Research Article

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Journal of Aquatic Pollution and Toxicology

2017
Vol. 1 No. 2: 13

Antibiotics in Three Chinese Coastal Systems: Huangpu River, East China Sea, Pearl River Estuary

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Citation: Fisch K, Waniek JJ, Zhou M, Xia Z Schulz-Bull DE (2017) Antibiotics in Three Chinese Coastal Systems: Huangpu River, East China Sea, Pearl River Estuary. J Aquat Pollut Toxicol. Vol. 1 No. 2:13

Abstract

The occurrence of antibiotics in the marine environment over a long time period can lead to development of antibiotic resistant bacteria, which is a major scientific concern. This study was carried out to investigate the occurrence of four antibiotics (sulfadiazine, sulfamerazine, sulfamethoxazole and trimethoprim) in the waters of Huangpu River and Pearl River Estuary and the East China Sea. To our knowledge, our results are the first findings of antibiotics in the East China Sea. The antibiotics were extracted from water and enriched via solid-phase extraction (Chromabond®EASY). The analysis was carried out with a liquid-chromatography tandem mass spectrometer equipped with a heated electrospray ionization source (HPLC-HESI-MS/MS). The selected antibiotics were detected in all three waters at low ng/L concentrations, which are comparable to literature data. In the Huangpu River the antibiotics occurred at a relatively stable concentration, with a slight decrease towards the Changjiang River. In the East China Sea antibiotics were mainly found in the surface water. Only sulfamethoxazole was found at three stations in bottom water. Detected concentrations in the Pearl River Estuary displayed an inverse correlation with salinity, confirming the dominant effect of dilution due to mixing of riverine and oceanic waters.

Keywords: Antibiotics; Huangpu River; Pearl River Estuary; East China Sea; Water

Received: June 09, 2017; **Accepted:** July 24, 2017; **Published:** July 31, 2017

Introduction

Coastal ecosystems are under threat because of climate change and anthropogenic pressure due to eutrophication and emerging contaminants like antibiotics. Those are ubiquitous contaminants and their occurrence in the ecosystem over a long time period can lead to development of antibiotic resistant bacteria, which is a major scientific concern [1,2]. Countries around the world including China enforce national and international measures to inspect pollution, to control and to improve the water quality and the surrounding environment [3].

Chinese waters are affected by pollutions from industrial, agricultural and domestical activities. Especially in dense metropolitan areas like Shanghai and Guangzhou/Hong Kong at the Pearl River Delta, the aquatic environment is exposed to heavy pollution [4-7]. For recent years a major concern has been the exposure of the aquatic environment to pharmaceuticals, in particular antibiotics. China is the largest producer (248,000 t, 2013) and user (162,000 t, 2013) of antibiotics in the world. In 2013 48% of the total amount was used for human applications and 52% were of veterinary usage. Sulfonamide (SA) antibiotics made up 5% of the total usage [8]. They are used to treat and prevent infections by gram positive and negative bacteria [9]. They are mostly used in combination with trimethoprim (TMP), because of their synergistic effects [10-13]. After application about 15-90% of antibiotics are excreted unchanged [8,14,15]. Based on these rates and the fact that SAs are hydrophilic, hydrologically stable and have low sorption to sediments, SAs can be found in aquatic environments almost everywhere [16-18]. Shanghai and the Pearl River Delta are the metropolitan areas in the east and south China regions, respectively. They are not only the most urbanized cities, but also are of great economical importance due to intensive livestock farming [2,19-21]. Both East and South China regions have a high consumption of SAs, 2270 t and 596 t, respectively. SAs are the second most used antibiotics in Chinese aquacultures [8,22]. As a result of metropolitan areas and high rates of antibiotic usage, the aquatic environments in these areas are under high pressure of pollution by antibiotics.

This study was carried out to determine the occurrences of Sulfonamide and TMP in two Chinese rivers flowing through metropolitan areas (Huangpu River and Pearl River) and to determine how far offshore they are transported via the Changjiang River into the East China Sea.

