukey's test was carried out for post hoc comparisons. For GLM, the post hoc paired comparisons were performed using a Sidak test to identify significant differences between treatments. 5% significance level was defined for all statistical tests.

To examine the effect of benthic macroinvertebrates presence and TCS on the sediment bacterial community structure as a whole , multivariate Monte Carlo permutation tests were conducted under the Redundancy analysis (RDA) option using the OTU table. The significance of the effect of benthic macroinvertebrates presence on the bacterial community structure was tested using microcosm system as explanatory variable and TCS treatments as covariable and by constraining the permutation to the covariable. The significance of the effect of TCS on the bacterial community structure was tested using TCS treatments as explanatory variables and microcosm system as covariable and by constraining the permutation to the covariable. If there was a significant difference in bacterial community structure between water control and acetone control, then the water control was excluded in further analyses. To test the interactive effects of TCS and the presence of benthic macroinvertebrates on the sediment bacterial community, a RDA was performed using the interaction between TCS treatments and systems as explanatory variable.

RDA analyses were also performed to determine the significance of the correlation between sediment variables (i.e., TCS concentration, the presence of benthic macroinvertebrates, OM, TN, TP and NH_4-N) and the bacterial community composition at both the phylum and the genus level. As there was no significant difference in bacterial community composition at the phylum and genus level between system A and B, the benthic macroinvertebrates presence was used as covariable in the above RDA analyses with constraining the permutation to the covariable. These analyses were performed using the CANOCO Software package, version 5 (Ter Braak and Šmilauer, 2012).

3. Results

3.1. Bacterial community composition in the sediment

A total of 61 phyla were found in all samples, and phyla with relative abundances > 0.5% are shown in Table S2 and Fig. 1. Overall, *Proteobacteria* was the most abundant phyla across all samples with the relative abundance in the range of 30-34%, followed by *Firmicutes* (9.6-23%), *Chloroflexi* (9.6-20%), *Actinobacteria* (5.6-11%), *Acidobacteria* (6.5-7.9%) and *Bacteroidetes* (2.3-5.1%) (Table S2). There was no significant difference in the relative abundance of *Proteobacteria* or *Acidobacteria* between system A and B microcosms or among treatments (two-way ANOVA, $p > 0.05$). The relative abundance of *Firmicutes* and *Bacteroidetes* was significantly lower and higher in system A than B microcosms, respectively (two-way ANOVA, $p < 0.05$). Besides, there was no significant difference in the relative abundance of *Chloroflexi* or *Actinobacteria* between system A and B microcosms (two-way ANOVA, $p > 0.05$). Comparison between treatments, *Actinobacteria*, *Firmicutes* and *Bacteroidetes* showed significantly lower relative abundance at T4, T3 and T4, T2, T3 and T4, respectively (two-way

ANOVA, $p < 0.05$). In contrast, *Chloroflexi* showed significantly higher relative abundance at T3 and T4 compared to the lower treatments (two-way ANOVA, $p < 0.05$).

A total of 711 genera were found in all samples, and genera with relative abundances > 0.5% are provided in Table S3. The six most abundant genera were *Bacillus* (*Firmicutes*), *Zoogloea* (*Proteobacteria*), *Gaiella* (*Actinobacteria*), *Clostridium sensu stricto 1* (*Firmicutes*), *Paenibacillus* (*Firmicutes*) and *unidentified Chloroplast* (*Cyanobacteria*). There was no significant difference in the relative abundance of *Bacillus* (two-way ANOVA, $p > 0.05$) or *Zoogloea* (GLM, $p > 0.05$) between system A and B microcosms. Besides, *unidentified Chloroplast* (GLM, $p > 0.05$) showed significantly higher relative abundance in system A than B microcosms, whereas *Gaiella*, *Clostridium sensu stricto 1* and *Paenibacillus* showed significantly lower relative abundances in system A than B microcosms (two-way ANOVA, $p < 0.05$). Comparison between treatments, *Bacillus*, *Clostridium sensu stricto 1*, *Gaiella* and *Paenibacillus* showed significantly lower relative abundance at T4 or T3 and T4 (two-way ANOVA, $p < 0.05$), whereas *Zoogloea* and *unidentified Chloroplast* showed significantly higher abundance at T3 and T4 (GLM, $p < 0.05$).

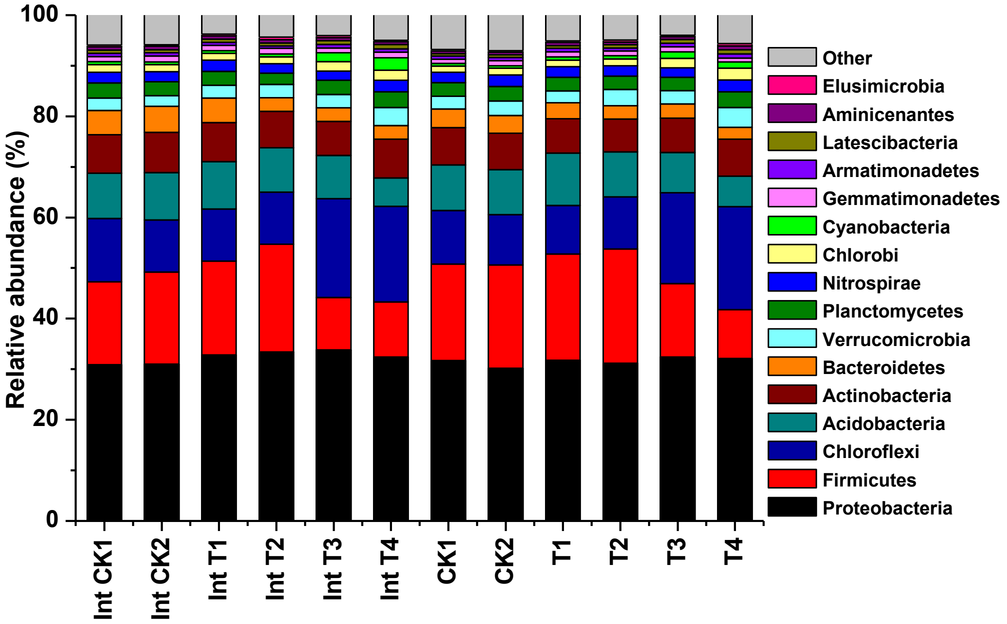

Figure 1. The relative abundance (%) of the dominant bacterial phyla (> 0.5%). Int means samples from system A microcosms. CK1, water control; CK2, acetone control; T1, 0.8 µg/g dw treatment; T2, 8 µg/g dw treatment; T3, 80 µg/g dw treatment; T4, 240 µg/g dw treatment.

3.2. Changes in α-diversity and sediment community structure

The results of alpha biodiversity of bacterial community in the sediment are presented in Table 1. The estimated Good's coverage of the datasets was higher than 90% across all treatments. The observed OTU numbers, Chao1 index and Shannon index were in the range of 3838-4345, 5098-6127 and 10.02-10.50, respectively. There was no significant difference in these indices between system A and B microcosms (two-way ANOVA, $p > 0.05$). In both system A and B microcosms these indices were significantly lower at T3 and T4 compared to the other treatments (two-way ANOVA, $p < 0.05$). Besides, the T2 in the system A microcosms (Int T2) also showed significantly lower observed OTU numbers and Shannon index (one-way ANOVA, $p < 0.05$; Table 1).

The results of Monte Carlo permutation showed that there was no significant difference in bacterial community structure between system A and B microcosms, but the bacterial community structure was significant different between water and acetone control (Table S4). Comparison between TCS treatments, there was a significant difference in bacterial community structure between acetone control and the remaining treatments ($p < 0.05$), except for T1 (NOEC = 0.8 mg/kg dw; Table S4). Besides, the sediment bacterial community structure was significantly different among T1, T2 and T3 while being similar between T3 and T4 (Table S4). In line with the above results of the Monte Carlo permutation tests, Fig. 2 shows that T3 and T4 in both microcosm systems and T2 in system A microcosms were placed separately from the remaining treatments. Also, among the 50 OTU's showing the most variation between treatments, most of them have the highest relative abundance in T3 and T4 of both system A and B microcosms (Fig. 2). There was a significant interaction effect between TCS and benthic macroinvertebrates presence on the microbial community structure ($p = 0.002$).

Table 1. The richness and diversity of sediment bacterial community.

Treatment	OTUs	Shannon	Chao1	Good's coverage
Int CK1	4274±205	10.46±0.19	5981±163	0.94±0.02
Int CK2	4225±176	10.37±0.17	5967±202	0.93±0.01
Int T1	4345±146	10.50±0.16	5960±138	0.93±0.01
Int T2	3968±278*	10.13±0.12*	5774±103	0.93±0.01
Int T3	3942±153*	10.03±0.15*	5405±184*	0.94±0.02
Int T4	3980±120*	10.10±0.09*	5195±174*	0.94±0.01
CK1	4185±146	10.35±0.13	5996±202	0.94±0.01
CK2	4272±178	10.44±0.16	6085±268	0.93±0.01
T1	4137±111	10.36±0.10	6127±281	0.94±0.02
T2	4315±87	10.44±0.24	6006±249	0.93±0.01
T3	3893±97*	10.06±0.14*	5355±83*	0.94±0.01
T4	3838±131*	10.02±0.11*	5098±128*	0.94±0.02

Three replicates for each treatment;

OTUs, Operational taxonomic units; Shannon, Shannon index; Chao 1, Chao 1 index;

Int means samples from system A microcosms.

CK1, water control; CK2, acetone control; T1, 0.8 µg/g dw treatment; T2, 8 µg/g dw treatment; T3, 80 µg/g dw treatment; T4, 240 µg/g dw treatment.

* treatment is significantly different from the acetone control at the 0.05 level.

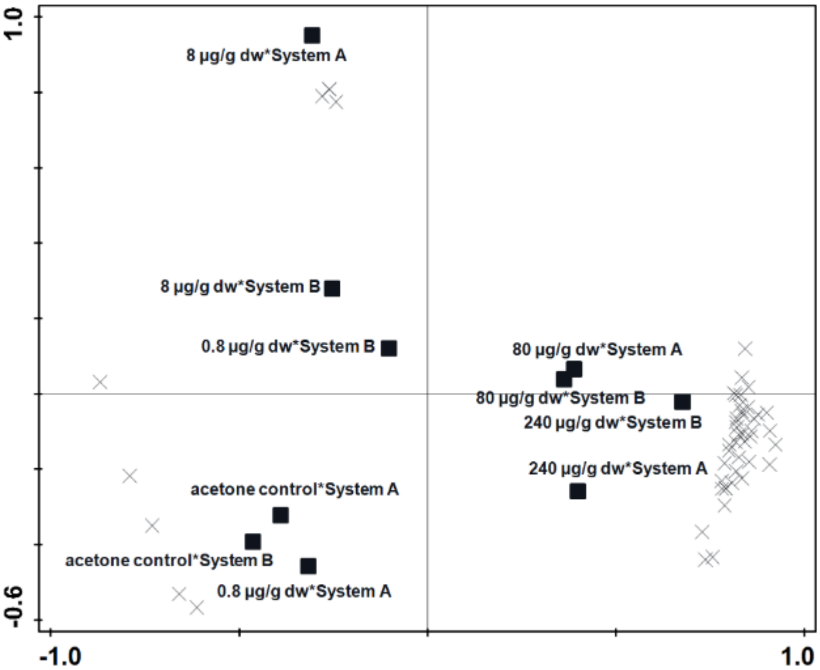

Figure 2. RDA biplot showing the interaction between TCS treatments and the presence of benthic macroinvertebrates for the OTU composition data. The horizontal axis shows 12% of the variation in OTU composition between all samples, the vertical another 7%. Only the 50 OTU's for which the most variation is displayed in the diagram are shown. The *p* values were both 0.002 for the permutation tests on the first axis and all axes.

3.3. Correlation of environmental variables with bacterial community composition

The results of the Monte Carlo permutation tests show that there was no significant difference in bacterial community composition at the phylum and genus level between system A and B. The RDA shows that the sediment bacterial community structure both at the phylum and genus level were significantly correlated with TCS and NH_4-N (Fig. 3, Table S5 and S6). Also, there was a strong positive correlation between TCS and NH_4-N (Fig. 3).

At the phylum level, the biplot scores show that the first and second axis explained 17.1% and 3.7% of the total variations in bacterial community composition between the microcosms, respectively (Fig. 3a). Phyla placed on the left-hand side of the diagram, such as *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*, were negatively correlated with TCS concentration and NH_4-N (Fig. 3a). In contrast, those placed on the right-hand side of the diagram such as *Chloroflexi*, *Chlorobi* and *Cyanobacteria* were positively correlated with TCS concentration and NH_4-N (Fig. 3a).

At the genus level, the biplot scores show that the first and second axis explained 16.1% and 5.1% of the total variations in bacterial community composition between the microcosms, respectively (Fig. 3b). Genera placed on the left-hand side of the diagram, such as *Acinetobacter*, *Bacillus*, and *Clostridium sensu stricto 1*, were negatively correlated with TCS concentration and NH_4-N (Fig. 3b). In contrast, those placed on the right-hand side of the diagram, such as *Anaerolinea*, *Leptolinea*, and *unidentified Chloroplast*, were positively correlated with TCS and NH_4-N (Fig. 3b).

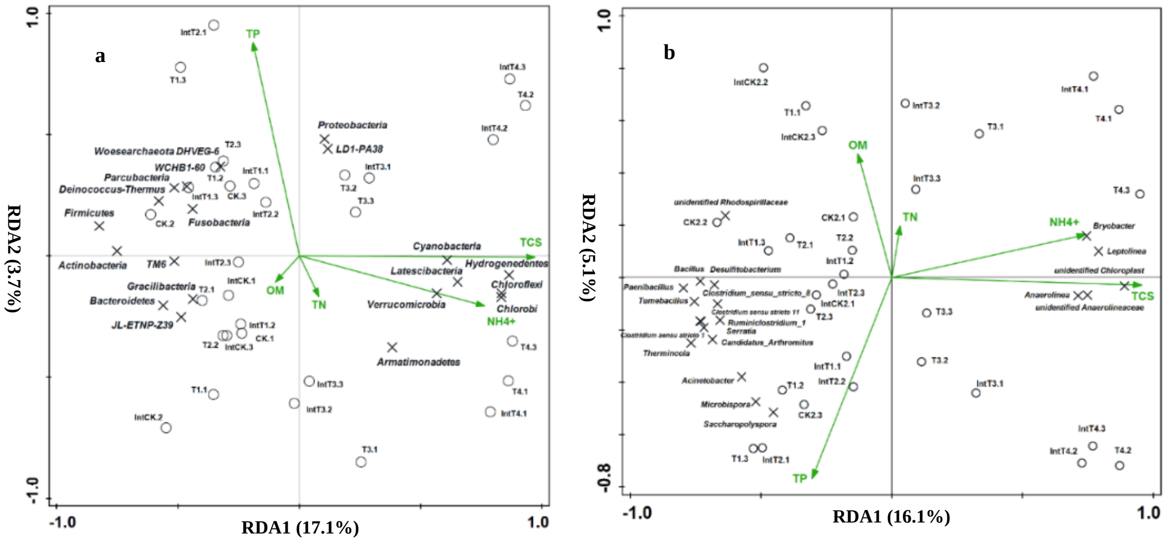

Figure 3. Ordination biplots of redundancy analysis (RDA) based on environmental variables and bacterial phyla (a) and genera (b) in the sediment of microcosms. Only the 20 phyla's (a) and genera's (b) for which the most variation is displayed in the diagram are shown. TP, total phosphorus; OM, organic matter; TN, total nitrogen; NH4-N, ammonia. TCS, triclosan. Int means samples from system A microcosms. CK, acetone control; T1, 0.8 μg/g dw treatment; T2, 8 μg/g dw treatment; T3, 80 μg/g dw treatment; T4, 240 μg/g dw treatment.

4. Discussion

In this study, we used Illumina high-throughput sequencing to quantify sediment bacterial community structures in microcosms mimicking subtropical shallow freshwater benthic ecosystems exposed to TCS. The results show that TCS at concentrations ≥ 80 μg/g dw significantly affected the relative abundance of several bacterial taxa in both microcosm systems. For example, the relative abundance of *Cyanobacteria* significantly increased in T3 and T4 in both microcosm systems (Table S2 and Fig. 1), which is in line with the findings from previous studies (Drury et al., 2013b; Lawrence et al., 2015). However, during the same period, TCS inhibited the growth of pelagic algae in T3 and T4 in both microcosm systems (Chapter 4). These findings correspond to the conclusion that some *cyanobacteria* are more tolerant or adapt to TCS exposure than other algae (Lawrence et al., 2009; 2015; Drury et al., 2013b). Indeed, there was a strong positive relationship between the TCS concentration and relative abundance of *Unidentified Chloroplast* (Fig 3b), a genus belonging to *Cyanobacteria*. Also, TCS significantly increased the relative abundance of *Chloroflexi* whereas decreased the relative abundance of *Firmicutes* in T3 and T4 (Table S2, Fig. 1 and 3a). Thus, similar to *Cyanobacteria*, *Chloroflexi* was also tolerant to TCS, whereas *Firmicutes* was susceptible to TCS in the sediment. The organohalide-respiring *Chloroflexi* contain several species involved in dechlorinating organochlorines (Krzmarzick et al. 2012), which likely explain the higher relative abundance of *Chloroflexi* at T3 and T4 in both microcosms systems. Besides, *Chloroflexi* are abundant and highly diverse in the aquifer sediments, and play roles in sediment carbon cycling and organohalide respiration (Hug et al., 2013). Therefore, *Chloroflexi* may contribute to the slow dissipation of TCS observed in the microcosms (Chapter 4). In accordance with our results, Suriya et al. (2017) found that the relative abundance of *Firmicutes* was dramatically reduced in sediment samples collected from a contaminated area. It should be noted that TCS significantly reduced the relative abundance of *Bacteroidetes* at 8 µg/g dw, suggesting that *Bacteroidetes* were very sensitive to TCS. This is in agreement with the findings of a previous study investigating bacterial biofilms exposure to TCS (Lubarsky et al., 2012). However, these TCS concentrations were higher than those typical environmental concentrations (Table S7).

The presence of benthic macroinvertebrates did not affect the bacterial richness, diversity and structure in the sediment, but significantly altered the relative abundance of a few bacteria, such as *Firmicutes*, *Bacteroidetes*, *Gaiella*, and *Clostridium sensu stricto 1* (Table S2 and S3). This is likely related to biological activities such as burrowing of oligochaete worm that can alter the oxygen in the sediment and across the sediment-water interface (Mermillod-Blondin et al., 2005; Zhang et al., 2010). Our results are partly in agreement with a previous study, which found that benthic macroinvertebrates (i.e., *Corbicula fluminea*, tubificid worms, and *Chironomidae* larvae) altered the dominant bacterial groups in sediments due to the bioturbation caused by benthic macroinvertebrates (Zeng et al., 2014). In fact, the density of oligochaete worm used in this study was similar to those found in the river ecosystem (Chapter 3). Benthic macroinvertebrates at certain environmental-related density, therefore, would not significantly affect sediment bacterial community structure but individual bacterium in a short term.

TCS at concentrations ≥ 80 µg/g dw remarkably altered the sediment bacterial community structure and reduced the bacterial richness and diversity in the microcosms (Table 1 and Table S4). This is comparable to the findings of McNamara et al. (2014), who demonstrated that anaerobic bacterial community structure was altered exposure to TCS at concentrations higher than 60 µg/g in bio-solids. However, TCS alone at concentration of 8 µg/g dw did not influence the richness, diversity and structure of bacterial community in the sediment after a 28 days exposure under the conditions in this study (Table 1 and Table S4). Unlike this study, in an artificial stream experiment, TCS significantly decreased the bacterial community diversity in the artificial stream sediment after 14 and 34 days exposure at concentration of few-17 µg/g (Drury et al., 2013b). The discrepancy between the two studies could be attributed to the different spike approaches. In fact, the sediment was directly spiked with TCS in this study, whereas they added the TCS into the water phase to reach a concentration of 8 mg/L. Therefore, TCS was more strongly bound to the sediment and, therefore, maybe less bio-available to benthic bacteria in the present study. Findings of these two studies further suggest that the binding between hydrophobic organic compounds and sediment can decrease their effect on sediment bacterial communities. Furthermore, as the exposure ran for 28 days, bacteria might have shown a short-term response to TCS at 0.8 and 8 µg/g dw, and followed by a rapid recovery (Drury et al., 2013b). Indeed, TCS at 1.8 µg/L altered bacterial community and affected algal-cyanobacterial abundance and diversity, but recovery and adaptation by the biofilm community were also observed during the 8 weeks exposure period (Lawrence et al., 2015).

There was a significant interactive effect of TCS and presence of benthic macroinvertebrates on the sediment bacterial communities. For example, in the presence of benthic macroinvertebrates, TCS significantly altered the microbial community structure and reduced the sediment bacterial richness and diversity at concentration of 8 µg/g dw (Table 1). This is likely to be associated with the increase in TCS bioavailability due to the disturbance at water-sediment interface caused by the presence of benthic macroinvertebrates. Therefore, more attention should be paid to the interaction effect between hydrophobic organic compounds and the presence of benthic macroinvertebrates in future studies assessing effect of chemicals on sediment bacterial communities.

Our data suggest that sediment-associated TCS alone would not impact the sediment bacterial communities at environmentally relevant concentrations. However, due to the interactive effect between TCS and the presence of benthic macroinvertebrate, TCS significantly reduced the richness and diversity of sediment bacterial community, when TCS concentrations were 5-fold higher than the currently detected maximum (1.33 µg/g dw) in the freshwater sediment (Zhao et al., 2010a). At such level, TCS alone also significantly reduced the relative abundance of some individual bacterium such as *Bacteroidetes*. Furthermore, when TCS concentration reached 80 µg/g dw, TCS alone altered the taxonomic composition and reduced the richness and diversity of sediment bacterial communities.

Acknowledgments

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Supporting information

Table S1. Summary of the aquatic eco-toxicity data for TCS.

Species	Trophic group	Duration Effect Endpoint	Value (μg/L)	Reference
Anabaena flos-aqua	Algae	96 h Biomass EC_{50}	0.97	(Orvos et al. 2002)
Dunaliella tertiolecta	Algae	96 h Cell density NOEC	3.55	(DeLorenzo and Fleming 2008)
Navicula pelliculosa	Algae	96 h Biomass EC_{50}	19.1	(Orvos et al. 2002)
Pseudokirchneriella subcapitata	Algae	72 h Growth inhibition IC_{50}	0.53	(Yang et al. 2008)
Pseudokirchneriella subcapitata	Algae	72 h Growth inhibition NOEC	0.2	(Yang et al. 2008)
Pseudokirchneriella subcapitata	Algae	72 h Growth inhibition LOEC	0.4	(Yang et al. 2008)
Pseudokirchneriella subcapitata	Algae	96 h Growth NOEC	8.3	(Harada et al. 2008)
Pseudokirchneriella subcapitata	Algae	72 h Growth inhibition EC_{50}	5.1	(Tamura et al. 2013)
Pseudokirchneriella subcapitata	Algae	72 h Growth inhibition NOEC	0.53	(Tamura et al. 2013)
Scenedesmus subspicatus	Algae	72 h Growth rate EC_{50}	2.8	(Orvos et al. 2002)
Scenedesmus subspicatus	Algae	72 h Growth rate NOEC	0.5	(Orvos et al. 2002)
Scenedesmus subspicatus	Algae	96 h Biomass EC_{50}	1.4	(Orvos et al. 2002)
Scenedesmus subspicatus	Algae	96 h Biomass NOEC	0.69	(Orvos et al. 2002)
Selenastrum capricornutum	Algae	96 h Biomass EC_{50}	4.46	(Orvos et al. 2002)
Skeletonema costatum	Algae	96 h Biomass EC_{50}	>66.0	(Orvos et al. 2002)
Anabaena flos-aquae	Bacterial	96 h Growth EC_{50}	1.0	(Orvos et al. 2002)
Anabaena flos-aquae	Bacterial	96 h Biomass EC_{50}	1.6	(Orvos et al. 2002)
Vibrio. fischeri	Bacterial	15 min Microtox EC_{50}	280	(Farré et al. 2008)
Ceriodaphnia dubia	Crustacean	7 d Survival NOEC	50	(Orvos et al. 2002)
Ceriodaphnia dubia	Crustacean	7 d Survival LOEC	339	(Orvos et al. 2002)
Ceriodaphnia dubia	Crustacean	7 d Reproduction NOEC	6	(Orvos et al. 2002)
Ceriodaphnia dubia	Crustacean	7 d Reproduction NOEC	182	(Orvos et al. 2002)
Ceriodaphnia dubia	Crustacean	8 d Reproduction NOEC	30	(Tamura et al. 2013)
Daphnia magna	Crustacean	21 d Reproduction NOEC	40	(Orvos et al. 2002)
Daphnia magna	Crustacean	21 d Reproduction LOEC	200	(Orvos et al. 2002)

Table S1. (Continued).