Materials and Methods

Sampling sites and sample collection

The Huangpu River is approximately 114 km long, originating in Tai Lake and flowing north-east through Shanghai with a discharge rate of 10 billion m^3/a into the Changjiang Estuary [23,24]. The upper river is influenced by suburbs, animal breeding farms and agriculture. The lower river (83 km) flows through an urban area, which is highly subjected to intensive industrial and residential activities [4,6]. The Huangpu River was sampled at 9 stations (HP-1-10, w/o HP-4) in the urban area in July 2016. Station HP-1 is located in the Minhang district and station HP-10 in the Changjiang River. The surface (0.5 m) water samples were taken close to the river bank using a pre-cleaned metal bucket (2 L). In July 2016 samples were taken in the East China Sea with the research vessel ZheHai Ke 1 (Zhejiang Ocean University). The East China Sea is dominantly influenced by the Changjiang River discharge and to a lower degree by the Hangzhou shelf. It is the largest marginal sea of the north western Pacific [25,26]. During the cruise discrete surface and bottom water samples were taken at 12 stations via go-flow bottles. At all stations conductivity, temperature and depth were measured with CTD-sensors (Sea Bird Scientific, Bellevue, WA, USA). The Pearl River is the largest water system discharging into the South China Sea via its 8 major outlets with a discharge rate of 336 billion m^3/a [27]. It is influenced by waste waters from intensive poultry, fish farming and industrial/domestic activities. The Pearl River Estuary was sampled in August 2016 with the research vessel Haiyang 4 (Guangzhou Marine Geological Survey) at 12 stations (PR-1-12) from Dongjiang towards the open South China Sea. The surface and bottom waters were sampled using go-flow bottles at 12 and 9 stations, respectively. At station PR-7-9 and PR-11 water samples were only taken directly from the surface. Conductivity, temperature and depth were measured using a Sea Bird Scientific (Bellevue, WA, USA) CTD-system at station PR-1-6, PR-10 and PR-12 **(Figures 1a and 1b and Table 1)**.

The samples of Huangpu River and Pearl River Estuary were collected in 2 L pre-cleaned amber glass bottles and taken to the laboratory where the solid-phase extraction was carried out. The samples were kept in the dark and cool at 4ºC during transport to the laboratory. Samples from the East China Sea were collected in 1 L pre-cleaned amber glass bottles and extracted on board. All extracted samples were stored at -20°C until further analysis in the laboratory at the Leibniz Institute for Baltic Sea Research in Germany.

Chemicals

All reference standards and chemicals were of analytical grade (>98%). Reference standards of the following pharmaceuticals: Sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethoxazole (SMX), trimethoprim (TMP) and the internal standard sulfamethoxazole-d4 (SMX-d4) were purchased from Dr. Ehrenstorfe GmbH (Germany). The solvents methanol, acetone and hydrochloric acid (HCl) in China were provided by Shanghai Jiao Tong University and Guangzhou Marine Geological Survey, in Germany methanol was obtained from Promochem (Germany). From Sigma-Aldrich (Germany) acetic acid and formic acid were purchased and water (LC-grade) was acquired from VWR (Germany).

Preparation and analysis of antibiotics

The 2 L water samples were divided into two 1 L glass amber bottles and spiked with an internal standard. The internal standard consisted of 20 ng/mL SMX-d4. The solid-phase extraction with 1 L of sample water was done in China accordingly to the pharmaceutical extraction method published by Fisch et al. [28]. For antibiotic analysis the pH was adjusted to pH=5 with 5 M HCl. Prior to the solid-phase extraction the cartridges (Chromabond® EASY, 3 mL, 200 mg, Marchery-Nagel GmbH) were conditioned

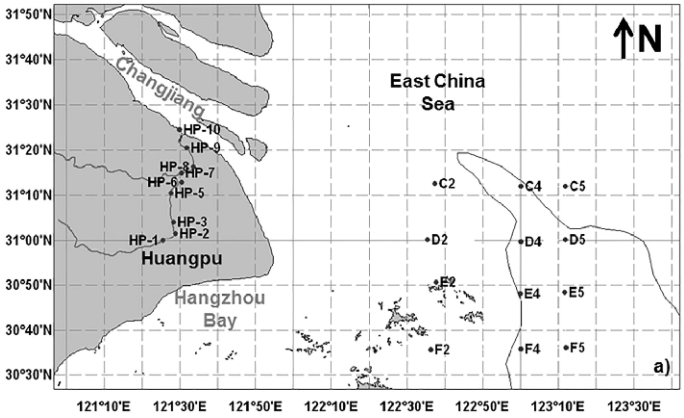

Figure 1a Sampling location: Huangpu River and East China Sea.

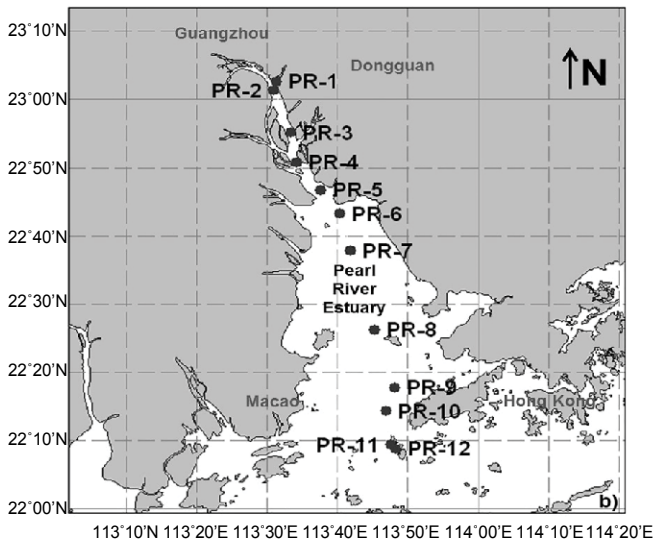

Figure 1b Sampling location: Pearl River Estuary.

Table 1 Station details with measured parameters: Depth, temperature, salinity and the concentrations of sulfadiazine (SDZ, LOD=0.6 ng/L), sulfamerazine (SMZ, LOD=0.6 ng/L), sulfamethoxazole (SMX, LOD=0.5 ng/L), trimethoprim (TMP, LOD=0.9 ng/L).