Species	Trophic group	Duration Effect Endpoint	Value (µg/L)	Reference
Daphnia magna	Crustacean	21 d Survival NOEC	200	(Orvos et al. 2002)
Daphnia magna	Crustacean	48 h Mobility EC_{50}	390	(Orvos et al. 2002)
Daphnia magna	Crustacean	48 h Immobilization EC_{50}	180	(Tamura et al. 2013)
Daphnia magna	Crustacean	48 h Mobility EC_{50}	338	(Wang et al. 2013)
Daphnia magna	Crustacean	21 d Reproduction EC_{10}	45	(Wang et al. 2013)
Hyalella azteca	Crustacean	10 d Survival LC_{50}	200	(Dussault et al. 2008)
Hyalella azteca	Crustacean	10 d Growth EC_{50}	250	(Dussault et al. 2008)
Neocaridina denticulata sinensis	Crustacean	96 h Mortality LC_{50}	772	(Wang et al. 2013)
Thamnocephalus platyurus	Crustacean	24 h Mortality LC_{50}	470	(Kim et al. 2009a)
Lemna gibba	Duckweed	7 d Biomass EC_{50}	>62.5	(Orvos et al. 2002)
Danio rerio	Fish	9 d hatching Survival NOEC	26	(Tamura et al. 2013)
Lepomis macrochirus	Fish	48 h Mortality LC_{50}	410	(Orvos et al. 2002)
Lepomis macrochirus	Fish	96 h Mortality LC_{50}	370	(Orvos et al. 2002)
Oncorhynchus mykiss	Fish	35 d Survival NOEC	34.1	(Orvos et al. 2002)
Oncorhynchus mykiss	Fish	35 d Survival LOEC	71.3	(Orvos et al. 2002)
Oryzias latipes	Fish	96 h Mortality LC_{50}	600	(Kim et al. 2009)
Oryzias latipes	Fish	96 h Larvae mortality LC_{50}	602	(Ishibashi et al. 2004)
Oryzias latipes	Fish	96 h Embryos mortality LC_{50}	399	(Ishibashi et al. 2004)
Oryzias latipes	Fish	96 h Mortality LC_{50}	210	(Tamura et al. 2013)
Pimephales promelas	Fish	24 h Mortality LC_{50}	360	(Orvos et al. 2002)
Pimephales promelas	Fish	48 h Mortality LC_{50}	270	(Orvos et al. 2002)
Pimephales promelas	Fish	72 h Mortality LC_{50}	270	(Orvos et al. 2002)
Pimephales promelas	Fish	96 h Mortality LC_{50}	260	(Orvos et al. 2002)
Chironomus riparius	Insect	10 d Survival LC_{50}	400	(Dussault et al. 2008)
Chironomus riparius	Insect	10 d Growth EC_{50}	280	(Dussault et al. 2008)
Chironomus plumosus	Insect	96 h Mortality LC_{50}	2890	(Wang et al. 2013)
Potamopyrgus antipodarum	Mollusca	28 d Reproduction NOEC	0.17	(Geiß et al. 2016)
Limnodrilus hoffmeisteri	Oligochaeta	96 h Mortality LC_{50}	2046	(Wang et al. 2013)
Tubifex tubifex	Oligochaeta	96 h Mortality LC_{50}	259	(Khatikarn et al. 2016)

Table S2. The average relative abundance of phylum in the sediment (> 0.5%).

Phyla	Int CK	Int T1	Int T2	Int T3	Int T4	CK	T1	T2	T3	T4
Proteobacteria	31.0	32.8	33.4	33.8	32.4	30.2	31.8	31.2	32.4	32.1
Firmicutes	18.2	18.6	21.3	10.4	10.9	20.4	21.0	22.6	14.5	9.67
Chloroflexi	10.3	10.3	10.3	19.5	18.9	9.97	9.56	10.3	18.0	20.4
Acidobacteria	9.39	9.36	8.78	8.57	5.62	8.91	10.4	8.88	7.96	6.00
Actinobacteria	7.94	7.71	7.21	6.73	7.65	7.18	6.75	6.48	6.77	7.33
Bacteroidetes	5.12	4.82	2.71	2.71	2.70	3.48	3.18	2.65	2.83	2.30
Verrucomicrobia	2.12	2.53	2.61	2.60	3.60	2.92	2.32	3.20	2.63	3.95
Planctomycetes	2.78	2.77	2.23	2.79	3.10	2.82	2.68	2.63	2.60	3.10
Nitrospirae	1.97	2.22	1.86	1.85	2.28	2.29	2.12	2.07	1.92	2.34
Chlorobi	1.43	1.37	1.37	1.87	1.96	1.34	1.31	1.32	1.86	2.34
Cyanobacteria	0.49	0.52	0.59	1.75	2.45	0.49	0.62	0.63	1.30	1.24
Gemmatimonadetes	1.21	1.06	1.11	0.93	1.15	0.99	0.99	0.95	0.95	0.77
Armatimonadetes	0.64	0.56	0.46	0.72	0.63	0.65	0.70	0.61	0.72	0.78
Latescibacteria	0.58	0.64	0.58	0.74	0.86	0.53	0.52	0.62	0.78	0.83
Aminicenantes	0.66	0.65	0.61	0.58	0.51	0.53	0.66	0.55	0.48	0.74
Elusimicrobia	0.30	0.33	0.53	0.43	0.33	0.30	0.32	0.41	0.35	0.47

Table S3. The average relative abundance of genus in the sediment (> 0.5%).

Genus	Int CK	Int T1	Int T2	Int T3	Int T4	CK	T1	T2	T3	T4
Bacillus	3.78	3.81	3.77	2.31	2.72	4.09	3.64	3.99	3.75	1.96
Zoogloea	1.76	1.69	1.25	2.83	2.79	1.48	1.43	0.95	3.13	2.81
Clostridium_sensu_stricto_1	1.62	1.99	3.21	0.95	0.98	1.91	2.32	3.07	1.28	1.05
Gaiella	1.59	1.32	1.41	1.76	1.17	2.00	2.13	1.96	1.71	1.09
Paenibacillus	1.20	1.18	1.30	0.75	0.71	1.44	1.46	1.62	0.99	0.61
unidentified_Chloroplast	0.23	0.38	0.45	1.50	2.25	0.29	0.36	0.37	0.94	1.56
Bryobacter	0.65	0.67	0.52	0.94	0.98	0.52	0.55	0.55	0.90	0.98
Cupriavidus	0.22	0.31	1.57	0.99	0.62	0.33	0.45	0.58	0.95	0.51
unidentified_Nitrospiraceae	0.68	0.76	0.82	0.88	0.97	0.70	0.64	0.85	0.68	0.83
Opitutus	0.59	0.44	0.63	0.48	0.95	0.72	0.58	0.56	0.56	0.63
Methylomonas	0.69	0.76	0.54	0.16	0.21	0.83	0.26	0.26	0.19	0.40
Longilinea	0.31	0.34	0.50	0.78	0.59	0.25	0.32	0.20	0.65	0.58
Anaerolinea	0.25	0.24	0.32	0.73	0.61	0.16	0.29	0.24	0.55	0.73
Ruminiclostridium_1	0.55	0.61	0.68	0.36	0.37	0.57	0.56	0.54	0.48	0.34
Tumebacillus	0.48	0.45	0.49	0.31	0.29	0.59	0.68	0.69	0.41	0.23
Piscinibacter	0.54	0.65	0.57	0.26	0.40	0.43	0.39	0.40	0.44	0.54
Clostridium_sensu_stricto_12	0.43	0.54	0.62	0.28	0.30	0.50	0.42	0.56	0.39	0.29
Haliangium	0.54	0.54	0.31	0.31	0.42	0.54	0.36	0.16	0.35	0.34

Table S4. The *p* values of Monte Carlo permutation tests under Redundancy analysis (RDA) at OTU level.

Permutation test	*p* values
System A versus B	0.850
water control versus acetone control	0.019
0.8 μg/g versus acetone control	0.272
8 μg/g versus acetone control	0.026
80 μg/g versus acetone control	0.004
240 μg/g versus acetone control	0.004
8 μg/g versus 0.8 μg/g	0.045
80 μg/g versus 0.8 μg/g	0.012
240 μg/g versus 0.8 μg/g	0.004
80 μg/g versus 8 μg/g	0.004
240 μg/g versus 8 μg/g	0.004
240 μg/g versus 80 μg/g	0.080

Table S5. Summary of simple environmental variable effect in redundancy analysis (RDA) with system as covariate at phylum level.

Environmental variables	Explains %	pseudo-F	*p*	*p* (adj)
TCS	16.3	5.3	0.002	0.005
NH_4^+	11.5	3.5	0.002	0.005
TP	4.1	1.1	0.304	0.507
TN	2.6	0.7	0.794	0.990
OM	1.9	0.5	0.99	0.990

Table S6. Summary of simple environmental variable effect in the redundancy analysis (RDA) based on the genera with relative abundance > 0.5%.

Environmental variables	Explains %	pseudo-F	*p*	*p* (adj)
TCS	15	4.7	0.002	0.01
NH_4^+	10.4	3.1	0.008	0.02
TP	5.5	1.6	0.104	0.17
OM	3.4	0.9	0.436	0.55
TN	2.4	0.7	0.886	0.89

Table S7. TCS concentrations in surface water and sediment.

Region	Surface waters (ng/L)	Sediment (μg/kg dw)	Reference
China	n.d-478	< LOQ-1329	1,2,3
Korea	n.d-82		4,5,6
Australia	14-75		7
Greece	3.00-98.0		8
Germany	<3-10.0		9
Romania	n.d-64.3		10
U.K.	n.d-95.0		11
Span	n.d-285	n.d-388	12,13,14
USA	0.5-28.3	<0.5-400	15,16,17,18

[1] Zhao et al., 2009; [2] Zhao et al., 2010a; [3] Chen et al., 2014a; [4] Kim et al., 2007; [5] Kim et al., 2009b; [6] Yoon et al., 2010; [7] Kookana et al., 2011; [8] Stasinakis et al., 2012; [9] Bester et al., 2005a; [10] Moldovan, 2006; [11] Kasprzyk-Hordern et al., 2008; [12] Kantiani et al., 2008; [13] Villaverde-de-Sáa et al., 2010; [14] Gorga etal., 2015; [15] Wilson et al., 2009; [16] Kumar et al., 2010; [17] Katz et al., 2013; [18] Gautam et al., 2014;

Chapter 6

Fate and effects of polycyclic musk HHCB in subtropical freshwater microcosms

Feng-Jiao Peng, Guang-Guo Ying, Paul J. Van den Brink

Abstract

Galaxolide (HHCB) is used as a fragrance ingredient in household and personal care products and ubiquitously persistent in the environment. Here we investigated the fate of HHCB in subtropical freshwater microcosms, and evaluated the effects of sediment-associated HHCB on several communities including benthic macroinvertebrates and bacteria under more ecologically realistic conditions compared to single species studies. During a 28 days exposure period, HHCB was persistent in the sediment of the microcosms, and accumulated in worms with biota sediment accumulation factor (BSAF) values in the range of 0.29-0.66 for *Branchiura sowerbyi* and 0.94-2.11 for *Limnodrilus hoffmeisteri*. 30 µg HHCB/g dry weight (dw) sediment did not significantly affect chlorophyll-a content, microbial community, and survival and growth of benthic macroinvertebrates. However, the presence of benthic macroinvertebrates altered the microbial community structure. The findings of this study suggest that a single high-dose of HHCB at environmentally relevant concentrations would not impose direct toxicological risks to aquatic organisms such as benthic macroinvertebrates within 28 days of exposure.

1. Introduction

Polycyclic musks (PCMs) are widely used as fragrances in household and personal care products, such as detergents, perfumes, body lotions and cosmetics (Reiner and Kannan, 2006). Due to their high lipophilicity, PCMs that end up in the waterways can bio-accumulate in aquatic organisms, such as fish and mussels, especially at low trophic levels (Seinen et al., 1999; Gomez et al., 2005; Díaz-Cruz and Barceló, 2015). Given the current high usage, there are concerns regarding environmental exposure and toxic effects of PCMs (Sun et al., 2014).

HHCB is a frequently used PCM, accounting for approximately 65% of total production (Balk and Ford, 1999a; Rimkus, 1999). Due to the widespread use and low rates of degradation, HHCB is detected throughout the environment (e.g., air, water and sediment) (Osenbrück et al., 2007; McDonough et al., 2016; Peng et al., 2017). In standard laboratory tests, HHCB generally has low acute toxicity on aquatic organisms (e.g. algae, crustaceans, and fish) (Breitholtz et al., 2003; Gooding et al., 2006). However, a recent study reported that, at environmentally relevant concentrations (0.5 µg/L), HHCB induced lipid peroxidation, protein carbonylation and primary genetic damage in the zebra mussel (Parolini et al., 2015). Additionally, with a log Kow value of 5.9 and log Koc value of 4.86 (Balk and Ford, 1999a), HHCB is expected to end up in the sediment and bio-accumulate in aquatic organisms. Despite the wide-usage of HHCB, little information is known about the effect of sediment-associated HHCB on benthic macroinvertebrates and microorganisms.

Oligochaete worms, such as *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*, are burrowing deposit feeders that are broadly distributed in the sediments of freshwater bodies such as lakes and rivers (Tyler, 2009). They ingest sediment particles, taking in large amounts of substrate (Wang and Matisoff 1997), and are therefore likely to be affected by sediment-associated organic contaminants. Because oligochaete worms play a key role in nutrient cycling and form important links in detritus food chains (Wang and Matisoff 1997), understanding the effects of sediment-associated organic chemicals on these organisms is a priority. For example, HHCB at concentrations of 26 µg/g dry weight (dw) significantly reduced the number of eggs produced per polychaete worm (*Capitella sp. I*) and increased the time between broods (Ramskov et al., 2009). Although this level is at environmentally elevated concentrations (e.g., Reiner and Kannan, 2011; Peng et al., 2017), these effects are of concern and we do not know the effects of HHCB on these organisms under more natural conditions.

HHCB is only partially biodegraded in the wastewater treatment plants (WWTPs), with removal occurring mainly via sorption onto sludge particles (Simonich, 2005), and are therefore frequently found in surface waters (e.g., Lee et al., 2014; Peng et al., 2017). Sediment-associated HHCB may go through accumulation and degradation in the body of oligochaete worms, which might affect the fate of HHCB. For example, HHCB accumulated in the bluegill sunfish (*Lepomis macrochirus*) and bio-degraded to at least one polar metabolite by the fish

(Balk and Ford, 1999a). The interaction between oligochaete worms and hydrophobic substances such as HHCB, is largely unknown, but is crucial in evaluating the environmental risk of sediment-associated compounds.

Microorganisms, such as bacteria, are also a major component of natural sediments and aquatic ecosystems, playing a key role in the processing of organic matter and nutrient cycling (Grünheid et al., 2005). Contaminated sediments are known to affect bacterial community composition and structure (Staley et al., 2015). For example, municipal wastewater discharging to streams resulted in an increase in the relative abundance of genes associated with restriction-modification systems, nitrogen, sulphur, purine and pyrimidine metabolisms (Li et la., 2016). However, the impact of individual sediment-associated hydrophobic contaminants, such as HHCB, on bacterial communities is largely unknown.

To understand the interaction between sediment-associated HHCB and benthic macroinvertebrates under more ecologically realistic conditions, we spiked HHCB into the sediment of 40 microcosms and introduced a model freshwater community (algae, *Daphnia*, clams, snails, midges, and benthic worms) that was representative of communities found in urban rivers of Guangzhou (sub-tropical South China) to half of them. Because of the importance of microbes for degradation of inorganic and organic contaminants, we also evaluated the effects of sediment-associated HHCB on microbial community in the sediment. The objective of this study was to (i) investigate the fate of HHCB in the microcosms with and without introduced organisms, (ii) to evaluate the effect of HHCB on a model freshwater community, and (iii) to assess the effect of HHCB and the presence of invertebrates on the microbial community in the sediment.

2. Material and methods

2.1. Test sediment and HHCB spiking

The test chemical was Galaxolide (HHCB; 1,3,4,6,7,8-hexahydro-4,6,6, 7, 8, 8-hexamethylcyclopenta (g) -2-benzopyran). Further details on reagents and standards are provided in the supporting information. Sediments used in the microcosms were collected from an uncontaminated reservoir (113˚47'42"N, 23˚46'01"E, Guangzhou, South China) in January 2016 (Zhao et al., 2010a; Peng et al., 2017). Sediment collection and pre-treatment were same as our previous study (Chapter 4). The sieved sediment contained 60% water (24 h at 105 ˚C; n = 4) and was composed primarily of clay (58.7%), silt (40.8%), and a small amount of sand (0.49%). It contained 20.6‰ organic matter (OM), 0.45‰ total phosphorus (TP), 1.45‰ total nitrogen (TN), and 0.11‰ ammonia nitrogen (NH_4^+) (Clesceri et al., 1998). The background HHCB concentration in the sediment was around 0.002 µg/g dw, and considered negligible for the purposes of this study.

For the sediment spiking procedure is referred to Chapter 4. Briefly, sediment was spiked with HHCB in acetone to obtain target concentrations of 30, 100, 200 and 300 µg/g dw. After spiking, sediments were further pre-equilibrated for 3 days. Two controls were used in the study: a water control and an acetone control, that were created by replacing the HHCB solution with the same volume of Mill-Q water and acetone, respectively.

2.2. Test organisms

To mimic shallow subtropical aquatic communities from an urban area of Guangzhou (South China), several key species were used in the experiment. These species were identified from biological monitoring of rivers in Guangzhou city in 2015 (Chapter 3). They were six species of algae (i.e., *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Scenedesmus quadricauda*, *Scenedesmus acuminatus, Selenastrum capricornutum* and *Chlamydomonas reinhardtii*), a filter-feeding cladoceran (*Daphnia magna*), and five species of benthic macroinvertebrates (i.e., clams (*Corbicula fluminea*), snails (*Viviparidae bellamya*), worms (*Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*), and midge (*Orthocladiinae*)). Clams were collected from a small uncontaminated headwater stream in Huizhou, Guangdong province (South China). The remaining benthic organisms were purchased from an aquatic market in Guangzhou, as insufficient numbers could be collected from the field. Benthic macroinvertebrates were, therefore, of an unknown age. For their culture methods is referred to Chapter 4. In general, microphytes (e.g., unicellular algae) are the preferred food for *D. magna* and benthic organisms used in this study. Further information of feeding habits and food preferences are presented in the supporting information (see Table S1).

The initial body wet weight of the midge larvae (4.5-6.2 mg) and worms (1.9-2.4 mg for *L. hoffmeisteri* and 16.8-18.5 mg for *B. sowerbyi*) were estimated using 100 randomly chosen individuals by weighing with a Sartorius balance (Germany). Furthermore, 30 individuals of each species were weighed to estimate the initial wet weight of clams (326-471 mg) and snails (598-766 mg) (see Table S1 for additional traits data).

2.3. Microcosm operation

To test the effects of HHCB on subtropical shallow freshwater communities, we used 40 microcosms that were placed in a climate controlled room (27±1 ˚C; white cool fluorescent light with a light intensity of approximately 2200 lux; photoperiod 12 h) (see Chapter 4). Of the 40 microcosms, 28 were used to test the effects of different concentrations of HHCB (30, 100, 200 and 300 µg/g dw). Because one microcosm per HHCB treatment was sacrificed at the start of exposure to measure HHCB concentrations in the overlying water, there were six replicates per HHCB treatment. The remaining 12 microcosms were used as controls (six for the water control and six for the acetone control).

Two different test systems were used for each treatment: A and B. Each microcosm was first filled with 4-cm spiked sediment and then 14-cm aerated tap water, and allowed to settle for 3 days. The aerated tap water had the following properties: 0.032% ammonia (NH_4^+), 0.175% nitrate (NO_3^-), 0.0002% nitrite (NO_2^-), 0.002% TP, 0.067% total organic carbon (TOC). One day prior to exposure, clams, snails, midges and worms were transferred from glass aquarium to glass beakers and allowed to clear their guts through defecating in aerated tap water overnight. At the beginning of the exposure (day 0), 12 *D. magna*, 6 *C. fluminea*, 6 *V. bellamya*, 50 *Orthocladiinae*, 240 *L. hoffmeisteri*, 30 *B. sowerbyi*, and algae with a total density of approximately 10^4 cells/mL in the systems, were added to the microcosms of system A. System B was identical to A, but did not contain any introduced organisms. As periphyton can develop in the microcosms, the effect of sediment-associated HHCB on periphyton communities was also investigated. In each microcosm, five microscopic glass slides (7.5 x 2.5 cm) were hung at a depth of 10cm using nylon sewing thread and left for the duration of the experiment. During the experiment, the microcosms were aerated using a glass pipette connected to an aeration system and any evaporated water was replenished with aerated tap water at weekly intervals. Nitrogen (0.7 mg/L as urea) and phosphorus (0.09 mg/L as triple super phosphate) were added biweekly to the systems to provide nutrients for algal growth (Rico et al., 2014).

To determine the abiotic degradation (i.e., photolysis and hydrolysis) during the exposure period, we performed two parallel fate experiments under similar conditions as the microcosm experiment. The specific procedure of the tests is provided in the supporting information.

2.4. Sampling, water and sediment quality parameter analysis

To detect possible changes in community metabolism, dissolved oxygen (DO), temperature (T), pH and conductivity were measured on days 0, 1, 3, 7, 10, 14, 21 and 28 in all microcosms. Turbidity was measured on day 1, 7, 14, 21 and 28.

At the end of the experiment, overlying water and sediment sampling can refer to our previous study (Chapter 4). We determined the concentrations of TOC, TP, NO_3^-, NO_2^-, and NH_4^+ in the overlying water and TN, NH_3, total phosphorus and OM in the sediment samples.

2.5. HHCB analysis

HHCB concentrations in the spiked sediment, original aerated tap water, overlying water, sediment and both species (*L. hoffmeisteri* and *B. sowerbyi*) of worms were determined at the beginning (day 0) and end of the exposure (day 28).