Station	Coordinates	Depth	Temperature	Salinity	Concentration [ng/L]			
		[m]	[°C]		SDZ	SMZ	SMX	TMP
Huangpu River (25.07.2016)								
HP-1	30.998545°N 121.427541°E	0.5			13.5 ± 0.4	n.d.	9.3 ± 0.1	4.4 ± 0.2
HP-2	31.025168°N 121.483560°E	0.5			14.5 ± 0.1	n.d.	9.6 ± 0.3	4.5 ± 0.1
HP-3	31.067856°N 121.472241°E	0.5			11.2 ± 0.4	n.d.	9.1 ± 0.2	4.1 ± 0.1
HP-5	31.174218°N 121.462442°E	0.5			9.2 ± 0.3	n.d.	7.9 ± 0.1	5.1 ± 0.2
HP-6	31.215413°N 121.508159°E	0.5			10.0 ± 0.1	n.d.	8.1 ± 0.1	5.0 ± 0.2
HP-7	31.249243°N 121.507880°E	0.5			9.8 ± 0.2	n.d.	8.1 ± 0.2	5.7 ± 0.1
HP-8	31.272169°N 121.562656°E	0.5			8.0 ± 0.4	n.d.	7.9 ± 0.2	6.3 ± 0.1
HP-9	31.340310°N 121.533086°E	0.5			10.0 ± 0.3	n.d.	8.3 ± 0.1	4.6 ± 0.1
HP-10	31.408173°N 121.500180°E	0.5			5.3 ± 0.3	n.d.	6.3 ± 0.2	3.0 ± 0.1
East China Sea (12-15.07.2016)								
F2	30.593361°N 122.605972°E	2.0	25.4	20.7	2.0 ± 0.07	n.d.	3.5 ± 0.1	1.3 ± 0.2
		24.8	20.3	33.6	n.d.	n.d.	<LOD	n.d.
F4	30.596722°N 123.002417°E	2.0	25.8	24.0	0.8 ± 0.01	n.d.	2.2 ± 0.02	1.1 ± 0.02
		57.5	19.9	34.4	n.d.	n.d.	n.d.	n.d.
F5	30.599528°N 123.201139°E	2.0	26.9	26.3	n.d.	n.d.	1.4 ± 0.04	1.3 ± 0.01
		51.7	20.2	34.4	n.d.	n.d.	n.d.	n.d.
E2	30.844022°N 122.630761°E	2.0	26.5	9.2	1.6 ± 0.1	n.d.	2.8 ± 0.06	0.9 ± 0.1
		23.9	20.6	32.5	n.d.	n.d.	0.8 ± 0.02	n.d.
E4	30.800833°N 123.001528°E	1.9	23.9	21.7	1.8 ± 0.03	n.d.	3.0 ± 0.02	0.9 ± 0.1
		43.0	20.3	34.2	n.d.	n.d.	<LOD	n.d.
E5	30.807314°N 123.197756°E	2.0	25.7	19.6	1.3 ± 0.05	n.d.	2.8 ± 0.1	1.5 ± 0.3
		54.5	20.3	34.2	n.d.	n.d.	n.d.	n.d.
D2	31.001389°N 122.593611°E	2.0	24.9	13.3	3.0 ± 0.04	n.d.	4.4 ± 0.08	1.1 ± 0.1
		14.9	20.5	31.3	<LOD	n.d.	1.0 ± 0.03	n.d.
D4	30.994981°N 123.004361°E	2.0	24.5	20.4	1.5 ± 0.03	n.d.	3.0 ± 0.04	1.1 ± 0.1
		46.8	20.0	33.9	n.d.	n.d.	<LOD	n.d.
D5	31.001444°N 123.199306°E	1.9	24.7	20.4	1.2 ± 0.03	n.d.	2.6 ± 0.08	<LOD
		51.0	20.6	34.2	n.d.	n.d.	<LOD	n.d.
C2	31.209667°N 122.624361°E	2.0	24.7	18.3	1.5 ± 0.03	n.d.	2.7 ± 0.03	<LOD
		26.8	20.6	33.7	n.d.	n.d.	0.6 ± 0.04	n.d.
C4	31.200417°N 123.003278°E	2.0	26.7	18.3	2.3 ± 0.05	n.d.	3.6 ± 0.2	1.0 ± 0.1
		59.7	20.0	34.1	n.d.	n.d.	<LOD	n.d.
C5	31.200167°N 123.200667°E	2.0	24.0	23.2	1.1 ± 0.01	n.d.	2.4 ± 0.2	0.9 ± 0.1
		51.0	20.6	34.2	n.d.	n.d.	<LOD	n.d.
Pearl River Estuary (29.08.2016)								
PR-1	23.043100°N 113.521020°E	1.5	30.1	0.1	8.3 ± 0.3	11.9 ± 0.2	13.9 ± 0.2	12.0 ± 0.4
		5.9	30.1	0.1	7.7 ± 0.2	11.8 ± 0.2	13.6 ± 0.4	15.2 ± 0.5
PR-2	23.020742°N 113.515870°E	2.0	30.0	0.1	7.5 ± 0.2	11.2 ± 0.2	12.1 ± 0.1	13.5 ± 0.2
		8.8	30.0	0.1	7.9 ± 0.2	11.1 ± 0.2	11.6 ± 0.3	11.4 ± 0.4
PR-3	22.918528ºN 113.557650°E	1.7	30.4	0.1	7.2 ± 0.1	9.3 ± 0.1	11.4 ± 0.4	7.4 ± 0.2
		17.9	30.3	0.1	7.1 ± 0.2	9.7 ± 0.1	11.4 ± 0.3	8.1 ± 0.3
PR-4	22.845817°N 113.570317°E	1.3	30.4	0.8	6.2 ± 0.2	8.0 ± 0.3	11.4 ± 0.2	11.1 ± 0.2
		16.4	30.2	3.9	6.4 ± 0.05	7.4 ± 0.2	11.3 ± 0.4	9.5 ± 0.5
PR-5	22.778505°N 113.628987°E	1.1	30.4	3.1	5.1 ± 0.1	4.5 ± 0.1	10.3 ± 0.1	10.6 ± 0.3
		9.6	30.0	8.8	4.1 ± 0.2	3.2 ± 0.1	9.6 ± 0.2	10.1 ± 0.3
PR-6	22.720088°N 113.672787°E	0.6	30.2	3.2	3.8 ± 0.1	2.9 ± 0.1	9.3 ± 0.3	13.9 ± 0.3
		12.1	29.1	12.6	3.5 ± 0.1	2.8 ± 0.04	9.1 ± 0.1	12.3 ± 0.1
PR-7	22.629917°N 113.697928°E				4.2 ± 0.2	9.2 ± 0.9	9.0 ± 0.7	22.1 ± 1.1
PR-8	22.437782°N 113.754897°E				1.6 ± 0.2	0.9 ± 0.2	5.1 ± 0.3	6.0 ± 0.1
PR-9	22.295800°N 113.802863°E				0.9 ± 0.1	n.d.	3.5 ± 0.1	4.5 ± 0.3
PR-10	22.237382°N 113.783957°E	0.5	27.4	26.3	<LOD	n.d.	2.5 ± 0.02	2.4 ± 0.1