Analytical methods used for the analysis of HHCB in water, sediment and worms tissue have been previously described (Chen et al., 2010; Chen et al., 2014b; Yao et al., 2016). Briefly, water samples were extracted by solid phase extraction using a HLB cartridges (6 mL, 200 mg) (Chen et al., 2010); sediment samples (Chen et al., 2014b) were extracted using an accelerated solvent extraction system (ASE) (Dionex 350, Dionex Corporation); and worm samples were extracted using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Yao et al., 2016). Detailed extraction methods are given in the supporting information. HHCB in extracts was analysed by using a gas chromatograph (Agilent 6890) coupled to a mass spectrometer (Agilent 5975B) with an electron impact ionization source (EI) according to Chen et al., 2014b (see supporting information). The limits of detection (LODs), limits of quantification (LOQ) and method recoveries of HHCB are listed in Table S2.

2.6. Community sampling and analysis

At the end of the experiment, *D. magna* and benthic macroinvertebrates were recruited from each microcosms of system A. Phytoplankton and periphyton chlorophyll-a concentration were analysed in both systems microcosms. For more details on this community sampling and analysis is referred to our previous study (Chapter 4).

2.7. Microbial analysis

The effect of HHCB on the microbial community structure and composition in the sediment was evaluated using deep16S rDNA sequencing. Because there is no information available on the effects of HHCB on microbial communities, microbial analysis was performed only on water controls, acetone controls, and sediments with the highest HHCB concentration, to ensure a detectable HHCB effect. Procedures for DNA extraction and amplicon sequencing were same as our previous study (Chapter 5).

2.8. Data analysis

Williams test (ANOVA, Williams, 1972) was used to compute the No Observed Effect Concentrations (NOECs) at taxon level ($p < 0.05$) with abundance values of the macroinvertebrate ln (2x+1) transformed, where x stands for the abundance value, to down-weigh high abundance values (Van den Brink et al., 2000). The analyses were performed with the Community Analysis computer program (Hommen et al., 1994). The acetone control was used in the Williams test, and there was no significant difference in counts of organisms between the water control and acetone control.

Differences in HHCB concentrations, HHCB dissipation, water and sediment quality parameters, and chlorophyll-a concentrations between systems or treatments were tested using SPSS version 23.0 (IBM, NY). Before analysis, the normality of data was tested by applying Shapiro-Wilk test, and the variance homogeneity of data was detected by using Levene's test. Accordingly, two-way ANOVA (factors: HHCB and presence of benthic macroinvertebrates), one-way ANOVA, Kruskal-Wallis test or paired-t test was conducted. When a significant main effect was detected by the ANOVA, Tukey’s test was carried out for post hoc comparisons. 5% significance level was defined for all of the statistical tests.

For microbial analysis, QIIME V1.7.0 and R software (Version 2.15.3) were used to analyse Shannon index, Chao1 index, and observed number of species. Differences in alpha diversity indices and relative abundance of the ten most abundant phyla between system A and B microcosms or among treatments were tested using two-way ANOVA (factors: HHCB treatment and the presence of benthic macroinvertebrates) or one-way ANOVA (factor: treatment).

The relative abundances of the microbial 16S rDNA gene at the phylum and OTU level were examined to determine the composition of the microbial community. To test the significance of the difference in microbial community composition between system A and B, Monte Carlo permutation tests under redundancy analysis (RDA) were performed on both phylum data set and OTU data set using microcosm system as explanatory variable and treatments as covariables and by constraining the permutation to the covariables. For each system, Monte Carlo permutation tests under RDA were performed to estimate whether there was a significant difference in community composition between acetone control and water control or 300 μg/g dw treatment. To test the interactive effects of treatments and the presence of benthic macroinvertebrates on the sediment bacterial community composition, Monte Carlo permutation tests under RDA were performed using the interaction between treatments and systems as explanatory variable. These analyses were performed using the CANOCO Software package, version 5 (Ter Braak and Šmilauer, 2012).

3. Results

3.1. Water and sediment quality parameters

Dissolved oxygen (DO) concentration remained stable during the experiment, at around 7 mg/L in the overlying water. pH levels were more stable in system B, without introduced organisms, than system A (Table S3). During the exposure period, while pH values decreased from ~ 7 to ~ 6 in system A, pH remained similar values of 6.7-7 in system B (Table S3). Conductivity remained at similar levels (90-110 µS/cm) in all microcosms during the 28 days exposure period (Table S4). Turbidity kept decreasing from day 0 (150-180 NTU) to day 28 (< 10 NTU) in system B (Table S5). However, there was an increase in turbidity between day 7 (50-60 NTU) and day 14 (110-140 NTU) in all HHCB treatments in system A (Table S5).

After 28 days, the overlying water from the microcosms was compared with the original aerated tap water to determine changes in nutrient concentrations. There was no difference in NO_2^- and TP concentrations, but TOC concentrations were higher and NO_3^- concentrations were lower by the end of the experiment (Table S6). In system A across all treatments, NH_4^+ concentrations had not changed, however they significantly decreased in system B after 28 days exposure ($p < 0.05$). Comparing systems A and B, significantly higher NH_4^+ and TOC concentrations were found in system A ($p < 0.05$), while significantly higher NO_3^- concentrations were found in system B ($p < 0.05$). There was no difference in NO_2^- and TP between the two systems ($p > 0.05$). In both system A and B, there was no effect of HHCB on the concentrations of NO_2^-, TP and NH_4^+ (Table S6). Although there was no effect of HHCB on the concentration of NO_3^- in system A, in system B NO_3^- concentrations were significantly lower in all HHCB treatments compared to controls (Table S6). In both system A and B, TOC concentrations were significantly higher in the two highest treatments compared to controls and lower treatments (30 µg/g dw and 100 µg/g dw) ($p < 0.05$) (Table S6).

After 28 days, there was no significant difference in any nutrients between the sediment from the microcosms and the original sediment (Table S7). There were no differences in OM, TN and TP sediment levels between system A and B ($p > 0.05$), but NH_4^+ showed significantly higher levels in system A ($p < 0.05$). OM, TN and TP concentrations in the sediment were not affected by the HHCB treatments in either systems, however NH_4^+ sediment concentrations were significantly lower in the two highest treatments in system B compared to the lower treatments (controls, 30 µg/g dw and 100 µg/g dw) ($p < 0.05$) (Table S7).

3.2. HHCB concentrations in water, sediment and worms

The recoveries of extraction methods were 100-110% and the method limit of quantitation was below 0.002 µg/L for overlying water, sediment and worms (Table S2). At the end of the experiment, HHCB concentrations in the overlying water were significantly higher in system A compared to system B ($p < 0.05$) (Fig.1 and Table 1). In both system A and B, HHCB concentrations in the overlying water of the two highest HHCB treatments (200 µg/g dw and 300 µg/g dw) dropped dramatically over the 28 days of exposure, from over a hundred µg/L to only few µg/L (Table 1).

Final sediment HHCB concentrations were similar to those at the start of the experiment ($p > 0.05$). Mass balance was assessed for HHCB by comparing the HHCB mass in the system on day 28 with the original added HHCB mass (Table S8 and Fig. 2). After 28 days exposure, the overall amount of HHCB in the HHCB treatments decreased by 0.36-1.54% in system A and 0.36-1.49% in system B (Table S8). However, there was no significant difference in HHCB mass between values from day 28 and from day 0 or between system A and B on day 28 ($p > 0.05$). Among the four HHCB treatments, the rates of HHCB dissipation in the microcosms followed the order: 30 µg/g dw treatment > 100 µg/g dw treatment > 200 µg/g dw treatment > 300 µg/g dw treatment ($p < 0.05$).

HHCB was detected in the tissue of both worms tested, *B. sowerbyi* and *L. hoffmeisteri*, with BSAF values of 0.29-0.66 and 0.94-2.11, respectively (Table 1). For the same treatment, *B. sowerbyi* showed higher HHCB body residues but smaller BSAF values relative to *L. hoffmeisteri* ($p < 0.05$) (Table 1).

The results of photolysis and hydrolysis experiments showed that HHCB remained at similar levels as the spiked HHCB concentration (1.2 mg/L) until day 21 when HHCB levels were slightly reduced until day 28 under both light (1.10 mg/L) and dark conditions (1.15 mg/L) in the aerated tap water (Table S9). Likewise, HHCB remained at similar concentration levels of 23.7-24.2 µg/L between day 0 and day 28 in the overlying water from the treatment with HHCB concentration of 30 µg/g dw (Table S9).

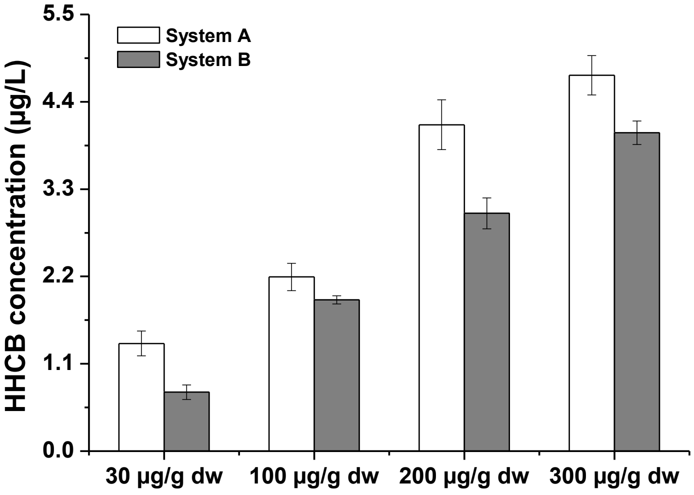

Figure 1. HHCB concentrations (µg/L) in the overlying water of the microcosms after 28 days of exposure to different concentrations of HHCB spiked sediment. Error bars represent standard error of the mean (n = 3). The white and grey bars represent treatment from systems with (System A) and without (System B) introduced organisms, respectively.

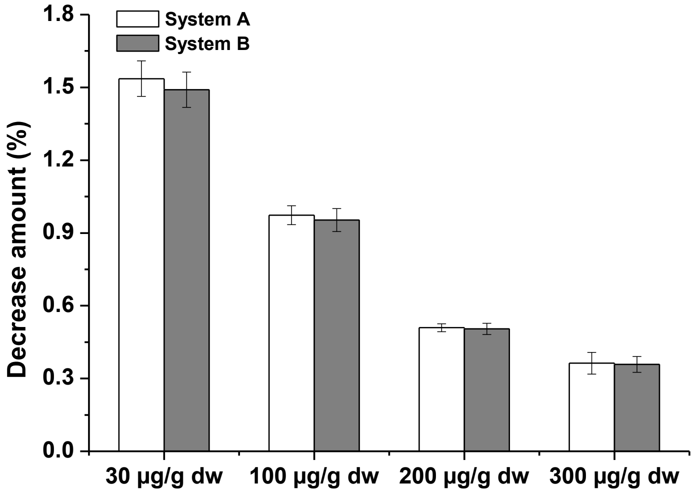

Figure 2. The decrease in HHCB (%) in the microcosms after 28 days of exposure. Error bars represent standard error of the mean (n = 3). The white and grey bars represent treatment from systems with (System A) and without (System B) introduced organisms, respectively.

Table 1. HHCB concentration in overlying water, sediment and worms at the start and end of exposure.

Treatment	Overlying water (µg/L)			Sediment (µg/g, dw) (day 28)			*B. sowerbyi* (day 28)		*L. hoffmeisteri* (day 28)	
	Day 0	Day 28		Day 0	Day 28		Concentrations (µg/g ww)	BSAF	Concentrations (µg/g ww)	BSAF
		System A	System B		System A	System B				
water control	<0.002	<0.002	<0.002	<0.003	<0.003	<0.003	<0.003	-	<0.003	-
acetone control	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	-	<0.003	-
30 µg/g dw	24.22±3.56	1.36±0.16	0.74±0.09	29.82±0.03	29.53±0.02	29.55±0.02	98.16±1.95	0.66±0.01	56.38±1.04	2.11±0.04
100 µg/g dw	67.79±5.19	2.19±0.17	1.90±0.05	99.51±0.04	99.01±0.04	99.03±0.05	240.88±4.44	0.49±0.01	135.04±1.56	1.37±0.02
200 µg/g dw	118.47±11.67	4.11±0.31	3.00±0.19	199.14±0.08	198.95±0.03	198.97±0.05	349.88±6.86	0.35±0.01	216.94±5.48	1.10±0.03
300 µg/g dw	163.98±13.97	4.73±0.25	4.01±0.15	298.81±0.10	298.88±0.14	298.90±0.10	431.21±3.65	0.29±0.00	278.55±2.86	0.94±0.01

dw, dry weight.

ww, wet weight.

$BSAF = (C_{org,WW}/f_{lip,\,WW})/(C_{sed,DW}/f_{OM,\,DW})$.

System A and B represent microcosms with and without introduced organisms, respectively.

Table 2. The number of organisms surviving after 28 days of exposure.

Treatment	*Corbicula fluminea*	*D. magna*	*Viviparidae Bellamya*	*Orthocladiinae*	*B. sowerbyi*	*L. hoffmeisteri*
water control	6±0	0±0	6±0	0±0	29.3±0.9	232.0±3.7
acetone control	6±0	0±0	6±0	0±0	29.5±0.6	232.5±3.9
30 µg/g dw	6±0	0±0	6±0	0±0	29.8±0.5	232.5±4.2
100 µg/g dw	6±0	0±0	6±0	0±0	25.0±0.8 *	231.8±5.0
200 µg/g dw	6±0	0±0	6±0	0±0	18.8±0.9 *	190.0±3.4 *
300 µg/g dw	6±0	0±0	6±0	0±0	16.0±0.8 *	166.5±3.9 *
Introduced number	6	12	6	50	30	240

* effect of HHCB on the survival of organism is significant at the 0.05 level.

3.3. Phytoplankton and periphyton chlorophyll-a

Chlorophyll-a levels in the overlying water were significantly higher in system B (0.82-1.10 μg/L) than A (0.31-0.50 μg/L) ($p < 0.05$) (Table S10 and Fig. 3a). In both systems A and B, microcosms with the highest sediment HHCB concentration had significantly lower chlorophyll-a concentrations compared to other treatments ($p < 0.05$) (Table S10). Similarly, chlorophyll-a levels of periphyton measured on the glass slides introduced to the microcosms were also higher in system B (4.99-6.64 μg/dm^2) than A (0.44-1.30 μg/dm^2) ($p < 0.05$) and were significantly lower at the highest HHCB treatment in both systems ($p < 0.05$) (Table S10 and Fig. 3b).

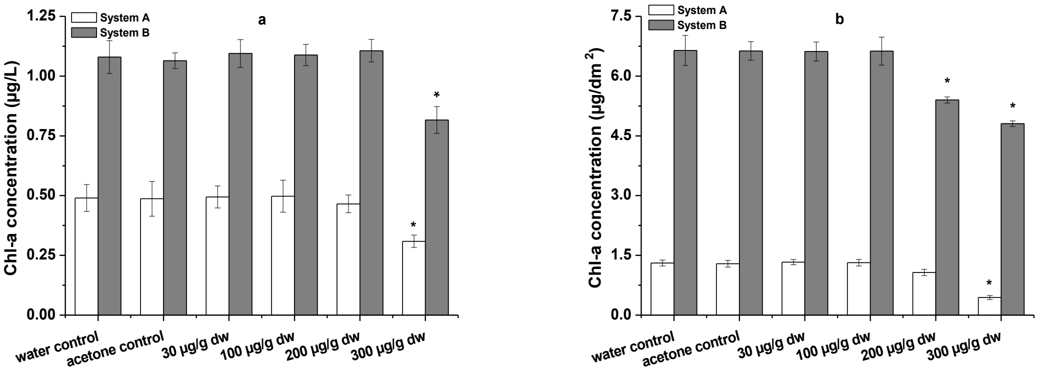

Figure 3. Chlorophyll a content in the water samples (a) and on the glass slides (b) at the end of exposure (day 28). Error bars represent standard error of the mean (n = 3). The white and grey bars represent treatment from systems with (System A) and without (System B) introduced organisms, respectively.

3.4. Daphnia and benthic macroinvertebrates

There were no surviving *Daphnia* or midge individuals in the microcosms of system A by the end of the experiment. However, the survival rates for snails and clams were close to 100% across all treatments (Table 2). There was no evidence of reproductive activity, such as clam or snail larvae, during the experiment. At low concentrations of HHCB (30 μg/g), there was no observable effect of HHCB on the survival of *B. sowerbyi* and *L. hoffmeisteri* (Table 2). However at higher concentrations, HHCB significantly reduced the survival of *B. sowerbyi* (at 100 μg/g dw) by 15.5% and *L. hoffmeisteri* (at 200 μg/g dw) by 21%. The NOECs based on the survival were 30 μg/g dw for *B. sowerbyi* and 100 μg/g dw for *L. hoffmeisteri* (Table S11). Similar to snails and clams, both worms showed no reproductive activity during the exposure period. There were no significant differences in the body wet weight of snails, clams and worms among treatments ($p > 0.05$) or between the beginning and end of the experiment ($p > 0.05$).

3.5. Microbial community

The richness, evaluated using OTUs (4237-4301) and Chao 1 parameter (5753-5956), was similar between system A and B (Table S12). Likewise, there was no significant difference in mean Shannon indices between system A (10.25-10.29) and B (10.19-10.23) (Table S12).

Moreover, there was no significant differences in the values of these indices between treatments.

A total of 61 phyla were observed in all sediment samples, and the top ten dominant phyla are presented in Fig. 4. Among all the identified phyla, *Proteobacteria* (26-29%), *Actinobacteria* (16-20%), *Chloroflexi* (11-16%), *Firmicutes* (9-15%), and *Acidobacteria* (5-6%) were the five most dominant microbial phyla in both controls and HHCB treatment. Among the top ten phyla, system A had a higher abundance of *Chloroflexi* ($p < 0.05$) and *Nitrospirae* ($p < 0.05$), whereas system B had a higher abundance of *Firmicutes* ($p < 0.05$). There was no significant difference in the abundances of the top ten phyla between HHCB treatment and controls, except that the abundance of *Nitrospirae* was significantly higher in the water controls of system A compared to acetone control and HHCB treatment ($p < 0.05$).

The Monte Carlo permutation test indicates that there was no significant difference in microbial community composition at the phylum level between system A and B ($p = 0.158$) (Table S13). In both systems, there was no significant difference in microbial community composition between acetone control and water control or 300 µg/g dw HHCB treatments (Table S13). However, there was a marginally significant interactive effect of the 300 µg/g dw HHCB treatment and the presence of benthic macroinvertebrates on microbial community composition at the phylum level ($p = 0.05$).

The Monte Carlo permutation test indicates that the microbial community composition as represented by OTU’s was significantly different between system A and B ($p = 0.039$) (Table 14). However, in both systems, there was no significant difference in microbial community composition at the OTU level between acetone control and water control or 300 µg/g dw HHCB treatments (Table S14). There was a significant interactive effect of the HHCB treatment and the presence of benthic macroinvertebrates on the microbial community composition at the OTU level ($p = 0.02$).

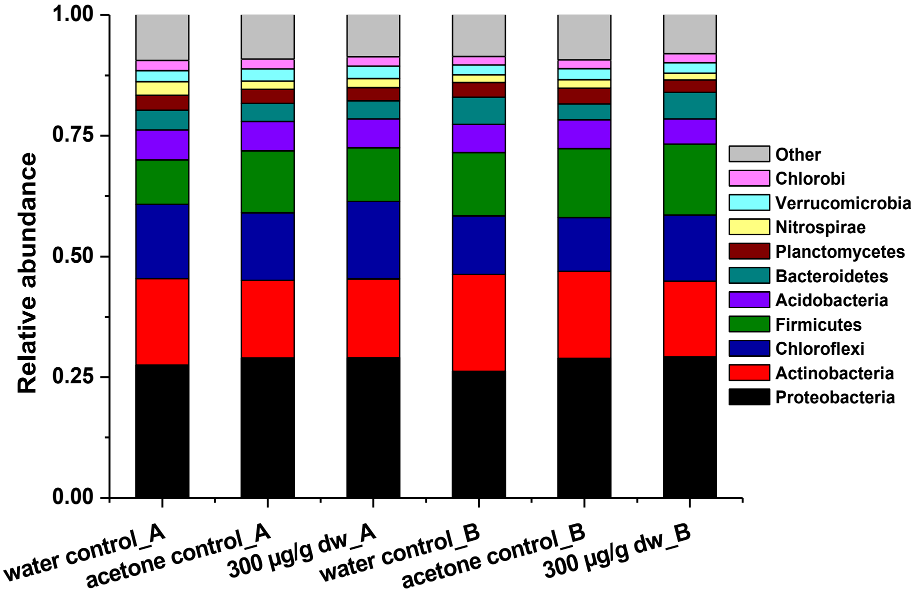

Figure 4. The relative abundance of the top ten phyla in the sediment after 28 days of exposure. The A and B represent treatment from systems with (System A) and without (System B) introduced organisms.

4. Discussion

Overall, the fate data of HHCB obtained in the present study is in agreement with data reported by other studies performed with HHCB (Bard and Ford, 1999, Chen et al., 2014b), all indicating that HHCB is not readily degradable. Similar to other aquatic organisms such as freshwater fish (Hu et al., 2011) and marine organisms (Nakata et al., 2005; 2007), oligochaete worms (*L. hoffmeisteri* and *B. sowerbyi*) can accumulate HHCB. The presence of oligochaete worms, however, did not significantly influence the dissipation of sediment-associated HHCB as there was no significant difference in HHCB levels between system A and B microcosms (Table 1). Sediment-associated HHCB significantly influenced the survival of *L. hoffmeisteri* and *B. sowerbyi* at concentration of 100 µg/g dw and 200 µg/g dw, respectively (Table 1). Although sediment-associated HHCB had no effect on the microbial community composition at concentration up till 300 µg/g dw, there was a significant interactive effect of HHCB treatment and presence of benthic macroinvertebrates on the microbial community composition. Although the concentrations of HHCB tested here are at environmentally elevated concentrations (see Table 15), our results can provide baseline data important for environmental risk assessments.

4.1. Fate of HHCB in the microcosms

HHCB concentrations decreased in the overlying water of both systems after 28 days exposure (Table 1), likely due to HHCB partition between the overlying water and sediment phase considering its hydrophobic property (log Koc value of 4.86) (Balk and Ford, 1999a). Furthermore, in the overlying water higher HHCB concentrations were found in the system A than system B. This is likely associated with the presence of introduced benthic macroinvertebrates, such as *L. hoffmeisteri* and *B. sowerbyi*, as demonstrated that *L. hoffmeisteri* can enhance sediment resuspension and raise the proportion of small size particles in suspended solids through their feeding and defecation activities (Zhang et al., 2014).

Because of the lipophilic properties, HHCB accumulated in *B. sowerbyi* and *L. hoffmeisteri* with BSAF values of 0.29-0.66 and 0.94-2.11, respectively (Table 1). This is in agreement with findings of Hu et al. (2011), who found that HHCB accumulated in the carps from the Haihe River (China) with similar BSAF values of 1.6-2.5. Furthermore, HHCB accumulation in other wildlife, such as marine benthic organisms, has also been reported in earlier studies (Table S15). For example, Nakata et al. (2007) found that HHCB accumulated in marine worm (lugworm) from the Ariake Sea (Japan) at concentrations of 0.003 µg/g wet weight.