		7.7	27.1	29.1	<LOD	n.d.	2.5 ± 0.05	2.8 ± 0.1
PR-11	22.154925°N 113.793738°E				<LOD	n.d.	1.4 ± 0.1	1.9 ± 0.1
PR-12	22.146218°N 113.804325°E	1.0	27.1	31.0	<LOD	n.d.	1.0 ± 0.1	1.4 ± 0.2
		10.1	26.6	32.3	<LOD	n.d.	0.9 ± 0.04	1.2 ± 0.1

±: Standard Deviation
n.d.: Not Detected
LOD: Limit of Detection

with 4 mL acetone and 4 mL water. Preceding the extraction, the samples were filtered through glass-fiber filters (GF/F, 0.7 µm, Whatman) and directly brought onto the cartridges. Following the extraction cartridges were cleaned with 4 mL pH=5 water and gently dried. All cartridges were stored at -20°C wrapped in aluminum foil and further analyzed in Germany. Samples were eluted with 10 mL acetone/methanol (1/1, v/v), evaporated to dryness at 40°C with a Turbo-Vap (LV, Zymark, USA) evaporator and afterwards reconstituted with 1 mL methanol/water (3/1, v/v). The analysis was carried out using a liquid chromatography heated electronspray ionisation tandem mass spectrometer (LC-HESI-MS/MS) from Thermo Fischer Scientific (Germany). Details of the method and quantification are described in Fisch et al. [28]. For liquid chromatography a reverse-phase Kinetex C-18 (Phenomenex, USA) column was used and 10 µL of sample were injected. Separation was achieved by a gradient program (flow rate: 250 µL/min) of the mobile phases A: water + 0.1% acetic acid and B: methanol + 0.1% formic acid. The temperature for the capillary and vaporizer were set to 275°C and 250°C, respectively. For method quantification the internal standard SMX-d4 and the most abundant transition (based on signal-to-noise ratio) were used, the second most abundant ion was used for qualification. As the antibiotics in this study belong to the group of sulfonamides it is appropriate to use SMX-d4 as an internal standard for all of them. The LODs (0.5-0.9 ng/L), LOQs (0.6-1.0 ng/L) and the recovery rates are displayed in **Table 2**.

Calculation of attenuation coefficient

Calculations of the attenuation coefficient (k) were done according from 3.0 to 14.5 ng/L, assuming a first order attenuation [29,30]. The attenuation of antibiotics involves e.g. dilution, adsorption, photolysis and biodegradation Zhang et al. As stated by the sources of pollution are diffused and the sea currents were not stable [29]. Therefore, the attenuation is the correlation of antibiotic concentration (ci, cI) at the stations (i, I) with distance (L) between the stations:

$$k = \left(\frac{1}{L}\right) ln\left(\frac{c_i}{c_I}\right)$$

Results and Discussion

Huangpu River

The surface water of the Huangpu River was sampled at 9 stations (HP-1-10, w/o HP-4) in July 2016 in the urban area of Shanghai. Only 3 out of 4 analyzed antibiotics were found in concentrations ranging from 3.0 to 14.5 ng/L. All values are uncorrected and could be corrected by a factor of 2, due to the low recovery rate of the internal standard SMX-d4. The antibiotics sulfadiazine (SDZ), sulfamethoxazole (SMX) and trimethoprim (TMP) were all detected with a frequency of 100%. Sulfamerazine (SMZ) was not found at any station. SMZ is only of veterinary usage and in China it is mostly used in aquacultures [22]. This may explain its absence in the urban area of Shanghai, which is an intensive industrial and residential area [19]. The salinity of the Huangpu River does not change significantly along the stream, which means the river is mostly influenced by fresh and waste water inputs [31]. SMX and TMP concentrations are mostly consistent along the river with a slight decrease from station HP-1 to HP-10. The attenuation coefficient for both is k=0.0007 km^{-1}, which indicates that the concentrations are only slightly diluted. SDZ displays a stronger decrease from the urban area towards station HP-10 (k=0.016 km^{-1}). In comparison with literature data by Jiang et al., this study shows that there is a slight increase in concentrations of approximately 10 ng/L [23]. The reason may be an increase in the usage of antibiotics in the last 7 years. Jiang et al. only found SMX at some stations. In this study it was detected at all stations, at a consistent concentration (mean 8.3 ± 1.0 ng/L) along the river [23]. At station HP-10 the concentration of SMX is similar to the concentration measured by Jiang et al. [23]. In this study TMP was detected with concentration of low variability. The increase of TMP (station HP-3-6) detected by Jiang et al. was not confirmed [23]. Like for SMX, TMP was detected at similar concentration at station HP-10 as reported in literature. The domestic waste water effluents are a major source of pollution in the urban area for the Huangpu River [32]. The waste water treatment rate in the urban area was approximately 88% by end of 2010. This corresponds to a direct discharge of approximately 419 million m^3 waste water into the Huangpu River every day [19]. However, the waste water is now supposed to be directly discharged into the East China Sea. Considering that most of the waste water is released to the East China Sea, the small increase of concentrations between 2009 and 2016 in the Huangpu River appears to be realistic. Additionally, all concentrations measured in this study are lower than reported concentrations for winter months and for the upstream area. Studies report, that higher concentrations of antibiotics can be found in winter months due to a higher usage, low flow conditions and lower photo and biodegradation [23,32-36]. An important loss pathway in water is the direct photolysis [37,38]. However, it is known that SA antibiotics are hydrologically stable, water soluble, have a low sorption to particles and soil and are easily transported in water over long distances, explaining the evenly distributed concentrations [17,37,39-41]. Furthermore, SA resistant genes (sulI, sulII) were detected near our sampling stations, which points towards an occurrence of SAs and their extensive usage

Table 2 Overview of selected antibiotics, their recovery rate, limit of detection (LOD) and the limit of quantification (LOQ).

Name	Abbreviation	CAS	Recovery [%]	LOD [ng/L]	LOQ [ng/L]
sulfadiazine	SDZ	68-35-9	85	0.6	0.7
sulfamerazine	SMZ	127-79-7	91	0.6	0.7
sulfamethoxazole	SMX	723-46-6	103	0.5	0.6
trimethoprim	TMP	738-70-5	85	0.9	1.0
sulfamethoxazole-d4	SMX-d4	1020719-86-1	56		

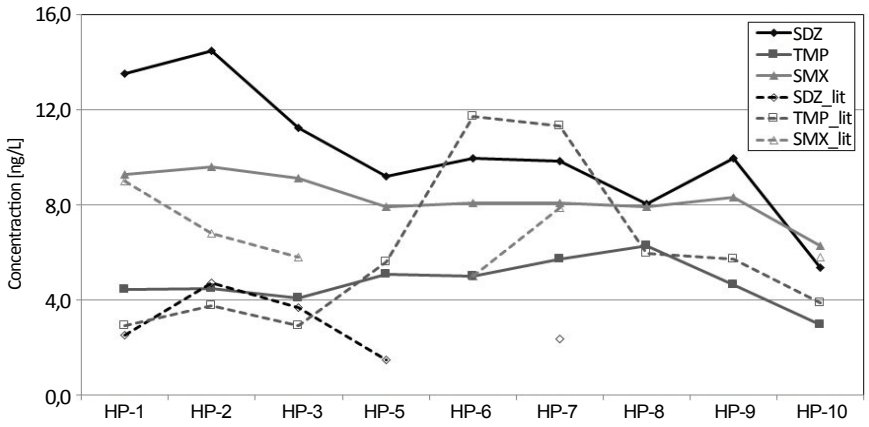

Figure 2 Concentration of sulfadiazine (SDZ), sulfamethoxazole (SMX) and trimethoprim (TMP) measured in Huangpu River with literature data for comparison.

Table 3 Comparison of detected concentration [ng/L] with literature data [ng/L] in surface water for the regions: a) Huangpu River and East China Sea, b) Pearl River estuary. Yang et al. [31,34,47] reported a temperature and salinity range of 10.9-14.8°C and 18.5-30.5, respectively. Liang et al. [32] reported a salinity range for wet and dry season of 0.4-21.7 and 0.3-32.1, respectively.