An earlier study found that the photochemical degradation of HHCB was negligible in lake Zurich (Switzerland) in both summer and winter (Buerge et al., 2003). Likewise, in this study, the results of HHCB photolysis and hydrolysis experiments indicate that these processes make a negligible contribution to HHCB reduction. Therefore, any decrease in HHCB during the experiment could be attributed to biodegradation by either microorganisms or introduced organisms. However, here we only found a minimal loss of HHCB from the microcosms (0.36-1.54% in system A and 0.36-1.49% in system B) (Table S8), indicating that HHCB was not readily degradable under the conditions in the present study. Similar results have been reported in other studies (e.g., Buerge et al., 2003; Balk and Fold, 1999a; Chen et al., 2014b). For example, Balk and Fold (1999a) found an average half-life value of about 4 months for HHCB dissipation in soil of microcosms. In fact, adsorption onto activated sludge particles has been demonstrated to be the major removal mechanism of HHCB in wastewater treatment plants (WWTPs), as HHCB is poorly biodegradable or susceptible to chemical degradation (e.g., Bester, 2004; Homem et al., 2015).

4.2. Effects of HHCB on algae

Although no algae were initially introduced into system B, higher levels of phytoplankton and periphyton chlorophyll-a were observed in system B than system A (Table S10), suggesting growth of microalgae. Compared to system B, in system A the negative effects of HHCB on the periphyton chlorophyll-a occurred at higher HHCB treatments, indicating that the toxic effects of HHCB on periphyton was mediated in system A. This is likely to be associated with the presence of snails and higher turbidity values in system A than B, as the turbidity and grazing activities of snail can impose an additional stress on the periphyton relative to HHCB. For phytoplankton chlorophyll-a, the toxic effect of HHCB was only observed at the 300 µg/g dw treatment in both systems, with the initial HHCB concentration of 164 µg/L in the overlying water. However, this level is slightly lower than the reported NOEC value (72 h algal growth inhibition) of 201 µg/L (Table S16) based on the *Pseudokirchneriella subcapitata* exposure to HHCB (Balk and Fold, 1999b). This could be attributed to the different sensitivity of different algae, the longer exposure duration (28 days) and more complicated growth conditions in this study (diverse organisms and water-sediment systems).

4.3. Sensitivity of Daphnia and benthic macroinvertebrates to HHCB stress

No *Daphnia* were found in any of the microcosms by the end of experiment (Table 2), which is likely related to food deficiency rather than HHCB stress, as *Daphnia* was also absent in the controls. The lack of effect of HHCB on survival and growth of clams and snails, even at quite high concentration (300 µg/g dw), builds on previous studies which found limited effects of HHCB on freshwater gastropods at 100 µg/g dw (Pedersen et al., 2009), and terrestrial gastropods at 289 µg/g dw (Wang et al., 2015). Unfortunately the effects of HHCB on midge larvae could not be assessed as all larvae emerged during the exposure period. Although there was no effect of HHCB on clams or snails, HHCB reduced the survival of *B. sowerbyi* and *L. hoffmeisteri* at 100 and 200 µg/g dw and higher, respectively (Table 2). However, these concentrations are higher than those occurring in the real environment (Table S15). Therefore, our results suggest that both oligochaete worms are highly tolerant to HHCB, which is in line with earlier findings that they tend to exist in organically polluted waters (Milbrink, 1973; Sang et al., 1987). Likewise, a previous study found that HHCB at a concentration of 168 µg/g dw had no detectable effects on a polychaete (*Capitella* sp.) in terms of adult survival and growth (Ramskov et al., 2009). Based on these findings, *B. sowerbyi* was more sensitive to sediment-associated HHCB than *L. hoffmeisteri* and *Capitella* sp., indicating the species-specific toxicity of HHCB. Therefore, it’s possible that other benthic organisms may suffered HHCB stress at lower concentrations.

4.4. HHCB effects on microbial community

HHCB at 300 µg/g dw had no effect on the sediment microbial community richness, diversity and composition at the phylum and OTU level in system B under the conditions in this study, indicating that HHCB alone would probably not affect microbial community richness diversity and structure at environmental-relevant concentrations. Other studies have found that HHCB had no effect on soil microbial richness and carbon sources utilization at a concentration of 100 µg/g dw (Lv et al. 2017).

Despite a lack of effect of HHCB, our results showed that the presence of benthic macroinvertebrates altered the relative abundance of some dominant bacteria in the sediment, most likely due to their reworking, irrigating, and burrowing activities (Lohrer et al. 2004; Bertics and Ziebis 2009). Similar results have been reported for microbial community composition in sediments inhabited by benthic macroinvertebrates (i.e., *C. fluminea*, *Chironomidae* larvae and tubificid worms) (Cuny et al. 2007; Zeng at al., 2014). The relative abundance of *Chloroflexi*, a common phylum in filed sediment that involved in carbon cycling (Hug et al., 2013), was significantly higher in the sediments of system A than system B. Thus, the presence of benthic macroinvertebrates may indirectly influence the degradation of organic contaminants via altering microbial communities. In contrast, the phylum *Firmicutes* showed statistically lower relative abundance in system A than system B, which is likely associated with the relatively aerobic conditions in the sediment of system A compared to system B, due to the bioturbation in system A. In line with this, a previous study has demonstrated that aerobic treatment caused approximately 8-fold decrease in the relative abundance of *Firmicutes* (McGarvey et al., 2007).

Furthermore, there was a significant interactive effect of HHCB and the presence of benthic macroinvertebrates on microbial community in the sediment, likely due to the increase in bioavailability of sediment-associated HHCB caused by bioturbation of these organisms. A similar phenomenon has been described for sediment-associated triclosan in such microcosms (Chapter 4 and 5).

4.5. Implications for environmental risk assessment

This study showed that HHCB at 30 µg/g dw had no effects on chlorophyll-a content, microbial community composition, and survival and growth of benthic macroinvertebrates. However, further work will be needed to elucidate whether HHCB would affect other benthic macroinvertebrates or not, given the species-specific response. Oligochaete worms at the density in this study made insignificant contribution to the loss of HHCB in the microcosms, but significantly changed the microbial community composition. However, this concentration is higher than those reported in field sediments (i.e., 0-1.48 µg/g dw), indicating that HHCB at environmentally relevant levels is unlikely to pose direct toxicological risks to algae, benthic macroinvertebrates and microbial communities. However, subtle effects, such as alterations in biochemical constituents and genetic damage, of HHCB on these organisms are unknown.

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Supporting information

Materials and methods

Reagents and standards

The standard Galaxolide (HHCB; 1,3,4,6,7,8-hexahydro-4,6,6, 7, 8, 8-hex-amethylcyclopenta (g) -2-benzopyran) was kindly provided by the Research Institute for Fragrance Materials (USA), while the internal standard *d_3*-Tonalide (d_3-AHTN) was purchased from Dr. Ehrenstorfer GmbH (Germany). All the organic solvents used were of HPLC grade. Methanol and acetonitrile were obtained from Merck Corporation (Germany), while ethyl acetate, acetone hexane and dichloromethane were purchased from CNW Technologies (Shanghai, China). Glass fiber filters (GF/F, pore size 0.7 µm) were purchased from Whatman (Maidstone, UK). Oasis HLB extraction cartridges (6 mL, 200 mg) were obtained from Waters Corporation (Milford, MA, USA). Acetic acid (HPLC grade) was supplied by Tedia Company (Tedia, USA). Anhydrous magnesium sulfate ($MgSO_4$) and anhydrous sodium sulfate (Na_2SO4) were purchased from Alfa Aesar (MA, USA). Anhydrous sodium chloride (NaCl), primary-secondary amine (PSA), ceramic homogenizer, and C_{18} bulk sorbent were obtained from Agilent (Santa Clara, USA). HPLC-grade water was from a Milli-Q water purification system (Millipore, Watford). HHCB standard solution was prepared in acetone at concentration of 90 g/L and stored at -18 ˚C for use.

Photolysis and hydrolysis experiment

We conducted 2 experiments in Erlenmeyer flasks to determine HHCB photolysis and hydrolysis under conditions in the present study. As de-chlorinated tap water was applied for the microcosm experiment, it was thus used as medium in the first experiment, to explore potential photolysis and hydrolysis of HHCB. Since water-sediment system is different from pure water phase, the second experiment was conducted with the overlying water from the microcosm with HHCB spiked concentration of 30 µg/g dw, to mimic HHCB photolysis and hydrolysis in the overlying water in the microcosm. For each experiment, we set two groups (with/without light). All related experiments were carried out in triplicates.

Experiment 1: 60 µL of HHCB dissolved in acetone (10 g/L) was added into a 1 L Erlenmeyer flask. Subsequently, 500 mL of de-chlorinated tap water was added into the flask to achieve final HHCB concentration of 1.2 mg/L after solvent evaporation. Erlenmeyer flasks were then placed in a shaker to equilibrate 2 h in the dark. Finally, flasks for HHCB photolysis and hydrolysis were kept under the same conditions as microcosm experiment, while flasks for HHCB hydrolysis were covered with aluminum foil to prevent possible photolysis of HHCB. On day 0, 3, 7, 14, 21 and 28, 1 mL of HHCB solution was sampled from each flask and was spiked with 50 ng of internal standard. Then, samples were immediately extracted by using liquid-liquid extraction method. Specifically, each sample was mixed well, added with 2 mL of n-hexane, manually shaken for 1 min and put at room temperature in the dark for 5 min. The supernatant of n-hexane phase was collected carefully into a 5 mL glass centrifugal tube using a glass pipette. The above procedure was repeated, and the extracts were combined and dried under a gentle nitrogen stream. The final extract was re-dissolved in 1 mL of methanol, transferred to a 2 mL amber glass and stored at -20 ˚C until analysis.

Experiment 2: Same as in the experiment 1, six replicates were set in this experiment with three of them covered with aluminum foil to keep solutions away from light. Specifically, each Erlenmeyer flask (volume: 500 mL) was added with 250 mL of overlying water collected from the microcosm with spiked HHCB concentration of 30 µg/g dw to determine HHCB concentration at the start of exposure. Then, Erlenmeyer flask with overlying water were placed under the same conditions as microcosm experiment. On day 28, the whole Erlenmeyer flask was sacrificed to determine HHCB concentrations. The extraction method was same as water samples from the microcosm. In addition, the particles on the glass fiber filters were also extracted by ultrasonic extraction using acetone/dichloromethane (1:1, v/v).

Extraction of HHCB in water samples

The water samples (500 L each) were extracted by solid phase extraction (SPE) following the method reported in a previous study (Chen et al., 2010). The collected water samples were filtered using 0.7-µm glass fiber filters, spiked with the internal standards (100 ng/L each), loaded onto Oasis HLB SPE cartridges (200 mg, 6 mL) preconditioned with 10 mL of methanol and 10 mL of Milli-Q water. After the filtered water samples passed through cartridges at a flow rate of 5-10 mL/min, each sample bottle was rinsed twice with 50 mL of Milli-Q water containing 5 % methanol (v/v) and passed through the SPE cartridge. The cartridges were then dried under vacuum for 3 h and eluted with 3×2 mL of dichloromethane, followed by 3×2 mL of ethyl acetate and 3×2 mL of methanol. The eluates were dried under a gentle nitrogen gas, re-dissolved in 1 mL of methanol, transferred to a 2 mL amber glass vial with filtering through a 0.22 µm nylon membrane filter, and finally stored at -18 ˚C prior to analysis.

Extraction of HHCB in sediment samples

Musk fragrances in sediment samples were extracted by an Accelerated Solvent Extractor (Dionex ASE 300) equipped with 34 mL stainless steel extraction cells (Chen et al., 2014b). Specifically, a cellulose filter was placed in the bottom of a steel extraction cell, followed by in-cell clean-up sorbents consisted of 2.0 g of silica and 2.0 g of neutral aluminum in sequence. Then 1 g of sediment sample was loaded, spiked with 200 ng of the internal standards AHTN-d_3, and mixed well. Cells were placed in a fume cupboard to remove the solvent, and then added with 5 g of quartz sand. The extraction solvent was acetone/dichloromethane (1:1, v/v). The extraction program was given as follows: extraction temperature (140 ˚C) with heat-up time of 5 min, 2 static cycles with static time of 5 min. The extracts were evaporated to about 2 mL by a rotary evaporator at 30 ˚C, dried under a gentle nitrogen gas and reconstituted in 2 mL of hexane. The final extract was filtered through a 0.22-µm nylon filter and transferred into a 2-mL amber glass vial and stored in -18 ˚C prior to analysis.

Extraction of HHCB in worm tissue

The quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction combined with dispersive solid-phase extraction (d-SPE) clean-up method reported by Yao et al. (2016) was applied to extract HHCB in the worms. Briefly, worms tissue sample was weighted into a 50-mL polypropylene centrifuge tube, spiked with internal standard (100 ng) into each tube and vortexed for 30 s. Then samples were stored at 4 ˚C for 30 min for equilibration. After that, 5 mL of Milli-Q water and two ceramic homogenizers were added into each tube and vortexed for 1 min to homogenize the sample sufficiently. Subsequently, 10 mL of acetonitrile containing 1 % acetic acid, a salt set containing 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous sodium acetate were added and hand shaken for 1 min immediately. Samples were then ultrasonic extracted for 15 min and centrifuged at 2364 *g* for 10 min. Supernatants were transferred to 15 mL d-SPE tube containing 900 mg anhydrous $MgSO_4$, 150 mg PSA, and 150 mg C18 and vortexed for 1 min, followed by centrifugation at 2364 *g* for 10 min. Then supernatants were dried under a gentle stream of nitrogen and re-dissolved by 1 mL methanol, transferred to a 2 mL amber glass vial with filtering through a 0.22 µm nylon membrane filter, and finally stored at -18 ˚C until instrumental analysis.

GC-MS analysis

Musks in the extracts were measured by using an Agilent 6890 N GC interfaced to a 5975B MSD (GC-MS), equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific Co., uSA), in selected ion monitoring (SIM) mode under electron-impact ionization (EI). The temperatures for the GC-MS interface, ion source, quadrupole and injector were 280 ˚C, 250 ˚C, 150 ˚C and 280 ˚C, respectively. Carrier gas: helium (purity > 99.999%) at a constant flow of 1.0 mL/min. Injection volume: 2 µL for each sample. The GC oven temperature was programmed as follows: 80 ˚C for 0 min, increased to 170 ˚C at 15 ˚C /min, from 170 ˚C to 185 ˚C at 1 ˚C/min, then to 300 ˚C at a rate of 20 ˚C/min for 5 min.

Table S1. Traits of *Corbicula fluminea*, *Limnodrilus hoffmeisteri*, *Branchiura sowerbyi*, *Viviparidae Bellamya* and *Orthocladiinae*.

Traits	*Corbicula fluminea*	*Limnodrilus hoffmeisteri*	*Branchiura sowerbyi*	*Viviparidae bellamya*	*Orthocladiinae*
					
Food type	detritus, living microphytes, living micro-invertebrates	microorganisms, living microphytes, mud, sand and detritus	microorganisms, detritus, living microphytes	living microphytes, living macrophytes, dead animal, living micro-invertebrates, dead plant and detritus	living microphytes, living macrophytes, living micro-invertebrates and detritus
Body length (mm) [a]	10.1-12.2	15-22	34-44	14.2-16.3	9.6-12.5
Weight (mg) [a]	326-471	1.9-2.4	16.8-18.5	598-766	4.5-6.2
Average lipid content (SD) (%) [a]	Not determined	1.62 ± 0.22	9.16 ± 0.35	Not determined	Not determined
Respiration type	tegument, gill	tegument	tegument, gill	tegument, gill	tegument, gill
Life cycle duration	> 1 year	> 1 year	> 1 year	> 1 year	≤ 1 year
Generation time (years)	< 0.5	0.5-1	< 1	1-2	<1
Life span (years)	> 1	> 1	> 1	> 5	0.6-1.9
Dispersal mode	passive, active	passive	passive	passive, active	passive, active
Dispersal ability	low-medium	low	low-medium	low	high
Aquatic stages	egg, larva, adult	egg, larva, adult	egg, larva, adult	egg, adult	egg, larva, nymph
Reproduction type	sexual reproduction	asexual reproduction	asexual reproduction	sexual reproduction	sexual reproduction
Feeding habits	filter-feeder	absorber, deposit feeder	absorber, deposit feeder	shredder, scraper, filter-feeder	deposit feeder, scraper, filter-feeder, parasite
Reproduction	ovoviviparity, isolated eggs, free	clutches, cemented or fixed	clutches, cemented or fixed	ovoviviparity	clutches, cemented or fixed, clutches free, clutches terrestrial

Table S1. (Continued).

Traits	*Corbicula fluminea*	*Limnodrilus hoffmeisteri*	*Branchiura sowerbyi*	*Viviparidae bellamya*	*Orthocladiinae*
Current velocity	Null-slow (< 25 cm/s)	null-fast (> 50 cm/s)	null-medium (25-50 cm/s)	null-medium (25-50 cm/s)	null-fast (> 50 cm/s)
Trophic status	mesotrophic, eutrophic	mesotrophic, eutrophic	mesotrophic, eutrophic	oligotrophic, mesotrophic, eutrophic	oligotrophic, mesotrophic, eutrophic
Temperature	warm (> 15°C)	eurythermic	eurythermic	eurythermic	eurythermic
Water depth	water surface	water surface, shallows, middle depths, bottom, buried	shallows, middle depths, bottom	shallows, middle depths, bottom	shallows, middle depths, bottom
Locomotion and substrate relation	full water swimmer, crawler, burrower	burrower, interstitial	burrower	crawler, burrower	full water swimmer, crawler, burrower, interstitial, temporarily attached
Transversal distribution	river channel, banks, connected side-arms, lakes	river channel, banks, connected side-arms, ponds, pools, disconnected side-arms, lakes	river channel, ponds, pools, disconnected side-arms, lakes	river channel, banks, connected side-arms, ponds, pools, disconnected side-arms, marshes, peat bogs, lakes	banks, connected side-arms, ponds, pools, disconnected side-arms, marshes, peat bogs, temporary waters, lakes, crenon
Substrate type	flags/boulders/cobbles/pebbles, gravel, sand, silt, mud	gravel, sand, silt, macrophytes, microphytes, twigs/roots, organic detritus/litter, mud	gravel, sand, silt, twigs/roots, organic detritus/litter, mud	flags/boulders/cobbles/pebbles, gravel, macrophytes, mud	flags/boulders/cobbles/pebbles, gravel, sand, silt, macrophytes, microphytes, organic detritus/litter, mud

[a] Traits determined in the present study at the start of the experiment. The remainder traits information was retrieved from the PondFX (Heneghan et al., 1999) and Tachet database (Tachet et al., 2000; Usseglio-Polatera et al., 1999; 2000).

Table S2. Recoveries and detection limits of HHCB in overlying water, sediment and worms by GC-MS.

Substrate	LOD [a] (ng/L)	LOQ [b] (ng/L)	Recoveries [c] (%)
Overlying water	0.35	1.01	109 ± 2.93
Sediment	0.41	1.36	515 ± 18.7
Worms	0.52	1.57	501±10.4

The spiked HHCB concentration in the water, sediment and worms were 100 ng/L, 500 ng/g dw and 500 ng/g ww, respectively.

[a] Mean ± standard deviation (n = 3).

[b] LOD, method limit of detection.

[c] LOQ, method limit of quantitation.

Table S3. pH values in the water phase during the exposure period.

System	Treatment	day0	day1	day3	day7	day10	day14	day21	day28
A	water control	6.91±0.02	6.86±0.01	6.65±0.04	6.65±0.02	6.50±0.05	6.45±0.09	6.26±0.03	6.06±0.02
	acetone control	6.92±0.02	6.82±0.01	6.70±0.02	6.74±0.03	6.62±0.04	6.57±0.06	6.36±0.07	6.09±0.03
	30 μg/g dw	6.94±0.02	6.81±0.01	6.69±0.03	6.89±0.06	6.87±0.04	6.68±0.06	6.28±0.06	6.08±0.03
	100 μg/g dw	6.93±0.02	6.89±0.01	6.78±0.04	6.89±0.02	6.90±0.03	6.70±0.03	6.33±0.23	6.16±0.04
	200 μg/g dw	6.99±0.01	6.86±0.03	6.81±0.01	6.85±0.04	6.87±0.03	6.66±0.03	6.40±0.07	6.14±0.04
	300 μg/g dw	6.92±0.03	6.83±0.03	6.82±0.03	6.96±0.05	6.95±0.03	6.68±0.03	6.67±0.02	6.41±0.08
B	water control	6.89±0.02	6.84±0.02	6.83±0.07	6.76±0.05	6.59±0.06	6.48±0.03	6.61±0.06	6.68±0.05
	acetone control	6.91±0.02	6.83±0.02	6.86±0.06	6.83±0.04	6.79±0.01	6.69±0.03	6.74±0.04	6.81±0.01
	30 μg/g dw	6.99±0.01	6.89±0.05	6.87±0.06	6.94±0.01	6.95±0.06	6.82±0.03	6.89±0.03	6.91±0.02
	100 μg/g dw	6.97±0.01	6.89±0.02	6.89±0.00	6.95±0.04	6.95±0.01	6.86±0.06	6.92±0.02	6.92±0.04
	200 μg/g dw	6.99±0.01	6.88±0.06	6.89±0.05	6.93±0.02	6.99±0.03	6.83±0.03	6.94±0.05	6.94±0.08
	300 μg/g dw	6.92±0.03	6.82±0.01	6.85±0.03	6.93±0.04	6.96±0.01	6.83±0.06	6.94±0.01	6.92±0.01

Table S4. Conductivity values (μS/cm) in the water phase during the exposure period.

System	Treatment	day0	day1	day3	day7	day10	day14	day21	day28
A	water control	96.95±1.10	106.88±1.65	105.65±2.10	101.20±1.78	98.55±0.79	95.63±1.51	100.13±1.08	92.60±1.86
	acetone control	97.50±0.95	107.60±4.75	106.80±1.64	102.43±6.16	100.20±1.48	95.93±1.66	100.15±1.50	90.68±0.85
	30 μg/g dw	97.18±1.26	106.25±5.65	105.45±3.13	104.75±4.70	99.75±4.33	94.95±1.17	100.55±1.77	90.18±2.24
	100 μg/g dw	97.93±1.18	109.38±2.65	104.03±4.26	100.55±1.68	98.80±1.13	96.65±2.22	99.23±1.30	98.23±1.51
	200 μg/g dw	97.40±0.88	106.38±2.13	102.60±1.31	98.88±1.21	101.28±3.69	99.63±3.03	103.20±2.90	103.53±2.13
	300 μg/g dw	97.25±1.39	107.98±3.15	104.25±2.33	100.20±3.29	101.08±4.57	99.60±1.50	101.40±1.22	105.68±2.69
B	water control	97.40±1.05	105.20±1.59	105.83±2.17	102.30±2.25	100.37±1.68	96.63±2.72	99.53±1.05	101.90±1.56
	acetone control	97.50±1.01	105.93±4.04	104.50±3.06	102.37±2.77	99.63±2.83	96.43±2.65	99.37±2.16	101.80±2.13
	30 μg/g dw	97.53±1.29	106.77±7.17	105.40±5.14	105.63±5.61	102.10±3.60	98.90±3.70	100.33±1.07	102.73±1.96
	100 μg/g dw	97.67±0.81	106.53±1.74	104.97±3.90	104.70±1.25	102.50±0.89	99.17±0.97	101.10±2.48	103.80±2.59
	200 μg/g dw	97.13±0.49	107.00±1.65	105.30±1.42	106.23±0.38	103.17±1.30	100.77±1.35	102.43±0.80	105.63±2.58
	300 μg/g dw	98.20±2.05	108.73±2.31	106.73±1.87	105.33±0.91	102.77±0.90	100.37±1.22	103.20±0.89	105.87±2.63

Table S5. Turbidity values (NTU) in the water phase during the exposure period.

System	Treatment	day1	day7	day14	day21	day28
A	water control	144.25±32.36	67.30±16.32	45.38±2.11	25.39±7.72	13.23±4.32
	acetone control	136.50±13.40	73.30±9.22	61.28±4.34	50.18±11.63	32.53±7.87
	30 µg/g dw	128.50±8.35	62.05±10.42	139.9±47.86	90.75±29.26	35.35±7.88
	100 µg/g dw	128.00±14.07	50.90±9.21	119.03±26.16	64.30±35.19	34.58±12.11
	200 µg/g dw	130.75±11.18	51.80±11.99	119.38±73.61	73.63±39.12	57.91±33.80
	300 µg/g dw	166±10.17	67.13±6.25	112.78±37.97	69.53±26.15	48.9±22.54
B	water control	145±9.54	41.93±10.52	14.52±5.39	5.50±6.25	5.35±3.38
	acetone control	156.33±8.02	30.5±20.42	3.57±1.07	2.11±1.10	1.75±0.27
	30 µg/g dw	157±12.77	23.40±2.98	3.46±1.25	1.21±0.30	1.37±0.60
	100 µg/g dw	156.33±6.51	22.01±6.16	8.29±3.76	3.24±1.88	2.78±1.06
	200 µg/g dw	158±10.15	37.63±17.87	22.77±7.98	13.37±5.36	10.00±3.65
	300 µg/g dw	183±11.79	59.33±8.69	8.38±3.89	2.53±0.87	4.20±1.52

Table S6. Nutrients concentrations in the water phase (mg/L) after 28 days of exposure.

System	Treatment	NH_4^+	NO_3-N	NO_2-N	TP	TOC
A	water control	0.33±0.04	1.21±0.15	0.002±0.00	0.02±0.00	4.71±0.26
	acetone control	0.34±0.03	1.18±0.15	0.002±0.00	0.02±0.00	4.68±0.25
	30 µg/g dw	0.33±0.04	1.23±0.10	0.002±0.00	0.02±0.00	4.79±0.08
	100 µg/g dw	0.34±0.07	1.22±0.13	0.002±0.00	0.02±0.01	4.96±0.08
	200 µg/g dw	0.32±0.03	1.19±0.05	0.002±0.00	0.02±0.00	5.23±0.14*
	300 µg/g dw	0.33±0.03	1.17±0.04	0.002±0.00	0.02±0.00	5.24±0.31*
B	water control	0.25±0.03	0.77±0.03	0.002±0.00	0.02±0.00	3.51±0.32
	acetone control	0.17±0.02	0.76±0.04	0.002±0.00	0.02±0.00	3.62±0.09
	30 µg/g dw	0.21±0.05	0.60±0.04*	0.002±0.00	0.02±0.00	3.58±0.14
	100 µg/g dw	0.22±0.03	0.44±0.08*	0.002±0.00	0.02±0.00	4.56±0.14*
	200 µg/g dw	0.22±0.02	0.45±0.00*	0.002±0.00	0.02±0.00	4.67±0.21*
	300 µg/g dw	0.22±0.03	0.44±0.03*	0.002±0.00	0.02±0.00	4.28±0.35*
Original water	-	0.32±0.03	1.75±0.06	0.002±0.00	0.02±0.00	0.67±0.12

* represents treatment showed significant difference ($p < 0.05$).

Table S7. Nutrients concentrations in the sediment (g/kg) after 28 days of exposure.

System	Treatment	OM	TN	TP	NH_4^+
A	water control	20.46±0.10	1.39±0.03	0.43±0.01	0.06±0.00
	acetone control	20.45±0.08	1.39±0.02	0.45±0.00	0.06±0.00
	30 μg/g dw	20.49±0.26	1.38±0.03	0.44±0.01	0.06±0.00
	100 μg/g dw	20.47±0.16	1.37±0.02	0.44±0.01	0.06±0.01
	200 μg/g dw	20.48±0.10	1.38±0.02	0.44±0.01	0.06±0.00
	300 μg/g dw	20.50±0.04	1.39±0.02	0.44±0.01	0.06±0.00
B	water control	20.47±0.03	1.39±0.03	0.44±0.01	0.06±0.00
	acetone control	20.48±0.06	1.38±0.01	0.44±0.01	0.06±0.00
	30 μg/g dw	20.49±0.02	1.39±0.01	0.45±0.01	0.06±0.00
	100 μg/g dw	20.49±0.07	1.38±0.02	0.44±0.01	0.06±0.00
	200 μg/g dw	20.51±0.05	1.39±0.03	0.44±0.01	0.05±0.00*
	300 μg/g dw	20.51±0.03	1.39±0.01	0.44±0.01	0.04±0.00*
Original sediment	-	20.58±0.02	1.45±0.01	0.45±0.01	0.11±0.01

* represents treatment showed significant difference ($p < 0.05$).

Table S8. HHCB decrease amount (%) in the microcosms on day 28, compared to the spiked HHCB amount.

Treatment	Decreased HHCB amount		Contribution of macroinvertebrates
	System A	System B	
water control	-	-	-
acetone control	-	-	-
30 μg/g dw	1.54±0.07	1.49±0.07	0.05±0.12
100 μg/g dw	0.97±0.04	0.95±0.05	0.02±0.06
200 μg/g dw	0.51±0.02	0.50±0.02	0.00±0.03
300 μg/g dw	0.36±0.05	0.36±0.03	0.00±0.03

Table S9. HHCB concentrations during the photolysis experiment.

Sampling points	De-chlorinated tap water (mg/L)		Overlying water (µg/L)	
	Light	Dark	Light	Dark
day 0	1.19±0.02	1.20±0.01	24.22±3.56	24.22±3.56
day 3	1.19±0.01	1.19±0.02	-	-
day 7	1.19±0.02	1.19±0.01	-	-
day 14	1.19±0.03	1.19±0.02	-	-
day 21	1.16±0.02	1.17±0.03	-	-
day 28	1.10±0.01	1.15±0.02	23.65±4.05	23.78±3.91

Table S10. Chlorophyll a concentrations of phytoplankton and periphyton after 28 days of exposure.

Treatment	Phytoplankton chl-a (µg/L)		Periphyton chl-a (µg/dm^2)	
	System A	System B	System A	System B
water control	0.49±0.06	1.08±0.07	1.30±0.07	6.64±0.38
acetone control	0.49±0.07	1.06±0.03	1.29±0.08	6.63±0.23
30 µg/g dw	0.49±0.05	1.10±0.06	1.29±0.04	6.62±0.24
100 µg/g dw	0.50±0.07	1.09±0.05	1.24±0.06	6.31±0.04*
200 µg/g dw	0.47±0.04	1.09±0.02	1.07±0.08	5.72±0.07*
300 µg/g dw	0.31±0.03*	0.82±0.06*	0.44±0.05*	4.99±0.07*

chl-a represents chlorophyll-a.

* represents treatment showed significant difference ($p < 0.05$).

Table S11. No observed effect concentrations (NOECs; Williams test, $p < 0.05$) in µg/g dw for daphnia and macroinvertebrates after 28 days of exposure.

Treatment	NOEC
Corbicula fluminea	> 300
D. magna	-
Viviparidae Bellamya	> 300
Orthocladiinae	-
B. sowerbyi	30
L. hoffmeisteri	100

- means insufficient data to obtain NOEC.

Table S12. The α-diversity indices among treatments.

Treatment	OTUs		Shannon		Chao1	
	System A	System B	System A	System B	System A	System B
water control	4257±83	4244±56	10.25±0.10	10.19±0.04	5835±152	5753±130
acetone control	4267±102	4237±67	10.29±0.11	10.23±0.07	5956±100	5910±120
300 µg/g dw	4273±87	4301±50	10.25±0.08	10.22±0.10	5807±95	5893±146

Table S13. Monte Carlo permutation tests on sequence data under redundancy analysis based on phylum data set.

Permutation test	*p*-values
System A versus B	0.158
System A: Control versus Acetone control	0.088
System A: 300 µg/g dw versus Acetone control	0.458
System B: Control versus Acetone control	0.412
System B: 300 µg/g dw versus Acetone control	0.088

Table S14. Monte Carlo permutation tests on sequence data under redundancy analysis based on OTU table.

Permutation test	*p*-values
System A versus B	0.039
System A: Control versus Acetone control	0.096
System A: 300 µg/g dw versus Acetone control	0.096
System B: Control versus Acetone control	0.402
System B: 300 µg/g dw versus Acetone control	0.193

Table S15. HHCB concentrations in surface water, sediment and biota in the literature.

Location	Surface water (ng/L)	Sediment (ng/g dw)	Biota (ng/g lipid weight (lw))	References
China		1.5-121.0		(Zeng et al., 2008)
China			0-2600	(Nakata et al., 2012)
China			0-7.5	(Zhang et al., 2013)
China	<0.35-753	<0.41-1480		(Peng et al., 2017)
China	3.5-32	1.5-32.3	107.9-823.3	(Hu et al., 2011)
China	20-93	3-78		(Zhang et al., 2008)
Korea	260-13920			(Lee et al., 2010)
Korea		0-6.3	56.2-1110	(Lee et al., 2014)
Korea			0-14000	(Nakata et al., 2012)
Korea			<2.3-187	(Moon et al., 2012)
Korea			11-76	(Moon et al., 2011)
Japan			0-7000	(Nakata et al., 2012)
Japan			0.45-75 (ng/g ww)	(Nakata et al., 2007)
Singapore	5.16-42.9	11.0-108		(Wang and Kelly, 2017)
Austria	0-32	0-120		(Clara et al., 2011)
France	52			(Mitjans and Ventura, 2004)
Germany	<5-678			(Quednow and Püttmann, 2008)
Germany	<10-180	<2-191		(Dsikowitzky et al., 2002)
Germany			4800-155000	(Franke et al., 1999)
Germany			<1.34-12000	(Subedi et al., 2012)
Germany	36-58			(Mitjans and Ventura, 2004)
Germany			4600-160000	(Gatermann et al., 2002)
Italy		<4	<4 (ng/g dw)	Casatta et al., 2015
Italy			<0.8-2.1 (ng/g ww)	(Nakata et al., 2007)
Romania	153-340			(Moldovan, 2006)
Spain	<0.05-1141	0-17.993		(Villa et al., 2012)
Spain	329			(Mitjans and Ventura, 2004)
Spain			15.8-367.3 (ng/g dw)	(Trabalon et al., 2015)
U.K.	<1-28			(Sumner et al., 2010)
USA	0-230			(Baldwin et al., 2016)
USA	3.95-25.1	3.2-388	<1-125	(Reiner and Kannan, 2011)
USA			0-2200	(Nakata et al., 2012)
USA			<0.8-19 (ng/g ww)	(Nakata et al., 2007)
USA			12-798	(Kanan et al., 2005)
USA			<1-25 (ng/g ww)	(Kanan et al., 2005)
USA	0-794	0-2.13		(Chase et al., 2012)

Table S16. Summary of the aquatic eco-toxicity data for HHCB.

Species	Trophic group	Duration, Effect, Endpoint	Value (µg/L)	Reference
Pseudokirchneriella subcapitata	Algae	72 h, Growth, NOEC	201	(Balk and Ford, 1999b)
Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	1000	(Carlsson and Norrgren, 2004)
Oryzias latipes	Fish	96 h Survival LC$_{50}$	950	(Yamauchi et al., 2008)
Lepomis macrochirus	Fish	21 d, Growth, NOEC	182	(Balk and Ford, 1999b)
Pimephales promelas	Fish	32 d, Growth, NOEC	68	(Balk and Ford, 1999b)
Pimephales promelas	Fish	33 d, Development, NOEC	68	(Balk and Ford, 1999b)
Pimephales promelas	Fish	34 d, survival, NOEC	68	(Balk and Ford, 1999b)
Danio rerio	Fish	ELS 48 h, Heart rate, NOEC	1,000	(Carlsson and Norrgren, 2004)
Daphnia magna	Crustacean	21 d, Reproduction, NOEC	111	(Balk and Ford, 1999b)
Daphnia magna	Crustacean	21 d, Reproduction, LOEC	205	(Balk and Ford, 1999b)
Daphnia magna	Crustacean	21 d, Mobility, EC$_{50}$	293	(Balk and Ford, 1999b)
Nitocra spinipes	Crustacean	96 h, Survival, LC$_{50}$	1900	(Breitholtz et al., 2003)
Acartia tonsa	Crustacean	5 d, Development, EC$_{50}$	59	(Wollenberger et al., 2003
Acartia tonsa	Crustacean	48 h, Development, LC$_{50}$	470	(Wollenberger et al., 2003
Lampsilis cardium	Mollusca	96 h, Growth, EC$_{50}$	153-831	(Gooding et al., 2006)
Lampsilis cardium	Mollusca	24 h, 48h, Survival LC$_{50}$	999-1750	(Gooding et al., 2006)

Chapter 7

Bioaccumulation and biotransformation of triclosan and galaxolide in the freshwater oligochaete *Limnodrilus hoffmeisteri* in a water/sediment microcosm

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Abstract

Personal care products are widely used in our daily life in considerable quantities and discharged through the down-the-drain route to the aquatic environments, resulting in potential risks to aquatic organisms. We investigated bioaccumulation and biotransformation of two widely used personal care products, triclosan (TCS) and galaxolide (HHCB) spiked to sediment, in the oligochaete *Limnodrilus hoffmeisteri* in water/sediment microcosms. After 7 days of sediment exposure to 3.1 μg TCS or HHCB /g dry weight (dw) sediment, the accumulation of TCS and HHCB in *L. hoffmeisteri* reached equilibrium, at which point the biota-sediment accumulation factors (BSAFs) were 2.07 and 2.50 for TCS and HHCB, respectively. The presence of *L. hoffmeisteri* significantly accelerated the dissipation of TCS and HHCB in the microcosms, with approximately 9.03% and 2.90% of TCS and HHCB eliminated from the water-sediment phases after 14 d exposure in presence of worms, respectively. Two biotransformation products, methyl triclosan and triclosan-O-sulfate, were identified for TCS in the worm tissue, whereas only methyl triclosan was identified in the sediment. Unlike TCS, no evidence of biotransformation products was found for HHCB in either worm tissue or sediment. These experiments demonstrate that *L. hoffmeisteri* biotransform TCS through methylation and sulfonation, whereas HHCB biotransformation was undetectable.

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1. Introduction

Personal care products (PCPs) are widely used in our daily life and can be a potential risk to the aquatic environment due to their release through the down-the-drain route and negative effects on aquatic ecosystems (Díaz-Cruz and Barceló, 2015). Sediments may act as 'sinks' and long-term reservoirs for hydrophobic PCPs released into the aquatic environment (Burton et al., 2002). Those hydrophobic organic chemicals can accumulate in aquatic organisms, which may further result in bio-magnification through dietary transfer in the food web (Janssen and Beckingham, 2013), or may potentially be biotransformed as observed in *Capitella teleta* exposed to sediment-associated acetyl cedrene (Dai et al., 2012) thus reducing the body burden. Despite this, oligochaete worms prevail in aquatic environments worldwide and are exposed to sediment-associated hydrophobic PCPs, little is known about their potential to biotransform these chemicals.

Triclosan (TCS) and galaxolide (HHCB) are two ingredients widely used in personal care products and have been found ubiquitous in a variety of aquatic environments (Ying, 2010). With their hydrophobic nature, these two chemicals likely sorb to settling particles and may thus be subject to bio-accumulation in deposit-feeding macroinvertebrates (Balk and Ford, 1999a; Bedoux et al., 2012). To date, laboratory degradation studies of TCS and HHCB have been limited to soil bacterial cultures (Ying et al., 2007), wastewater microorganisms (Lee et al., 2012), fungi (Martin et al., 2007; Vallecillos et al., 2017), diatom (Ding et al., 2018), algae (Wang et al., 2018), activated sludge (Chen et al., 2011, 2015; Armstrong et al., 2017, 2018) and iron and manganese oxides (Ding et al., 2015). For example, TCS can be transformed into methyl triclosan (Me-TCS) in activated sludge under aerobic conditions (Chen et al., 2011) and in biosolid-amended agricultural soil by earthworms (Macherius et al., 2014). Similarly, the biological oxidation of HHCB into HHCB-lactone has been reported in wastewater treatment processes (Bester, 2005b) and fish samples (Hühnerfuss et al., 2004). However, little to no research has been performed to investigate their degradation in water/sediment systems with the presence of oligochaete worms that may efficiently biotransform organic contaminants. Indeed, *Lumbriculus variegatus* (Oligochaeta) was reported to biotransform pyrene into 1-hydroxypyrene (Navarro et al., 2011).

Oligochaete worms are an important group of freshwater benthic macroinvertebrates, ubiquitous and abundant in sediments of freshwater ecosystems, such as rivers, ponds and lakes (Vivien et al., 2004). They are thus widely used to evaluate the toxicity and accumulation of sediment-associated hydrophobic organic contaminants (Lotufo and Fleeger, 1996; Di et al., 2016; Yang et al., 2016). *Limnodrilus hoffmeisteri* (Naididae, Oligochaeta) is the dominant taxon within oligochaete worms in the Pearl River, and it can achieve a density of up to 50.000 ind./m^2 (Jiang et al., 2011). As conveyor-belt feeder, *L. hoffmeisteri* ingests small particles in sediments and egests them as faecal pellets on the sediment surface (Kaster et al., 1984; Dafoe et al., 2011). Sediment-associated hydrophobic organic contaminants may go through bioaccumulation and biotransformation in the body of *L. hoffmeisteri*, producing more water-soluble by-products. For example, 2,4-dichlorophenol, a metabolite of TCS, is more water-soluble but less toxic than its parent compound (Yin et al., 2003; Ying et al., 2007). However, Macherius et al. (2014) reported that *Eisenia fetida* can biotransform TCS into Me-TCS that is more environmentally persistent, lipophilic (James et al., 2012; Pycke et al., 2014) and toxic to *Vibrio fischeri* than TCS (Farré et al., 2008). Therefore, it is important to understand the metabolic pathway of hydrophobic contaminants such as TCS and HHCB by *L. hoffmeisteri* in order to evaluate the persistence and risk of these contaminants in the real environment.

This study aims to evaluate the importance of *L. hoffmeisteri* in the dissipation of sediment-associated TCS and HHCB in microcosms simulating static water systems. The microcosms were divided into two treatment groups: with and without addition of *L. hoffmeisteri*. The exposures lasted for 14 d, and the worms were sampled on day 1, 3, 7, 10 and 14 to investigate the bioaccumulation kinetics of TCS and HHCB. The results from this study will help understand the dissipation kinetics of TCS and HHCB in a water/sediment system, and the accumulation and biotransformation of sediment-associated TCS and HHCB in *L. hoffmeisteri*.

2. Materials and methods

2.1. Standards and Reagents

Standards of triclosan (TCS), methyl triclosan, 2,4-dichlorophenol, 4-chlorocatechol, and d_3-Tonalide (d_3-AHTN) were purchased from Dr. Ehrensorfer (Germany), while triclosan-O-β-D-glucuronide sodium salt (TCSG), triclosan-O-sulfate sodium salt and galaxolidone (HHCB-lactone) were obtained from TRC (Canada). The standard galaxolide (HHCB; 1,3,4,6,7,8-hexahydro-4,6,6, 7, 8, 8-hexamethyl cyclopenta (g)-2-benzopyran) was kindly provided by International Flavors & Fragrances (USA). The internal standards ^{13} C_{12}-triclosan and ^{13} C_{12}-methyl triclosan were obtained from Cambridge Isotope Laboratories (Andover, USA). Sylon BTZ containing trimethylchlorosilane, N,O-bis(trimethylsilyl) acetamide, and N-trimethylsilylimidazole was obtained from Supelco. Further details are provided in the supporting Information.

2.2. Test sediment and spiking

The experimental sediment was collected from an uncontaminated reservoir (113˚47'42"N, 23˚46'01"E) (Zhao et al., 2010a; Peng et al., 2017), a drinking water source of Guangzhou city (South China). The natural sediment was wet-sieved (300 µm) with deionized water, and then allowed to settle overnight. After removing the overlying water, the resultant sediment was kept frozen at -20 °C until use. The sediments used in the microcosms consisted of 0.49% sand, 40.82% silt, and 58.69% clay, and they had a water content of 57% (24 h at 105 °C; n = 4), an organic matter (OM) content of 20.6‰, a total nitrogen (TN) content of 1.45‰, a total phosphorus (TP) content of 0.45‰ and an ammonia (NH_4^+) content of 0.11‰ (Clesceri et al., 1998). The background TCS and HHCB concentrations in the sediment were around 0.002

µg/g dry weight (dw), and considered negligible for the purposes of this study. Before chemical application, sediment was thawed at 27 ± 1 °C in the dark and rinsed with Milli-Q water.

To spike each test compound into sediment, 15 g wet sediment was weighed into a centrifuge tube (50 mL), producing a sediment height of approximately 2.5 cm, and amended with 10 µL of TCS or HHCB stock solution to achieve a final concentration of 3.1 µg/g dw sed. Two controls were used in the experiments: a water control and an acetone control, which were created by replacing the chemical solution with the same volume of Milli-Q water and acetone, respectively. Tubes were wrapped with aluminium foil to minimize photolysis of TCS and HHCB. After 15 min of solvent evaporation in the fume hood under darkness, each tube was vortexed for 5 min and then shaken on a horizontal shaker for 12 h in the dark at 16 °C to achieve homogeneity.

2.3. Test organisms

The *L. hoffmeisteri* was obtained from an aquarium market (Guangzhou, China). It was acclimatized in a 18-L glass tank containing aerated deionized water and thawed sediment (27 ± 1 °C, dark). The acclimatization phase lasted three weeks before the start of exposure. The culturing water and sediment were renewed once during the acclimatization. The total lipids were extracted with acetone/hexane (1/1, v/v) and quantified gravimetrically (Bligh and Dyer, 1959).

2.4. Experimental design

TCS and HHCB biotransformation experiments were performed separately in water/sediment microcosms. After sediment spiking, 30 mL of aerated Milli-Q water was gently pipetted into each glass vial along the wall, and stored at 4 °C in the dark for 2 days to enable equilibrium between water and sediment. Then 30 *L. hoffmeisteri* (length: 20.48±3.17 mm; width: 0.375±0.032 mm; wet weight: 0.0021±0.0006 g) at larval stage were introduced into each tube belonging to system with worms. A parallel set of vials without worms were also included to assess microbial degradation. Constant gentle aeration was provided through a glass Pasteur pipette in each tube of both systems. Microcosms were incubated statically at 27±1 °C in the dark. Water evaporation was minimized by covering the tubes with parafilm during the exposure period. According to our previous experience, the evaporation was negligible after 14 d culturing. To measure the abiotic loss of TCS and HHCB during the exposure period, blanks were prepared by adding 30 mL of aerated Milli-Q water containing TCS or HHCB at concentration of 2 µg/L into microcosms. All experiments were performed in four replicates, thus there were 72 tubes in total for each experiment. During the exposure period, no food was added into the microcosms as worms live on the organic matter associated with the sediment particles. To analyse bioaccumulation and dissipation kinetics of TCS or HHCB, 8 tubes were sacrificed on days 0, 1, 3, 7, 10 and 14, respectively. Blank and control vessels were sacrificed only at the start and end of exposure. The TCS and HHCB concentrations in the overlying water were determined only at the start of exposure. As Me-TCS and HHCB-lactone have been reported as the main product of TCS (Macherius et al., 2014) and HHCB (Martin et al., 2007), their concentrations were also measured on each sampling date in the worms and water-sediment phases. The pH value in the overlying water was measured at the start and end of exposure. The biotransformation products were determined on days 0, 7 and 14 in worm tissue and in water-sediment phases.