	Area	Sampling Season	SDZ	SMZ	SMX	TMP	Reference
a)	Huangpu River	June 2009	1.39-17.19 (1.3)[(a)]	n.d. (0.8)	4.86-14.32 (4.5)	2.23-11.73 (2.2)	Jiang et al. [23]
		December 2009	4.19-40.5	n.d.	16.92-55.24	6.75-62.39	
	Huangpu River	July 2012	4.9-112.5	<LOQ	2.2-764.9	-	Chen and Zhou [56]
	(upstream)		(0.03-1.7)	(0.03-1.7)	(0.03-1.7)	(0.03-1.7)	
	Huangpu River	July 2016	5.3-14.5	n.d.	6.3-9.3	3.0-6.3	This study
	(downstream)		(0.7)	(0.7)	(0.6)	(1.0)	
	East China Sea	November 2009	-	-	4.2-40	-	Yang et al. [47]
	(Changjiang Estuary)				(0.08)		
	Changjiang Estuary	July 2011-May 2012	0.3-71.8 (0.41)	n.d. (0.04)	0.3-56.8 (0.3)	-	Yan et al. [35]
	Changjiang Estuary (C16)	Nov-Dec 2012	-	-	11.2	n.d.	Wu et al. [58]
	East China Sea	July 2016	n.d.-3.0	n.d.	1.4-4.4	< LOQ-1.5	This study
b)	Victoria Harbor (HK)	Dec 2004/Feb 2005	n.d. (1.0)	-	n.d. (1.0)	-	Xu et al. [36]
	Pearl River (upstream)	June 2005	max. 141	-	max. 165	-	Xu et al. [36]
	Major Pearl River	Feb/June 2006	<MQL (70)	-	<MQL-510 (80)	<MQL (100)	Peng et al. [21]
	Stonecutters Island (HK)	June-August 2008	-	-	n.d. (0.5)	n.d. (0.4)	Minh et al. [57]
	Pearl River	September 2008	4.9-26.7 (0.2)	-	211-616 (1.0)	81.2-179 (0.3)	Yang et al. [34]
	(upstream, Shijing)						
	Pearl River Estuary	June 2009	10 (1.0)	-	2.3-11.1 (1.0)	-	Xu et al. [2]
	East River (Dongjiang)	July 2009	n.d.-8.2 (1.0)	-	7.9-30.4(1.0)	n.d.-15.5	Zhang et al, [48]
	Pearl River Estuary	August 2011	max. 2.1 (0.5)	-	max. 9.4 (0.6)	-	Jiang et al. [1]
		January 2011	max. 18	-	max. 30.1	-	
	Pearl River Estuary	August 2016	<LOD-8.3	n.d.-11.9	1.0-13.9	1.4-22.9	This study

- not Analyzed
n.d.: not detected
LOQ: Limit of Quantification
LOD: Limit of Detection
Max: Maximum
MQL: Method Quantification Limit
(a) Values in parentheses are quantification limits (ng/L)

[1] **(Figure 2 and Table 3)**.