2.5. Sample pre-treatment

Tubes from system without worms were directly frozen (-20 °C) until lyophilization. Tubes from system with worms were gently vortexed, the resultant water-sediment mixture was then sieved (300 µm). Worms were transferred to glass beakers with 400 mL of aerated tap water, left to depurate overnight, weighted into a 50-mL polypropylene centrifuge tube for chemical extraction. Water and sediment were separated by centrifugation at 4000 rpm. The resultant overlying water was immediately filtered through 0.7-µm glass fibre filters, combined with the above Milli-Q water, diluted to 1000 mL and extracted using solid-phase extraction (SPE) as previously described (Chen et al., 2012). The collected sediment and filters were frozen (-20 °C), lyophilized and stored at 4 °C in the dark until extraction. The detailed explanation for SPE is given in the supporting information.

Sediment samples were extracted by ultrasonic extraction combined with purification by SPE cartridges. Briefly, 15 mL of methanol (for TCS extraction) or acetone/dichloromethane (1:1, v/v) (for HHCB extraction) was added into each tube with dry sediment, vortexed for 5 min and further shaken on a horizontal shaker for 2.5 h at 16 °C to thoroughly mix the sediment and solvent. Samples were then extracted in an ultrasonic bath for 0.5 h, and centrifuged at 3000 rpm for 10 min. The clear supernatant was transferred to a 300-mL flat-bottomed flask using a glass pipette. The extraction procedure was repeated three times. For the fourth extraction of TCS, 15 mL of methanol containing 0.1 % (v/v) formic acid was used as extraction solvent. Extraction procedures for particles on the filters were the same as the sediment samples. The supernatants of the sediment and filter from the same microcosm were combined, allowed to evaporate at 37 °C to about 20 mL for TCS whereas to almost dry and reconstituted in 20-mL methanol for HHCB, and diluted with Milli-Q water to a volume of 300 mL. Each diluted extract was then purified and enriched on an Oasis HLB cartridge (200 mg, 6 mL) using the same procedures for the extraction of water samples.

Worms in the tubes were first spiked with 100 ng of d_3-AHTN in case of HHCB samples and 100 ng of $^{13}C_{12}$-triclosan and $^{13}C_{12}$-methyl triclosan in case of TCS samples, vortexed for 30 s, and equilibrated at 4 °C for 30 min. Worm tissue was then homogenized in 4 mL of acetonitrile with two ceramic homogenizers. The homogenates were ultra-sonicated (30 min, 20 °C) and centrifuged (10 min, 4000 rpm). The clear supernatants were transferred to 15 mL d-SPE tubes containing 900 mg anhydrous $MgSO_4$, 150 mg PSA, and 150 mg C_{18} to remove lipids. The extraction procedure was repeated twice for each sample. In the third extraction, acetonitrile was replaced by acetonitrile containing 0.2 % acetic acid. The d-SPE tubes containing supernatants were shaken for 2 min and centrifuged (15 min, 4000 rpm). The final supernatants were transferred to 15-mL glass tubes, dried under gentle nitrogen stream, re-dissolved in 1 mL of methanol, filtered through 0.22-μm membrane filters into 2-mL amber glass vials and stored at -20 °C until instrumental analysis.

To identify biotransformation products of HHCB, the extracts were derivatized following the procedure described by Martin et al. (2007). The details of derivatization are given in the supporting information.

2.6. Instrumental analysis

TCS in the extracts was quantified using an Agilent 1200 high performance liquid chromatograph (Agilent, USA) coupled to an Agilent 6460 triple quadrupole mass spectrometer with electrospray ionization under negative ionization modes (HPLC-MS/MS, ESI-). Me-TCS, HHCB and HHCB-lactone in the extracts were determined by an Agilent 6890N gas chromatograph (Agilent, USA) connected to an Agilent 5975B MSD mass spectrometer (GC-MS), equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific Co., USA), in the selected-ion-monitoring (SIM) mode under electron-impact ionization (EI). Qualification of TCS biotransformation products in worm tissue and sediment was performed using Waters ACQUITY UPLC-I Class with Xevo G2-XS QTOF, whereas Agilent 7250 GC/Q-TOF was used to analyse biotransformation products of HHCB. The detailed procedures used for the quantitative and qualitative analysis are provided in the supporting information.

2.7. Quality assurance, quality control, and data analysis

Solvent blanks and procedural blanks were determined successively for each batch of samples to check background contamination and ensure the performance of the analytical procedure. The method quantification limits (MQLs) were defined as 10 times the ratio of the signal to instrument noise (Table S1). The recoveries of TCS, Me-TCS, HHCB and HHCB-lactone in each compartment were separately assessed by spiking a standard solution at three levels (0.1, 0.5, and 2) in clean Milli-Q water (μg/L), sediment (μg/g dw), sediment particles on the filters (μg/g dw) and worm tissue (μg/g ww), respectively. All recoveries were in the range of 60% to 110% (Table S2). Concentration data below MQLs were treated as not detected (ND). TCS, Me-TCS, HHCB and HHCB-lactone were below the MQLs in the clean Milli-Q water and unexposed worm tissue and in the controls at the end of experiment.

The dissipation kinetics of TCS and HHCB in the water/sediment systems were described using a first-order kinetic model: $C_{(t)} = C_{(t=0)} \times \exp^{(-kt)}$, where $C_{(t)}$ (μg/g dw) is the TCS or HHCB concentration in the sediment at sampling time t (days) and k is the elimination rate constant. The half-life $t_{1/2}$ was calculated as: $t_{1/2} = \ln(2) / k$.

The biota-sediment-accumulation-factor (BSAF) was calculated at each sampling point using the following equation (Ankley et al., 1992): $BSAF = (C_o / f_l) / (C_s / f_{OM})$, where C_o is the chemical concentration in the organism (µg/g wet weight (ww)) at each sampling point, f_l is the lipid fraction of the organism (g lipid/g ww), C_s is the chemical concentration in the sediment (µg/g dw) at the corresponding sampling point, and f_{OM} is the organic matter fraction of the sediment (g organic matter/g dw).

Statistical analyses were performed with the software SPSS Statistics (Ver 23.0.0). Two-way ANOVA (factors: presence of *L. hoffmeisteri* and sampling time) with Tukey's multiple comparison tests was used to determine the statistical differences in the chemicals concentrations between systems with and without worms or among sampling dates. Data were checked for normality and variance homogeneity with Shapiro-Wilk test and Levene's test, respectively. Statistical significance was accepted at $p < 0.05$ level.

3. Results

3.1. Concentrations and dissipation kinetics of TCS and HHCB in the microcosms

The concentrations of TCS and HHCB in the sediment were measured on days 0, 1, 3, 7, 10 and 14 and are shown in Table S3 and Figure 1. TCS and HHCB concentrations in the blank samples remained at 2 µg/L during the 14 d incubation period. At the start of exposure, TCS and HHCB concentrations in the overlying water were 0.59 µg/L and 0.48 µg/L, respectively. Over the course of experiment, TCS and HHCB gradually disappeared from the microcosms, with faster dissipation found for TCS relative to HHCB (Table S3 and Figure 1). After 14 d exposure, the TCS concentrations decreased from 3.1 µg/g dw to 2.8 µg/g dw (9.03%) and 3.0 µg/g dw (3.23%) in systems with and without worms, respectively (Table S3). The HHCB concentrations declined slightly from 3.10 µg/g dw to 3.02 µg/g dw (2.90%) and 3.08 µg/g dw (0.65%) in systems with and without worms, respectively (Table S3). Furthermore, there was a significant difference in TCS and HHCB concentrations between systems with and without worms (two-way ANOVA, $p < 0.05$). Compared to the original spiked concentration, TCS and HHCB concentrations significantly decreased from day 3 and 10 onwards, respectively (two-way ANOVA, $p < 0.05$).

In both systems with and without worms, dissipation kinetics of TCS and HHCB in the sediment followed the first-order reaction kinetic model (Table S4 and Figure 1). The estimated half-lives ($t_{1/2}$) for TCS were 103 d and 301 d, and for HHCB were 433 d and 1386 d in systems with and without worms, respectively.

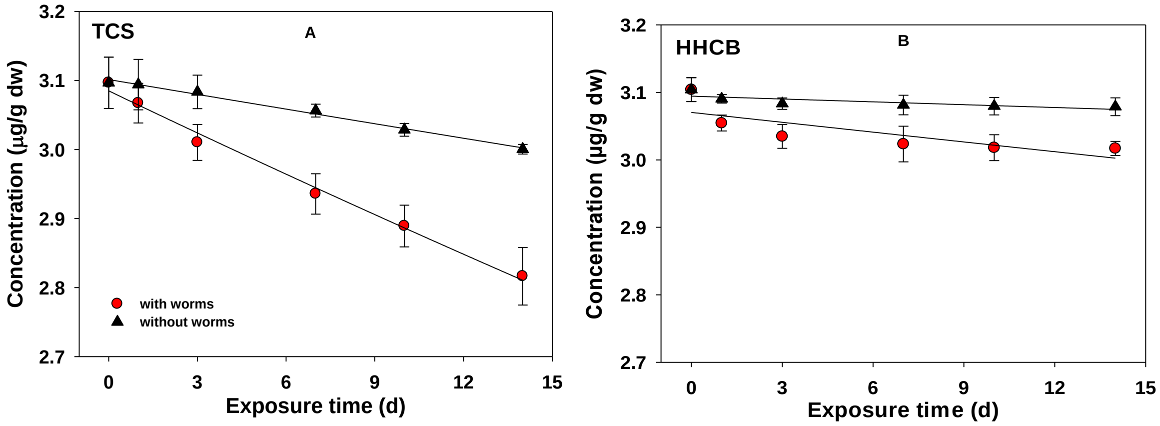

Figure 1. Time courses of TCS (A) and HHCB (B) concentrations in the sediment from microcosms with and without *Limnodrilus hoffmeisteri*. Red circle symbols and black up triangle symbols represent averages of chemical concentrations in systems with and without worms, respectively.

3.2. Identification of biotransformation products in the sediment

The concentrations of Me-TCS increased in both systems during the exposure period, with significantly higher concentrations in systems with compared to without worm presence (two-way ANOVA, $p < 0.05$) (Table S3 and Figure 2). HHCB-lactone concentration remained at similar levels throughout the exposure period in both systems, with values around 0.34 µg/g dw (two-way ANOVA, $p > 0.05$) (Figure 2). However, HHCB stock solution was found to contain about 10% HHCB-lactone and resulted in a 0.34 µg/g dw spiked HHCB-lactone concentration in the sediment. Me-TCS concentrations significantly increased from day 7 onwards (two-way ANOVA, $p < 0.05$), whereas there was no significant difference in HHCB-lactone between sampling dates (two-way ANOVA, $p > 0.05$). No other products were found for TCS or HHCB in the sediment by UPLC- QTOF and GC-QTOF, respectively.

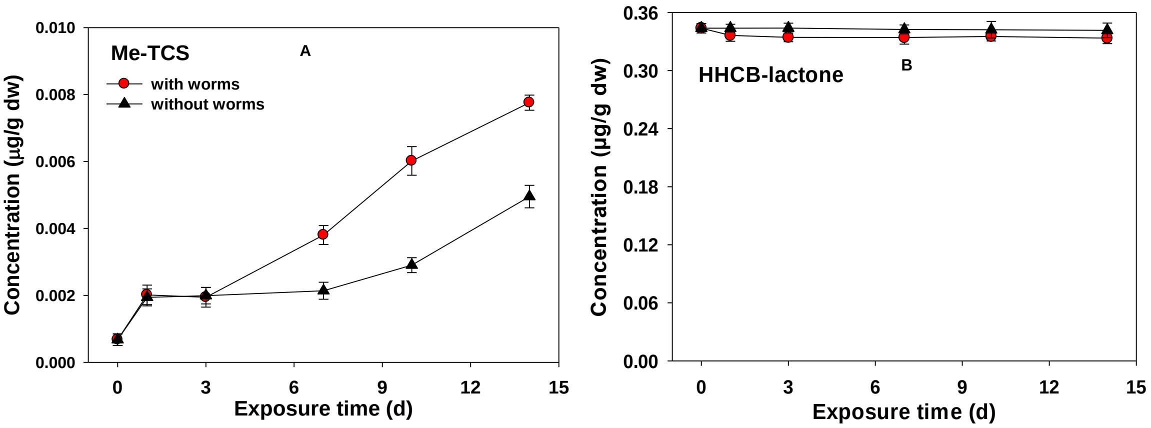

Figure 2. Time courses of Me-TCS (A) and HHCB-lactone (B) concentrations in the sediment from microcosms with and without *Limnodrilus hoffmeisteri*. Red circle symbols and black up triangle symbols represent averages of chemicals concentration in systems with and without worms, respectively.

3.3. Bioaccumulation and biotransformation products of TCS and HHCB in the worm tissue

The lipid content of *L. hoffmeisteri* was 2.26% ww. During the 14 d exposure period, there was no mortality of *L. hoffmeisteri* in any treatments, suggesting that neither HHCB- or TCS-associated sediment was toxic at the concentrations used. The pH was around 6.6 in the overlying water at the start and end of exposure. TCS and HHCB concentrations showed similar change trends in the worm tissue, i.e., increasing from day 0 to day 3 and remaining stable from day 7 onwards (Figure 3). After 1 d exposure, the TCS and HHCB concentrations were 2.4 µg/g ww and 6.5 µg/g ww, respectively. After 3 d exposure, the TCS and HHCB concentrations reached 6.5 and 8.4 µg/g ww, respectively (Table S3 and Figure 3). The BSAF values of TCS and HHCB were in the range of 0.70 to 2.07 and 1.84 to 2.50 during the exposure period, respectively (Table S3).

As was observed in the sediment, Me-TCS and HHCB-lactone were also detected in the worm tissue, with concentrations in the range of 0.06-0.15 µg/g ww and 0.70-0.81 µg/g ww, respectively (Table S3 and Figure 3). Me-TCS and HHCB-lactone concentrations reached the steady state in *L. hoffmeisteri* on day 7 and day 10, respectively.

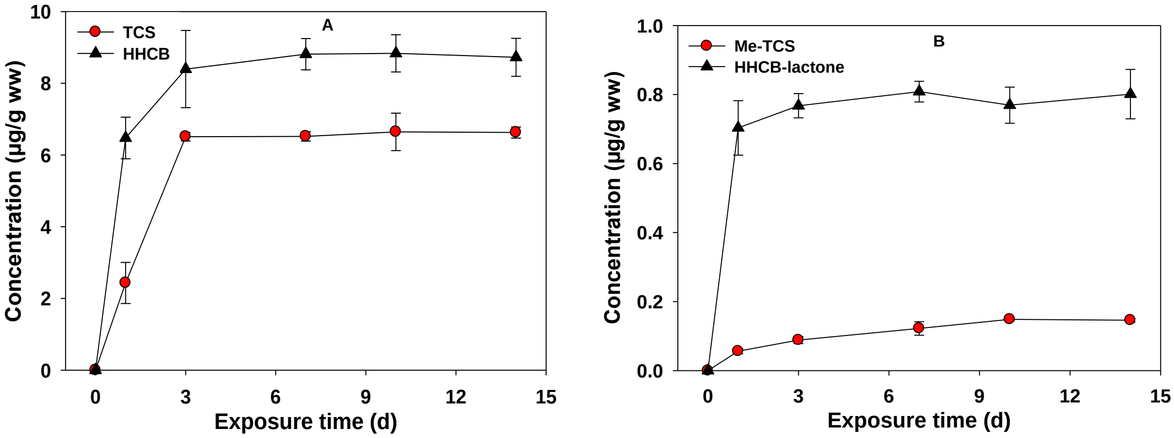

Figure 3. Time courses of TCS, HHCB (A), Me-TCS and HHCB-lactone (B) concentrations (µg/g ww) in the tissue of *Limnodrilus hoffmeisteri*.

In addition to Me-TCS, triclosan-O-sulfate (TCS-O-sulfate) was detected by LC-Q-TOF (Figure 4 and Figure S1). The identification of TCS-O-sulfate was further confirmed by its authentic standard. However, no biotransformation product was identified for HHCB in worm tissue by GC-QTOF, except for HHCB-lactone.

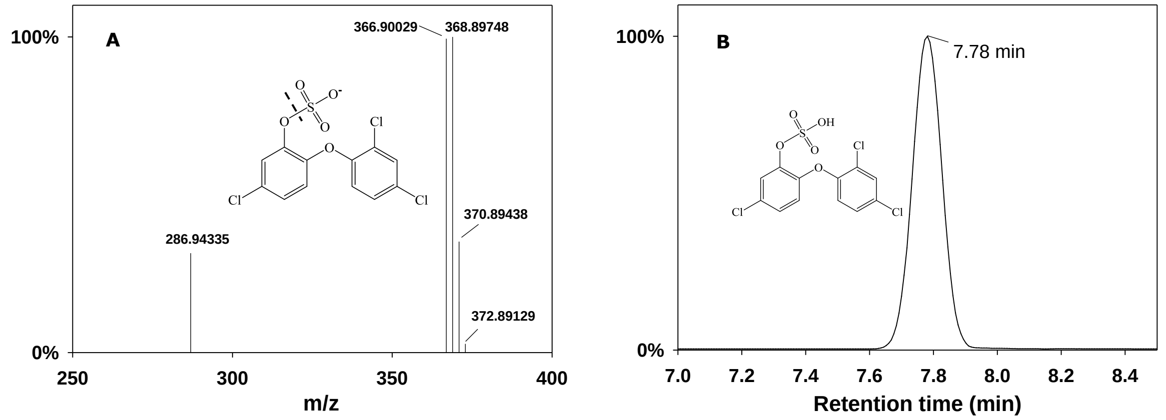

Figure 4. UPLC-Q-TOF product ion spectra and chromatogram of sulfonated metabolite of TCS in worm tissue. (A) Product ion spectra of the m/z 368.89748 peak (7.78 min), the product was identified as TCS-O-sulfate. (B) Extracted ion chromatogram of TCS-O-sulfate in the worm tissue.

4. Discussion

This study showed that the TCS and HHCB dissipation in the microcosms followed the first-order exponential decay model. The same model has been described for TCS (Chen et al., 2011; Armstrong et al., 2018) and HHCB (Martin et al., 2007) dissipation by wastewater microorganisms and fungi, respectively. TCS dissipated slowly in systems without worm presence with a $t_{1/2}$ value of 301 d which is larger than the $t_{1/2}$ value of 58 d detected in the pond water-silty clay loam sediment system under aerobic conditions (EU, 2012) and 239 d in the lake water-silty clay sediment system with dissolved oxygen levels above 3 mg/L (Huang et al., 2015). These differences is likely related to different microbial communities and sediment properties including organic matter and clay content between studies (Marshall, 1975; Mashtare et al., 2013). In addition, although the oxygen was supplied in the overlying water during the incubation in this study, the sediment in systems without worms was likely under reducing condition due to the lack of bioturbation and microbial respiration (Boros et al., 2011), which might hamper the dissipation of TCS due to the faster dissipation of TCS under aerobic than anaerobic conditions (Ying et al., 2007; Bedoux et al., 2012). The estimated $t_{1/2}$ for sediment-associated HHCB were > 400 d in both systems, suggesting that HHCB was persistent in the water/sediment system under the conditions in the present study. However, in the EU Risk Assessment Report (EU RAR) for HHCB, $t_{1/2}$ of 79 d in the sediment was deemed most relevant for modelling the fate of HHCB in sediment using the European Union System for the Evaluation of Substances (EUSES) model (EC, 2008). These differences could be attributed to differences in sediment properties, microbial communities and exposure scenarios. The estimated $t_{1/2}$ values of HHCB were $\sim$3.2 and $\sim$3.6 times longer than those of TCS in systems with and without the presence of worms, respectively, indicating that HHCB is more persistent than TCS in the water-sediment system. Likewise, a longer $t_{1/2}$ value of HHCB (900 d) relative to TCS (258 d) has been reported in biosolid-amended soils in field (Chen et al., 2014b). However, a faster dissipation of HHCB in biosolid-amended soils has been described by DiFrancesco et al. (2004). The corresponding $t_{1/2}$ values were 141 and 144 d for the spiked and unspiked biosolids-amended soils, respectively (EC, 2008). The dissipation of TCS and HHCB were faster in systems with than without worms, suggesting that *L. hoffmeisteri* stimulated the dissipation of the two hydrophobic compounds in the water/sediment systems in this study. This is likely to be associated with the bioaccumulation and biotransformation in *L. hoffmeisteri*, and the enhanced microbial degradation due to the sediment reworking by worms (Madsen et al., 1997; Kristensen and Holmer, 2001).

TCS, Me-TCS, HHCB and HHCB-lactone were detected in the worm tissue, with concentrations increasing from exposure day 1 to 7 and reaching the steady state from then onwards (Table S3), which indicates that *L. hoffmeisteri* can accumulate these hydrophobic compounds. However, it should be noted that the initial HHCB-lactone concentration was 0.34 µg/g dw in the sediment, which is likely responsible for the bioaccumulation observed here. Similar time to reach steady state has previously been observed for sediment-associated polybrominated diphenyl ether (PBDE) accumulation in the oligochaete *Lumbriculus variegatus* (a similar species to *L. hoffmeisteri* to) (Leppänen and Kukkonen, 2004; Ciparis and Hale, 2005). The stabilized BSAF values of TCS (~2.07) in *L. hoffmeisteri* were larger than the 28-day BSAF value (1.4) reported by Dang et al. (2016), who studied the bioaccumulation of TCS in *L. variegatus*. However, another study has reported a greater BSAF (9.04) of TCS in *L. variegatus* than the present study (Karlsson et al., 2016). These differences are most likely related to the differences in sediment characteristics and species traits between the studies (Diepens et al., 2015; Karlsson et al., 2016). The stabilized BSAFs of HHCB were around 2.50 in *L. hoffmeisteri*, similar to the values (1.5-2.5) reported in carps from the Haihe River (China) (Hu et al., 2011). The higher BSAF values of HHCB relative to TCS in *L. hoffmeisteri* is likely to be associated with the higher log K_{ow} value of HHCB (5.9) than TCS (4.8) and biotransformation of TCS in the worm tissue. In fact, a previous study reported that BSAF values of PBDE in *Tubifex tubifex* (a freshwater Naididae) declined with the decrease of log K_{ow} value (Tian et al., 2012).

Me-TCS was detected in both the sediment and worm tissue whereas TCS-O-sulfate was only detected in the worm tissue. These two metabolites were products from phase II reaction, i.e., methylation and sulfation. However, no formation of products from phase I transformation (e.g., oxidation, reduction and hydrolysis reactions) were observed in this study. This may be related to the fast transformation of phase I to phase II products, as described by Malmquist et al. (2013) who investigated the biotransformation of pyrene by the benthic invertebrate *Nereis diversicolor*. Also, analyses of the overlying water would have provided more information on the fate of phase I products. In the future work, we therefore recommend to analyse metabolites in the overlying water. Yet, the formation of Me-TCS via biological methylation has been reported for different stages of wastewater treatment plants (Lozano et al., 2013; Chen et al., 2011). Besides, Macherius et al. (2014) found that TCS was transformed into Me-TCS by earthworms in biosolid-amended agricultural field. However, compared to TCS, Me-TCS is more persistent and also more prone to bio-accumulate in aquatic organisms (Pycke et al., 2014). The formation of TCS-O-sulfate has been reported in activated sludge (Chen et al., 2015), plants (Macherius et al., 2012), rats (Wu et al., 2010) and human urine (Ranganathan et al., 2015).

Unlike TCS, no product was identified for HHCB, except for HHCB-lactone. In previous studies, HHCB-lactone has been described as a HHCB degradation metabolite in activated sludge (Balk and Ford, 1999a) and cultures of fungi such as *Myrioconium* sp (Martin et al., 2007) and *Phanerochaete chrysosporium* (Balk and Ford, 1999a). However, we found that HHCB-lactone also existed in the HHCB stock solution, resulting in a concentration of 0.34 µg/g dw in the spiked sediment. In line with this, Best, (2005) reported that HHCB-lactone was contained as a by-product in technical HHCB formulations accounting for up to 10%. After 14 d exposure, the final HHCB-lactone concentration in the sediment was slightly lower in system with (~0.33 µg/g dw) than without (~0.34 µg/g dw) worms (Table S3). However, the results of mass balance show that HHCB-lactone accumulation in worms was responsible for the loss of HHCB-lactone in systems with worm presence. This suggests that HHCB-lactone was not produced from HHCB in sediment (i.e., microbial degradation) or worms as it was stable in the systems under the present conditions. Similarly, the total HHCB concentration was also stable during the exposure period in both systems. These results suggest that *L. hoffmeisteri* and microorganisms in the sediment do not degrade HHCB or HHCB-lactone to a measurable degree. Unlike HHCB, Dai et al. (2012) found that sediment-associated acetyl cedrene (another fragrance material) was reduced by 88-99% and 13-31% in the sediment with and without *C. teleta*, respectively, after 14 days of exposure. However, another study reported that acetyl cedrene in the sediment decreased 72% in both treatments with and without *C. teleta* after 16 days (Ellegaard-Petersen et al., 2010). One explanation for these findings is that the microbial activity was very low initially in the present study due to the freezing of the sediment, which would potentially decrease microbial degradation compared to a full-active microbial community in previous studies. In addition, it seems that macrofaunal biotransformation is both species- and chemical specific (e.g., Malmquist et al., 2013).

In conclusion, the findings from the present study show that oligochaete worm presence significantly accelerated HHCB and TCS dissipation in water-sediment systems. *L. hoffmeisteri* either cannot or have a very low ability to biodegrade HHCB but can biotransform TCS through methylation and sulfonation. However, currently little information is available for TCS-O-sulfate. Further work is therefore needed to evaluate the (eco)toxicity and persistence of TCS-O-sulfate.

Acknowledgments

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Supporting information

Materials and Methods

Standards and Reagents

All solvents used for chemical analysis, including methanol, ethyl acetate, n-hexane, acetone, dichloromethane, and acetonitrile were of high-performance liquid chromatography (HPLC) grade and purchased from CNW Technologies (Shanghai, China) or Merck (Germany). Acetic acid and ammonium acetic were bought from Sigma-aldrich (St.Louis, USA), while formic acid was obtained from Tedia (USA). Oasis HLB cartridges (60 mg, 3 mL) and Oasis HLB cartridges (200 mg, 6 mL) were supplied by Waters Corporation (Milford, MA, USA). Glass fiber filters (GF/F, pore size 0.7 µm) were obtained from Whatman (Maidstone, UK). Ceramic homogenizer, Z-Sep tube, anhydrous magnesium sulfate ($MgSO_4$), primary-secondary amine (PSA) and C_{18} bulk sorbent were purchased from Agilent (Santa Clara, USA). Ultrapure water was provided by a Milli-Q system from Millipore (Watford, UK). TCS and HHCB were dissolved in acetone to make a stock solution of 2 g/L. The remaining stock solutions with concentration of 100 mg/L in methanol were individually prepared, and stored at -18 ˚C until use.

Solid phase extraction

Oasis HLB SPE cartridges were preconditioned with 10 mL of methanol and 10 mL of Milli-Q water. Samples were passed through cartridges at a flow rate of 5-10 mL/min. Each sample bottle was rinsed twice with 50 mL of Milli-Q water containing 5 % methanol (v/v) and passed through the SPE cartridge. The cartridges were then dried under vacuum for 3 h. The cartridges were eluted with 3×3 mL of methanol followed by 3×3 mL of ethyl acetate, 3×3 mL of dichloromethane and 3×3 n-hexane. The eluates were dried under a gentle nitrogen gas, re-dissolved in 1 mL of methanol, transferred to a 2 mL amber glass vial with filtering through a 0.22 µm nylon membrane filter, and finally stored at -18 ˚C until analysis.

Derivatization of HHCB and its biotransformation products

The derivatization method for HHCB and its biotransformation products reported by Martin et al.[1] was used in this study. Specifically, 100 µL of an extract in methanol was transferred to a 2 mL amber glass vial with polytetrafluoroethylene (PTFE) screw cap and dried under a gentle nitrogen stream, added with 100 µL of Sylon BTZ and derivatized at 60 ˚C for 1 hour. Then, 200 µL of Milli-Q water at pH 3.0 was added to remove the excess derivatization reagent. The derivatization products were extracted with 500 µL of n-hexane, dried over anhydrous sodium sulfate, concentrated to a final volume of 100 µL, and analysed by GC-Q-TOF.

Instrumental analysis

LC-MS/MS for TCS quantification: TCS was analysed by an Agilent 1200 rapid resolution liquid chromatograph coupled to Agilent G6460A triple quadrupole mass spectrometer under electrospray negative ionization (ESI) mode[2]. A 10 µL aliquot of each extract was injected into an Agilent SB-C18 column (3.0 mm × 100 mm ID, 1.8 µm particle size) at temperature of 40 ˚C with an RRLC in-line pre-column filter (4.6 mm, 0.2 µm filter), with Milli-Q water containing 0.01% acetic acid (v/v) (solvent A) and acetonitrile : methanol (1:1, v/v) (solvent B) as the mobile phase at a flow rate of 0.3 mL/min. The gradient program was given as follows: 60% B at 0 min, then increased to 90% B at 3 min and kept at 90% B for 4 min, then returned to the initial 60% B at 9 min and let column re-equilibrate for 6 min. The capillary was maintained at 3500 V. Dry and sheath gas flows were kept at 8 and 12 mL/min, respectively. Both dry and sheath temperatures were kept at 350 ˚C.

GC-MS for Me-TCS quantification: Me-TCS was analysed using an Agilent 6890N GC interfaced to a 5975B MSD (GC-MS), equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific Co., USA), under electron-impact ionization (EI) mode. Helium (purity > 99.999%) was used as the carrier gas at a constant flowrate of 1.0 mL/min. Splitless mode was applied for injection, with injection volume of 1 µL for each samples. The temperatures for the GC-MS interface, ion source, quadrupole and injector were kept at 300 ˚C, 230 ˚C, 150 ˚C and 250 ˚C, respectively. The column temperature was programmed as follows: from 100 ˚C (2 min) to 180 ˚C at 5 ˚C/min (2 min), from 180 ˚C to 300 ˚C at 10 ˚C/min (2 min), and then to the temperature 310 ˚C at 10 ˚C/min (10 min). The characteristic ions were 314, 264 and 243.9 for $^{13}C_{12}$-Me-TCS, 301.9, 251.9 and 232 for Me-TCS.

ACQUITY UPLC-I Class with Xevo G2-XS QTOF: the qualification of TCS biotransformation products were analysed by an Waters ACQUITY UPLC-I Class with Xevo G2-XS QTOF under a negative ion mode. For chromatographic conditions, a 2 µL aliquot of each extract was injected into a HSS T3 column (2.1 × 100 mm ID, 1.8 µm particle size) at temperature of 40 ˚C, with Milli-Q water containing 10 mM ammonium acetate (solvent A) and methanol (solvent B) as the mobile phase at a flow rate of 0.4 mL/min. The gradient program was given as follows: kept 2% B from 0 to 0.25 min, increased to 98% B at 12 min and kept at 98% B for 3 min, then returned to the initial 2% B at 18 min and let column re-equilibrate for 6 min. For mass spectrometry conditions, the capillary and cone voltage were maintained at 2500 V and 20 V, respectively. The cone gas flow was kept at 50 L/h, and the source temperature was 120 ˚C. The desolvation temperature and desolvation gas flow were set as 500 ˚C and 800 L/h. The samples were scanned using MSE scan mode at a range of 50-1000 m/z with scan time of 0.2 s. Leucine-enkephalin was used as reference for mass correction. The data were processed using the UNIFI Scientific Information System to identify the putative compounds present in the extracts.

GC-MS for HHCB and HHCB-lactone quantification: HHCB and HHCB-lactone in the extracts were measured using an Agilent 6890 N GC interfaced to a 5975B MSD (GC-MS), equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific Co., USA), in selected ion monitoring (SIM) mode under electron-impact ionization

(EI). Helium (purity > 99.999%) was used as carrier gas at a constant flow of 1.0 mL/min. Splitless mode was used for injection, with injection volume of 2 μL for each samples. The temperatures for the GC-MS interface, ion source, quadrupole and injector were 280 ˚C, 250 ˚C, 150 ˚C and 280 ˚C, respectively. The GC oven temperature was programmed as follows: 80 ˚C for 0 min, increased to 170 ˚C at 15 ˚C/min, from 170 ˚C to 185 ˚C at 1 ˚C /min, then to 300 ˚C at a rate of 20 ˚C /min for 5 min.

GC/Q-TOF for HHCB biotransformation qualification: HHCB biotransformation products qualification was analysed using an Agilent 7890B GC interfaced to a 7250 QTOF, equipped with a HP-5MS UI column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific Co., USA) under electron-impact ionization (EI). Helium (purity > 99.999%) was used as carrier gas at a constant flow of 1.0 mL/min. Splitless mode was applied for injection, with injection volume of 2 μL for each samples. The temperatures for the GC/Q-TOF interface, ion source, quadrupole and injector were 300 ˚C, 200 ˚C, 150 ˚C and 280 ˚C respectively. The GC oven temperature was programmed as follows: 80 ˚C for 0 min, increased to 170 ˚C at 15 ˚C/min, from 170 ˚C to 185 ˚C at 1 ˚C /min, then to 300 ˚C at a rate of 20 ˚C /min for 5 min. The samples were scanned using full scan TOF mode at a range of 50-550 m/z with scan time of 0.20 s.

Table S1. Method quantification limits of target compounds in surface water, sediment, particles and worms by HPLC-MS/MS or GC-MS.

Compound	Surface water (ng/L)	Sediment (ng/g)	Particles (ng/g)	Worm (ng/g)
Triclosan	0.08	0.10	0.14	0.16
Methyl triclosan	0.94	1.17	1.28	1.55
Galaxolide	1.01	1.36	1.41	1.78
Galaxolidone	1.40	1.72	2.05	2.36

Table S2. Recoveries of target compounds in surface water (μg/L), sediment (μg/g dw), particles (μg/g dw) and worm (μg/g ww).

Compounds	Spiked concentrations in water			Spiked concentrations in sediment			Spiked concentrations in particles			Spiked concentrations in worm		
	0.1	0.5	2	0.1	0.5	2	0.1	0.5	2	0.1	0.5	2
Triclosan	105 ± 5.31	98.1 ± 4.84	94.1 ± 4.66	102 ± 4.15	95.3 ± 5.16	91.9 ± 2.28	102 ± 4.17	97.5 ± 4.82	92.5 ± 3.94	102 ± 5.17	105 ± 4.36	95.7 ± 3.09
Methyl triclosan	97.0 ± 4.19	93.9 ± 4.07	85.9 ± 5.65	101 ± 5.14	90.6± 5.38	82.5 ± 3.16	103 ± 5.43	89.4 ± 5.39	80.7 ± 4.62	105± 4.31	100 ± 4.75	97.9 ± 4.82
Galaxolide	90.4 ± 4.97	82.4 ± 4.11	75.8 ± 3.96	79.6 ± 4.36	70.6 ± 4.47	62.7 ± 2.43	81.3 ± 4.37	74.2 ± 4.19	64.9 ± 4.42	99.3 ± 4.97	103 ± 3.76	106 ± 4.24
Galaxolidone	92.3 ± 5.12	85.7 ± 4.65	77.6 ± 3.77	82.3 ± 4.18	75.4 ± 3.53	68.1 ± 3.16	83.0 ± 5.13	77.9 ± 5.77	70.5 ± 3.46	105 ± 4.83	98.5 ± 3.77	94.7 ± 4.98

Three replicates were used to determine recovery.

Table S3. TCS, Me-TCS, HHCB and HHCB-lactone concentrations in sediment (μg/g dw) and worm tissue (μg/g ww) during the exposure period.

Exposure time (d)	Sediment								Worm				BSAFs	
	TCS		Me-TCS		HHCB		HHCB-lactone		TCS	Me-TCS	HHCB	HHCB-lactone	TCS	HHCB
	with worms	without worms	with worms	without worms	with worms	without worms	with worms	without worms						
0	3.10±0.04	3.10±0.04	< MQL	< MQL	3.10±0.02	3.10±0.02	0.34±0.01	0.34±0.01	< MQL	< MQL	< MQL	< MQL	-	-
1	3.07±0.03	3.09±0.04	0.0020±0.0003	0.0019±0.0003	3.05±0.01	3.09±0.01	0.34±0.01	0.34±0.01	2.43±0.57	0.06±0.01	6.47±0.58	0.70±0.08	0.70±0.17	1.84±0.16
3	3.01±0.03	3.08±0.02	0.0019±0.0003	0.0020±0.0002	3.03±0.02	3.08±0.01	0.33±0.01	0.34±0.01	6.51±0.12	0.09±0.01	8.40±1.08	0.76±0.04	1.90±0.04	2.38±0.31
7	2.94±0.03	3.06±0.01	0.0038±0.0003	0.0021±0.0003	3.02±0.03	3.08±0.01	0.33±0.01	0.34±0.01	6.52±0.13	0.12±0.02	8.81±0.44	0.81±0.03	1.95±0.04	2.50±0.12
10	2.89±0.03	3.03±0.01	0.006±0.0004	0.0029±0.0002	3.02±0.02	3.08±0.01	0.33±0.01	0.34±0.01	6.65±0.52	0.15±0.02	8.84±0.52	0.77±0.05	2.02±0.16	2.50±0.15
14	2.82±0.04	3.00±0.01	0.0078±0.0002	0.0050±0.0003	3.02±0.01	3.08±0.01	0.33±0.01	0.34±0.01	6.63±0.15	0.15±0.01	8.72±0.53	0.80±0.07	2.07±0.04	2.47±0.15

MQL means method limit of quantitation.

with and without worms represent microcosms with and without worm, respectively.

BSAFs: biota-sediment accumulation factor.

Table S4. Estimates of kinetic parameters for TCS and HHCB elimination.

Compound	R^2		k		*p*	
	group A	group B	group A	group B	group A	group B
TCS	0.992	0.991	0.0067	0.0023	< 0.0001	< 0.0001
HHCB	0.629	0.627	0.0016	0.0005	0.06	0.06

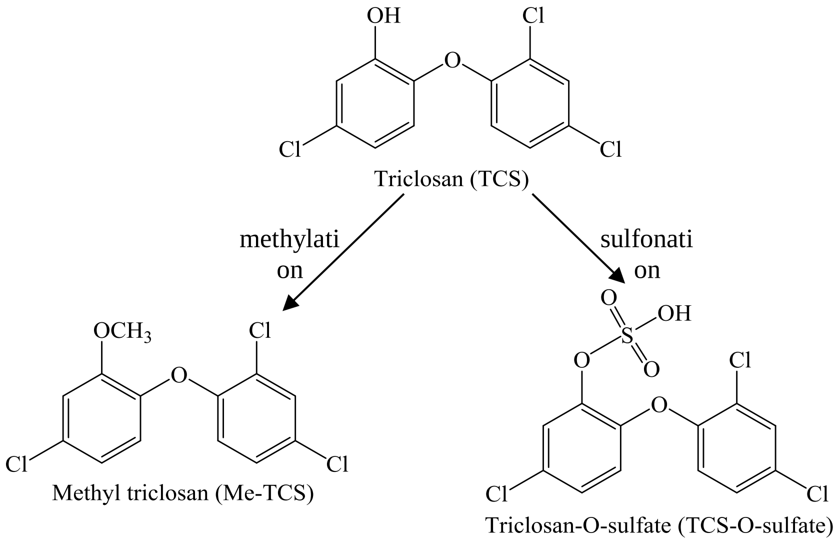

Figure S1. Predicted biotransformation pathways of TCS in *Limnodrilus hoffmeisteri*.

Chapter 8

General discussion and conclusions

China has been among the top three countries with the largest PCPs consumption (ChinaIRN, 2012), which may result in serious PCPs contamination in aquatic environments (Liu and Wong, 2013). As down-the-drain chemicals, most PCPs used in households enter WWTPs that have been well constructed in urban areas like Guangzhou (China). However, a small proportion of the produced domestic sewage with its associated PCPs is still directly released into urban rivers of Guangzhou. Also, the incomplete removal for some organic contaminants such as PCPs in WWTPs can result in residuals of these compounds entering surface waters.

Sediments are sinks for organic contaminants and serve as potential sources for release back into the surface water (Burton, 2002). Sediment-dwellers, ubiquitous and abundant in the freshwater sediment, are exposed to hydrophobic organic contaminants through feeding on pore water, overlying water and sediment particles (Selck et al., 2012; Diepens et al., 2014), and may bioaccumulate these chemicals because of their lipophilic property (Karlsson et al., 2016). They may thus suffer from stress posed by sediment-associated PCPs, affect the persistence of hydrophobic PCPs in the sediment via promoting microbial degradation through reworking of the sediment, bioaccumulation and biodegradation, and disrupt the potential equilibrium established between sediment pore-water and overlying water in the aquatic system.

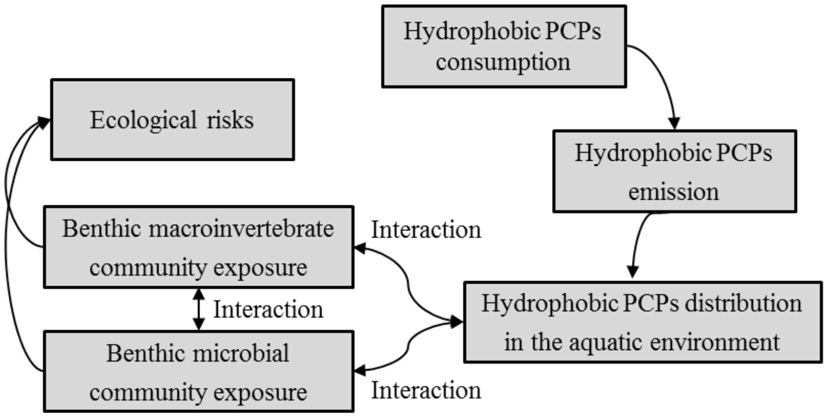

Figure 1. Ecological risks to benthic biological communities arising from the consumption of hydrophobic PCPs in relation to the causality chain of ‘consumption → emission → distribution in the environment → exposure → effect’.

Given the lack of a broad accepted framework for prospective environmental risk assessment of sediment-bound organic chemicals, recently a tiered approach has been proposed (Diepens et al., 2017). However, the sediment risk assessment of hydrophobic PCPs is a rather unexplored field. The general aim of this thesis thus was to increase our understanding on the potential ecological risks posed by sediment-associated PCPs on benthic communities in subtropical regions using Guangzhou as a case study. Research carried out in this thesis followed the causality chain described in Figure 1. This section discusses the results of this thesis in light of the research objectives proposed in **Chapter 1**.

1. Correlations between the benthic community distribution and exposure to hydrophobic PCPs and conventional contaminants in urban rivers

Guangzhou (South China) is a highly urbanized megacity and has a humid subtropical climate with a population of 13.1 million (http://www.guangzhou.gov.cn/), resulting in huge consumption of PCPs. Therefore, Guangzhou city was chosen as a case study for this thesis to examine the presence, fate and ecological risks of hydrophobic PCPs and conventional contamination and their correlations with biological communities distribution in the subtropical region of China. This section provides an overview of the chemical exposure patterns observed in the sediment, distribution patters of benthic macroinvertebrate and microbial communities in urban rivers, and their correlations.

Due to the treated and untreated industrial and domestic sewage and waste discharges, hydrophobic PCPs reached much higher levels in urban rivers in the city centre of Guangzhou compared to the suburban river and Pearl River, and even higher than previously reported data worldwide (**Chapter 2**). Such trend was also observed for nutrients (i.e., total organic carbon (TOC), total phosphorous (TP), total nitrogen (TN), and ammonia-nitrogen (NH_3-N)) (**Chapter 2**). Likewise, sediment from the sampling sites in the Zhujiang River and Shijing River showed higher concentrations of heavy metals compared to the remaining sites (**Chapter 3**). Moreover, several sampling sites showed heavy metals contamination exceeding the toxic effect threshold (TET) values suggested by the sediment quality guidelines for freshwater ecosystems (MacDonald et al., 2000) (**Chapter 3**).

From the upstream part of the Liuxi River (minimally influenced) to the downstream part of the Liuxi River, Pearl River and the central urban rivers (contaminated), the prevalent benthic macroinvertebrates shifted from gastropoda and bivalvia to the contamination-tolerant annelids *L. hoffmeisteri* and *B. sowerbyi* (**Chapter 3**). In accordance with this, results of laboratory experiments showed that *L. hoffmeisteri* was tolerant to sediment-associated TCS (NOEC = 8 µg/g dw, **Chapter 4**) and HHCB (NOEC = 100 µg/g dw, **Chapter 6**), and *B. sowerbyi* was also tolerant to sediment-associated HHCB with a NOEC value of 30 µg/g dw (**Chapter 6**). However, *Orthocladiinae* was the predominant benthic macroinvertebrate at the site in the Sha River in both seasons (**Chapter 3**). In addition, absence of benthic macroinvertebrate was observed at sites in the Shijing River, Liede River and Chebei River in the wet season (**Chapter 3**). This is likely related to the elevated bioavailability of contaminants caused by sediment resuspension (resulted from elevated flows in the wet season relative to the dry season) and hypoxic conditions caused by organic contamination in these rivers during the wet season. Therefore, measures (e.g., improve civil awareness, build new WWTPs, improve removal efficiency of contaminants in WWTPs and enhance law enforcement) should be taken to improve the water and sediment quality in these central urban rivers.

There was a significant correlation between the environmental variables (i.e., Cr, TP, HHCB and TCS) and benthic macroinvertebrate community composition (**Chapter 3**). Likewise, there was a significant correlation between environmental variables (i.e., Cr and TP) and traits composition (i.e., Reproduction: asexual reproduction) (**Chapter 3**). Therefore, species changing along the environmental gradients also caused changes in the ecological functioning of the species assemblages, which have previously been reported by other authors (Oug et al., 2012; Rico et al., 2016).

Same as the benthic macroinvertebrates community, the sediment bacterial community composition shifted along the rural-urban gradient (**Chapter 3**). Also, significant correlations were observed between the environmental variables (e.g., HHCB, TCS, Cu, TOC and NH_3-N) and sediment bacterial phyla that were significantly different among rivers (**Chapter 3**). This has also been demonstrated in the microcosm experiment conducted with TCS (**Chapter 5**). For example, TCS at concentrations ≥ 80 μg/g dw significantly altered the taxonomic composition and decreased richness and diversity of sediment bacterial communities in the absence of benthic macroinvertebrates while at concentrations ≥ 8 μg/g dw affected the bacterial community in the presence of macroinvertebrates (**Chapter 5**). However, there was no HHCB-associated effect on microbial community structure, richness, diversity and composition in the microcosm experiment at concentration of 300 µg/g dw (**Chapter 6**). Such different patterns between field and laboratory data could be explained by the properties of HHCB and the more complicated conditions in the field environment relative to the microcosm experiment. Among all sampling sites, several sewer infrastructure-associated bacterial genera (e.g., *Acinetobacter*, *Arcobacter* and *Trichococcus*) showed higher relative abundances at the site in the Sha River where chironomids were abundant (**Chapter 3**). Thus, it seems that there were interactions among contaminants, benthic macroinvertebrate and sediment microbial communities in the Sha River. Indeed, in the microcosm experiments conducted with TCS and HHCB, the microbial community degraded sediment-associated TCS and HHCB. In addition, there was an interactive effect of TCS and the presence of benthic macroinvertebrates on the microbial community composition in the sediment (**Chapter 5** and **6**). Denitrifiers have been found in the activated sludge of WWTPs (Tsai et al., 2012; Wang et al., 2014) and showed higher relative abundances in the samples from the central urban rivers than the Liuxi and the Zhujiang River (**Chapter 3**). This indicates that sites in the central urban rivers were affected by WWTPs effluence.

2. Ecological risks of sediment-associated PCPs for subtropical aquatic organisms

The results of the screening-level risk assessment showed that all selected hydrophobic PCPs posed low ecological risks to aquatic organisms at all sampling sites, except for TCS (**Chapter 2**). This is related to the low acute toxicity of PCPs to aquatic organisms, resulting in high PNEC values. Risk quotient values higher than 1 were recorded in 83% and 75% of sediment samples for TCS in the dry season and wet season, respectively (**Chapter 2**). This is likely due to the high toxicity of TCS to algae with an associated PNEC value of 0.026 µg/L (Chen et al., 2014a). Therefore, algae are likely to suffer adverse effects of TCS in the urban rivers of Guangzhou. It is unknown, however, whether the PNEC values used here are too conservative or not. Furthermore, little is known about the chronic toxicity of PCPs to benthic macroinvertebrates.

Although sediment risk assessment has been developed over two decades (Diepens et al., 2014), the effect assessment of PCPs has mainly focused on the water phase using standard test species under standard laboratory conditions (e.g., Orvos et al. 2002; Tamura et al. 2013; Balk and Fordb, 1999; Carlsson and Norrgren, 2004). To assess the ecological risks of sediment-associated hydrophobic PCPs for subtropical aquatic organisms, we performed microcosm experiments with TCS and HHCB that focused on benthic macroinvertebrates and sediment microbial communities.

Results of **Chapters 4** and **5** showed that sediment-associated TCS is unlikely to affect, at least in the short term (28 days), the density of algae, the survival and growth of snails and worms, and bacterial community structure and composition in the sediments at the concentration of 0.8 µg/g dry weight (dw) and lower (**Chapter 4**). In line with this, benthic macroinvertebrates such as *L. hoffmeisteri* are tolerant to organic contamination in the field (**Chapter 3**). However, according to the screening-level risk assessment, TCS at concentration of around 0.06 µg/g dw (based on corresponding concentrations in pore water) would pose potential risks to aquatic organisms, especially algae (**Chapter 2**). Therefore, the SSD approach is more conservative than the microcosm approach. This is in agreement with the tiered effect assessment approaches proposed by Diepens et al. (2017) (second-tier versus third-tier).With the presence of *L. hoffmeisteri* at a density of ~2667 ind/m^2, sediment-associated TCS significantly affected the microbial community composition and diversity at 8 µg/g dw and higher, whereas the microbial community was affected at much higher concentrations (NOEC = 8 µg/g dw) when invertebrates were absent (**Chapter 5**). This is likely due to the elevated bioavailability of sediment-associated TCS caused by bioturbation and/of feeding. Therefore, we recommend to include benthic macroinvertebrates when assessing the response of sediment bacterial communities to exposure to environmental stress such as organic contaminants (Figure 2). The density of *L. hoffmeisteri* used in the microcosm experiment has been found in the urban rivers of Guangzhou (**Chapter 3**). Thus, the disturbance caused by benthic macroinvertebrates at the water-sediment interface may elevate the effects of sediment-associated organic contaminants on sediment microbial communities in the field environment. In this respect, the results of

Chapter 3 also showed that the sediment microbial community composition in the downstream part of the Liuxi River was similar to those in the Zhujiang River, despite the totally different environmental conditions (e.g., hydrological conditions, chemical and biological characteristics), but a similar pollution level. This suggests that the contamination in the aquatic environment may have altered the microbial community in the sediments in a similar way in both rivers.

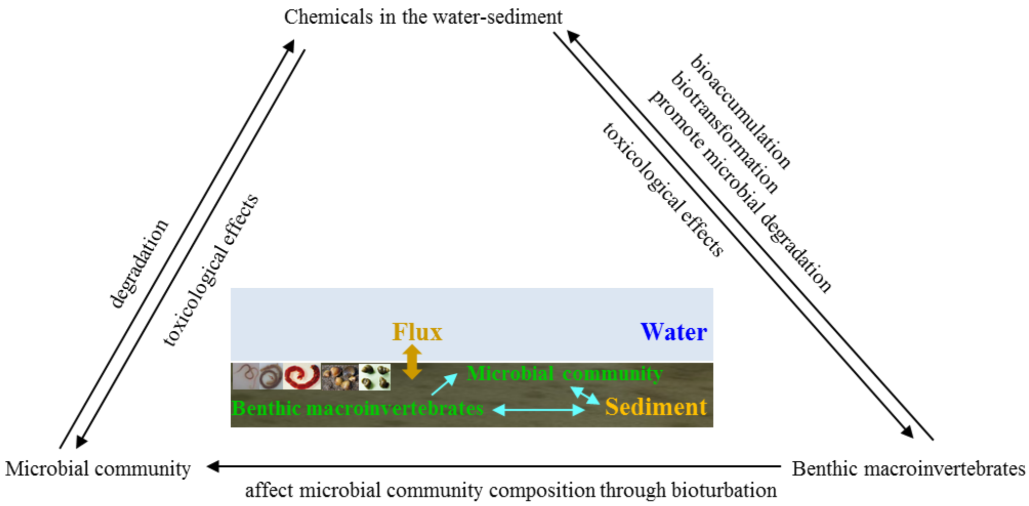

Figure 2. Proposal for incorporating the interactive effects between contaminants and benthic macroinvertebrates into sediment risk assessment.

HHCB proved to be less toxic than TCS with effects observed at concentrations higher than 30 μg/g dw on chlorophyll-a content, microbial community composition, and survival and growth of benthic macroinvertebrates (**Chapter 6**). This is in agreement with the results of the screening-level risk assessment of HHCB in the urban rivers of Guangzhou (**Chapter 2**).

Based on the results of this thesis and published literatures, it can be concluded that a single high-dose of sediment-associated TCS and HHCB at environmentally relevant concentrations would not pose any direct toxicological threat to algae, benthic macroinvertebrates and benthic microbial community within a 28-d exposure. However, it is unknown whether there is an adverse effect on benthic macroinvertebrates or not during the continuous exposure for generations in field.

3. The fate of sediment-associated PCPs in water-sediment systems with and without the presence of sediment-dwellers

Results of **Chapters 4** and **6** showed that TCS and HHCB concentrations in the overlying water were very low (ng/L-few μg/L) in both water-sediment systems with and without the presence of sediment-dwellers at the end of exposure. This can be attributed to their hydrophobicity (log Koc = 4.3 and 4.86 for TCS and HHCB, respectively) and the sediment spiking approach used in this thesis. However, due to the disturbance caused by sediment-dwellers at the water-sediment interface, the chemicals concentrations were significantly higher in the overlying water of systems with the presence of sediment-dwellers than without (**Chapters 4** and **6**).

The mass balance calculations performed in **Chapters 4** and **6** indicated that TCS and HHCB decreased by 3.43-11.4% and 0.36-1.54% in the microcosms during the 28-d exposure period, respectively, suggesting that TCS and HHCB were persistent in the water/sediment systems under the conditions used in this thesis. In line with this, the reported half-life values of TCS and HHCB in water/sediment systems were in the range of 55-239 d and 120-900 d, respectively (Balk and Fold, 1999a; Chen et al., 2014b; Huang et al., 2015). As TCS and HHCB showed negligible photolysis and hydrolysis during the exposure period, the decrease found for TCS and HHCB in the water/sediment systems could be attributed to bioaccumulation in aquatic organisms and biodegradation caused by either microorganisms or introduced organisms. Indeed, TCS and HHCB can accumulate in sediment-dwellers (**Chapters 4, 6 and 7**). These experiments confirmed the bioaccumulation of hydrophobic organic compounds in aquatic organisms that haven been reported by other authors (Nakata et al., 2007; Hu et al., 2011; Dang et al., 2016; Karlsson et al., 2016). TCS was detected in *L. hoffmeisteri* with BSAF values in the range of 0.38-3.55 (**Chapters 4 and 7**). Higher and lower BSAF values have previously been reported for TCS in another, similar, benthic species, i.e. *Limnodrilus variegatus* (Dang et al., 2016; Karlsson et al., 2016). Similar to TCS, HHCB also accumulated in sediment-dwellers with BSAF values of 0.29-0.66 in *B. sowerbyi* and 0.94-2.50 in *L. hoffmeisteri* (**Chapters 6 and 7**). The differences in BSAF values between species or chemicals are likely related to the sediment characteristics such as organic matter content and pH value, physical-chemical properties of chemicals (e.g., log Kow value), chemical concentrations, species traits (e.g., lipid content, diet, ingestion rate), or possible transformation of chemicals in the test system or in the worms themselves into other more hydrophobic products (Diepens et al., 2015; Karlsson et al., 2016). Indeed, TCS was degraded into methyl-triclosan and triclosan-O-sulfate by *L. hoffmeisteri* (**Chapter 7**). However, currently little toxicity information is available for TCS-O-sulfate. Further work is therefore needed to evaluate the (eco)toxicity and persistence of TCS-O-sulfate. Unlike TCS, no degradation was found for HHCB in *L. hoffmeisteri* (**Chapter 7**).This is due to the physicochemical property of HHCB, as demonstrated by Balk and Ford, (1999a), who reported that no mineralization of HHCB occurred in soil- and sediment-containing microcosms after 28 days incubation.

4. The contribution of sediment-dwellers to the dissipation of sediment-associated PCPs

Results of **Chapters 4** and **6** indicated that there was no significant difference in TCS and HHCB dissipation between the water-sediment systems with and without addition of sediment-dwellers. Therefore, the contribution of benthic macroinvertebrates to the loss of TCS and HHCB in the water/sediment systems were negligible during a 28-d exposure period. However, the results of **Chapter 7** showed that the presence of *L. hoffmeisteri* significantly accelerated the dissipation of sediment-associated TCS and HHCB in the microcosms in a 14-d exposure. This difference is likely a result of the much lower abundance of sediment-dwellers used in experiments of **Chapters 4** and **6** (~2667 ind/m^2) relative to **Chapter 7** (~42000 ind/m^2). Actually, a *L. hoffmeisteri* density of ~30000 ind/m^2 has previously been observed in the Lake Taihu (Cai et al., 2010). This indicates that *L. hoffmeisteri* may make significant contribution to the dissipation of some organic contaminants in the field environment. Likewise, Dai et al. (2012) found that *Capitella teleta*, a species of polychaetes typically inhabiting organically enriched sediment, decreased the concentration of sediment-associated acetyl cedrene (another personal care product) by more than 88% in a 14-d exposure at population density of 44000 worms/m^2. Therefore, there are many factors (e.g., density and traits of sediment-dwellers, chemicals properties, sediment properties) that can influence the dissipation of organic contaminants in the environment.

5. Conclusions and outlook

According to the results of this thesis it can be concluded that urban rivers in megacity of China (at least in Guangzhou) suffered both conventional and emerging contamination (**Chapter 2**), which was confirmed by the absence of benthic macroinvertebrates in the central urban rivers and the similarity in microbial community composition between the downstream part of the Liuxi River and Zhujiang River of Guangzhou (**Chapter 3**). The toxicological assessment of sediment-associated TCS on a benthic microbial community in the microcosm experiment has also shown that with the presence of sediment-dwellers, TCS significantly altered its composition and diversity at the concentration of 5-fold higher than the currently detected maximum (1.33 μg/g dw) in the freshwater sediment (**Chapter 5**). Furthermore, the bioturbation caused by sediment-dwellers can elevate the effects of sediment-associated contaminants on the sediment microbial community (**Chapter 5**). Therefore, it is suggested to include benthic macroinvertebrates when assessing the response of sediment bacterial communities to exposure to environmental stress such as organic contaminants. Besides, to overcome contamination problems, Zhang et al. (2015c) recommended that the Chinese government should improve environmental law enforcement, strengthen and institutionalize public participation and information disclosure. Furthermore, efficient removal techniques, such as microbiological removal technologies and constructed wetland that have been proven to efficiently remove pharmaceuticals (Langenhoff et al., 2013; He et al., 2018), should be developed to improve the removal efficiency of hydrophobic PCPs in WWTPs, as they are mainly removed through sorption onto biosolids in WWTPs (Lozano et al., 2013; Homem et al., 2015).

Environmental variables, i.e. Cr, TP, HHCB and TCS, significantly explained 60.6% of the total variation in benthic macroinvertebrates composition between samples (**Chapter 3**). Furthermore, the results of microcosm experiments showed that local dominant benthic macroinvertebrates in the urban rivers of the subtropical region in China were highly tolerant to sediment-associated TCS and HHCB during a 28-d exposure period, with the corresponding NOEC values greater than those concentrations occurring in the field environment (**Chapters 4, 6 and 7**). However, lifetime effects on aquatic organisms are unknown. Likewise, there were significant correlations between microbial community composition and the selected emerging organic contaminants, nutrients and heavy metals (**Chapter 3**). However, there was no significant correlations between traits of benthic macroinvertebrates and the selected emerging organic contaminants (**Chapter 3**).

Local dominant sediment-dweller *L. hoffmeisteri* cannot efficiently degrade HHCB but degrade TCS during a 14-d exposure period (**Chapter 7**). *L. hoffmeisteri* can significantly accelerate the dissipation of sediment-associated TCS and HHCB at really large density under the conditions used in this thesis.

Further work is required to (1) assess the toxicological risks for aquatic organisms exposure to chemicals during their whole life-time, as no reproduction occurred in the microcosms after an exposure period of 28 days to hydrophobic PCPs, (2) assess the ecological risks for aquatic organisms of more realistic exposure scenarios, (3) incorporate the interactive effects of contaminants and benthic macroinvertebrates into the ecological risk assessment, and (4) evaluate the persistence and eco(toxicity) of metabolites of PCPs.

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Summary

China is among the top three countries with the largest personal care product (PCP) consumption, and possesses the fastest growing rate of personal care product market in the world. After use, most PCPs enter WWTPs where their removal is often incomplete, and finally enter the environment. In the natural environment, they may undergo sorption, photolysis, hydrolysis, volatilization and biodegradation, and even transform into more persistent and toxic compounds. For example, triclosan can be conversed to 2,8-dibenzodichloro-p-dioxin (2,8-DCDD) in the water when exposed to natural sunlight. The residual PCPs in the environment may have detrimental effects on aquatic organisms. Furthermore, some PCPs such as triclosan, triclocarban, galaxolide, tonalide, musk xylene and musk ketone are endocrine disruptors. However, it is unknown whether there is hydrophobic PCPs-associated ecological risks to subtropical benthic biological community or not.

The aims of this thesis were to 1) investigate the PCPs contamination profile in urban rivers in China with Guangzhou as a case study and identify key benthic macroinvertebrates, their taxa and traits distribution patterns and microbial community composition in urban rivers; 2) evaluate their correlations with conventional contamination and PCPs contamination; 3) assess the toxicological effects of sediment-associated hydrophobic PCPs on the subtropical benthic biological community; 4) investigate the fate of sediment-associated hydrophobic PCPs in subtropical aquatic ecosystem; and 5) evaluate the contribution of sediment-dwellers to the dissipation of sediment-associated hydrophobic PCPs.

Chapter 2 shows the outcomes of the chemical monitoring program in the urban rivers of Guangzhou (South China). Among all the selected emerging organic chemicals, 4-nonylphenol, bisphenol A, triclocarban, triclosan and galaxolide were the most frequently detected and predominant target compounds in both water phase and sediment phase. The concentrations of 4-nonylphenol, triclocarban and triclosan in urban rivers in the city centre of Guangzhou are relatively high compared to previously reported data worldwide, which is likely associated with the treated and untreated industrial and domestic sewage and waste discharges. Risks assessment showed that 4-NP and TCS had RQs >1 in more than 70% of samples and might pose various ecological risks to aquatic ecosystems, especially for algae. Also, it is better to consider the ionization of ionizable chemicals and water quality status when conducting environment risk assessment. Further investigations of eco-toxicological effects and potential ecological risks of these chemicals are therefore required to protect aquatic ecosystems and biodiversity in urban rivers of megacities in China.

Chapter 3 describes the results of biological monitoring program. The input of domestic and/or industrial sewage effluents influenced the sediment quality and the distribution patterns of the local benthic macroinvertebrates, their functional attributes and bacterial communities

composition in the rivers of Guangzhou (South China). Selected hydrophobic PCPs such as galaxolide and triclosan, heavy metals such as chromium, and nutrients such as total phosphorus were strongly correlated with the biological variance in the sediment of urban rivers. Among the four central urban rivers, the Sha River had different benthic macroinvertebrate and bacterial community composition from the other three rivers. Therefore, interactions likely existed among contaminants, benthic macroinvertebrate and bacterial communities.

In **Chapter 4**, **5** and **6**, the fate and effects of sediment-associated triclosan and galaxolide were investigated in the subtropical freshwater benthic dominated microcosms. These studies confirmed the high tolerance of planktonic and benthic macroinvertebrates communities to the triclosan and galaxolide at concentrations that have been measured in the environment. These studies also revealed that the richness and diversity of sediment bacterial community were reduced by TCS at concentrations only 5-fold higher than the currently detected maximum environmental concentration (1.33 µg/g dw) with the presence of benthic macroinvertebrates. At such level, TCS alone also significantly reduced the relative abundance of some individual bacterium such as Bacteroidetes. However, sediment-associated galaxolide had no significant effect on the microbial community composition in the sediment even at 300 µg/g concentration. These studies also confirmed that triclosan and galaxolide can accumulate in sediment-dwellers, such as *L. hoffmeisteri* and *B. sowerbyi*. However, the presence of these organisms did not make significant contribution to the dissipation of sediment-associated TCS and HHCB in the microcosms at the density used in this study. Based on the chemical and biological monitoring programs performed in the urban rivers of Guangzhou and the toxicity data reported in the literature, it can be concluded that the short-term risks of sediment-associated PCPs appear to be low for subtropical biological community in the field environment.

In **Chapter 7**, the bioaccumulation and biotransformation of triclosan and galaxolide in the freshwater oligochaete *L. hoffmeisteri* were investigated using water/sediment microcosms. This study confirmed that TCS and HHCB can accumulate in the sediment-dweller *L. hoffmeisteri* and reached the equilibrium after 7 days of exposure. This study demonstrated that the presence of *L. hoffmeisteri* significantly accelerated the dissipation of TCS and HHCB in the microcosms. This study also revealed that *L. hoffmeisteri* cannot biodegrade HHCB but biodegrade TCS through methylation and sulfonation. However, there is little to no toxicity data or persistence data of products like triclosan-O-sulfate. Therefore, toxicological evaluation of these products is therefore warranted.

In **Chapter 8**, the overall results from previous chapters are summarized and discussed. We propose to incorporate benthic macroinvertebrates when assessing effects of contaminants on benthic microbial community to reach a more realistic exposure scenario.

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Curriculum vitae

Fengjiao Peng was born on 29th of December 1987 in Hunan province, China. In 2007 she started to follow the BSc study program *Hydrology and Water Resources* at the University of Jinan in China. After completing her BSc studies, she moved into the field of environmental research in 2011. She got a degree on Environmental Engineering from the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences in 2014. For her MSc thesis she studied the sorption behavior of several typical antibiotics in different types of soil and their related antibacterial activity. Meanwhile, she participated in a few projects such as endocrine disrupting compounds determination and pharmaceuticals and personal care products determination in surface water samples and sediment samples. Consequently, during her master study she has acquired plenty valuable knowledge about analytical chemistry, environmental chemistry, environmental microbiology and environmental toxicity. Also, she gained a lot of experience in experimental practice and instrumental analysis. More importantly, she became interested in environmental research and decided to continue her research. In August of 2014, she started her PhD project at Wageningen University under the supervision of Prof. Paul van den Brink and Prof. Guang-Guo Ying. In the last two years, she participated in two presentations at international conferences. Currently, her research interests lie on assessing the fate and effect of personal care products in urban rivers in the subtropical region.

List of publications

Published

Peng, F.-J., Diepens, N.J., Pan, C.-G., Bracewell, S.A., Ying, G.-G., Salvito, D., Selck, H., Van den Brink, P.J., 2018. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquatic Toxicology. https://doi.org/10.1016/j.aquatox.2018.07.008.

Peng, F.-J., Ying, G.-G., Pan, C.-G., Selck, H., Salvito, D., Van den Brink, P.J., 2018. Bioaccumulation and biotransformation of triclosan and galaxolide in the freshwater oligochaete Limnodrilus hoffmeisteri in a water/sediment microcosm. Environmental Science & Technology. DOI: 10.1021/acs.est.8b02637

Peng, F.-J., Pan, C.-G., Zhang, M., Zhang, N.-S., Windfeld, R., Salvito, D., Selck, H., Van den Brink, P.J., Ying, G.-G., 2017. Occurrence and ecological risk assessment of emerging organic chemicals in urban rivers: Guangzhou as a case study in China. Science of the Total Environment 589, 46-55.

Peng, F.-J., Ying, G.-G., Liu, Y.-S., Su, H.-C., He, L.-Y., 2015. Joint antibacterial activity of soil-adsorbed antibiotics trimethoprim and sulfamethazine. Science of the Total Environment 506, 58-65.

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In preparation

Peng, F.-J. et al., Sediment associated biodiversity in typical urban rivers: correlations with environmental variables.

Peng, F.-J. et al., Response of sediment bacterial community to triclosan in subtropical freshwater benthic microcosms.

Peng, F.-J. et al., Fate and effects of polycyclic musk HHCB in subtropical freshwater microcosms.

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The SENSE Research School declares that **Fengjiao Peng** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 49.0 EC, including the following activities:

SENSE PhD Courses

- Environmental research in context (2014)
- Research in context activity: 'Creating and publishing Wikipedia internet page on *Limnodrilus hoffmeisteri* (red worm) and communicating accessible press release with dissertation results' (2018)

Other PhD and Advanced MSc Courses

- Multivariate Analysis, Wageningen University (2014)
- Chemical stress ecology and risk assessment, Wageningen University (2014)
- The Essentials of scientific writing and presenting, Wageningen University (2014)
- PhD peer consultation - as support in academic skills development, Wageningen University (2014)
- Ecological methods I, Wageningen University (2017)
- Ecological models and data in R, Wageningen University (2017)
- Risk assessment, Postgraduate Education in Toxicology (PET) (2017)
- Mixed linear models, Wageningen University (2018)
- Generalized linear models, Wageningen University (2018)
- Meta-analysis, Wageningen University (2018)
- Presenting with impact, Wageningen University (2018)

External training at a foreign research institute

- Training in the use of Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), gas chromatography and mass spectrometry (GC/MS), Guangzhou Institute of Geochemistry, China (2015)

Management and Didactic Skills Training

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Oral Presentation

- Occurrence and ecological risk assessment of emerging organic chemicals in urban rivers: Guangzhou as a case study in China. Society of Environmental Toxicology and Chemistry, 16-19 September 2016, Singapore, Singapore

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