East China Sea

The surface and bottom water of the East China Sea near the coast was sampled at 12 stations in July 2016. The sampling stations were all located in the Changjiang Diluted Water (CDW), which covers most of the northern East China Sea during flooding and is defined by salinity <31 [42]. The antibiotics SDZ, SMX and TMP were detected in all surface water samples with a detection frequency of 92%, 100% and 83%, respectively. Only SMX was detected in 3 bottom water samples closest to the coast (E2, D2, C2, F2<LOD). To our knowledge, these are the first results of antibiotics in the open East China Sea. SMZ was not detected in any samples. It is an unauthorized antibiotic, but still used in aquacultures. The output of aquatic products for the Shanghai-Zhejiang region is approximately 2.08 mio t per year [22]. It can be assumed that the input of SMZ is too low in order for its detection in the open sea. Only low concentrations of SDZ, SMX and TMP up to 4.4 ng/L were detected, which are lower than the measured concentrations in the Huangpu River. No correlation was found between the occurrence of the antibiotics and particulate organic carbon (POC) and chlorophyll a (ChlA). POC and ChlA data was obtained from satellite images provided by NASA. The concentrations decrease from stations near the coast towards stations in the open sea. An inverse correlation was calculated for SMX (r=-0.7) and SDZ (r=-0.6) with salinity. Only TMP (r=0.4) displayed a positive correlation. Due to the inverse correlation, it can be assumed that the antibiotics are diluted while being transported from the estuary to the open sea [29]. SAs and TMP have a half-life of about 100 days (relatively persistent) in seawater and are not inclined to degrade in surface water, indicating that they can be transported in water for a long distance, which explains their occurrence in the open sea [43-45]. From 1942 to 2002 aquacultures increased by 14% in the Changjiang Delta [46]. SAs are directly added into the water while feeding and are used/applied in larger dosages in order for them to reach the effective inhibition concentration within the animal [22]. The salinity demonstrates that the sampled water originated from the Changjiang River. The CDW and the direct input of the Shanghai waste water are possibly the major sources for these antibiotics in the East China Sea. SAs have a low sorption affinity to soil, sediment and suspended particular matter and are hydrophilic, explaining their occurrence in surface water and their absence in bottom water [16,39-41,47]. In comparison to Changjiang River, lower concentrations were measured during this study. However, the reported higher concentrations in the Changjiang River were detected during the dry season, when the dilution effect is much lower than in the wet season. Additionally, the distance to the coast is much larger than in previous studies, which explains the lower concentrations detected in this study **(Figures 1a and 3 and Table 1)**.

Pearl River Estuary

The surface water of the Pearl River Estuary was sampled at 12 stations and the bottom water at 8 stations. All 4 analysed antibiotics were detected in the surface and bottom water. SMX and TMP were detected at a frequency of 100%. SDZ and SMZ were detected in surface water with a frequency of 75%, 66.6%, respectively and 75% in bottom water. Highest concentrations (∑SA=46.2 ng/L) were measured at station PR-1 which is located in the East River (Dongjiang). The East River is mostly influenced by domestic waste water discharge. It is the most economically developed region in China and has a high livestock production rate [48,49]. The detected concentrations are in similar range as those measured by Zhang et al. [48]. All concentrations decrease with distance from the city center (PR-1) towards the South China Sea (PR-12). Concentrations in surface and bottom water only differ slightly, it can be explained by the mixing between different water bodies [36]. SDZ and SMZ have higher attenuation factors (k=0.028 km^{-1} and k=0.036 km^{-1}, respectively) than SMX and TMP (k=0.024 km^{-1} and k=0.02 km^{-1}, respectively). Only SMZ and TMP have an increased concentration at PR-7 (9.2 ng/L and 22.1

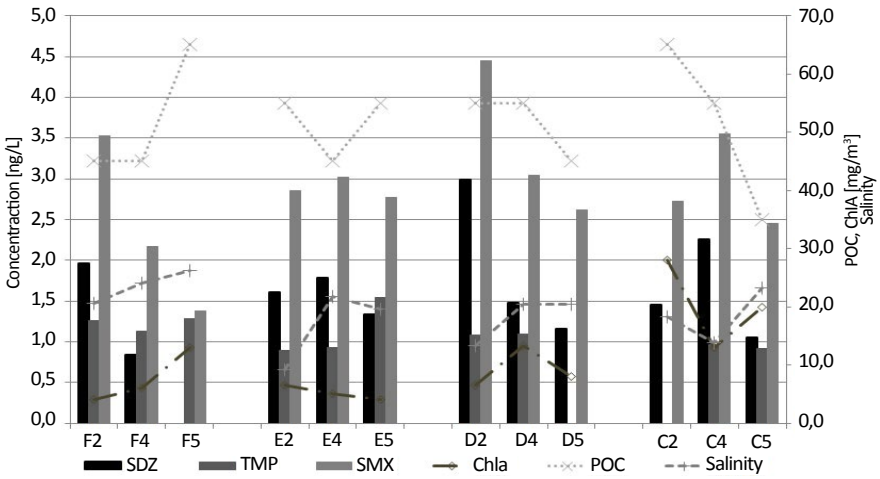

Figure 3 Concentration of sulfadiazine (SDZ), sulfamethoxazole (SMX) and trimethoprim (TMP) measured in East China Sea with particulate organic carbon (POC), chlorophyll a (ChlA) and salinity.

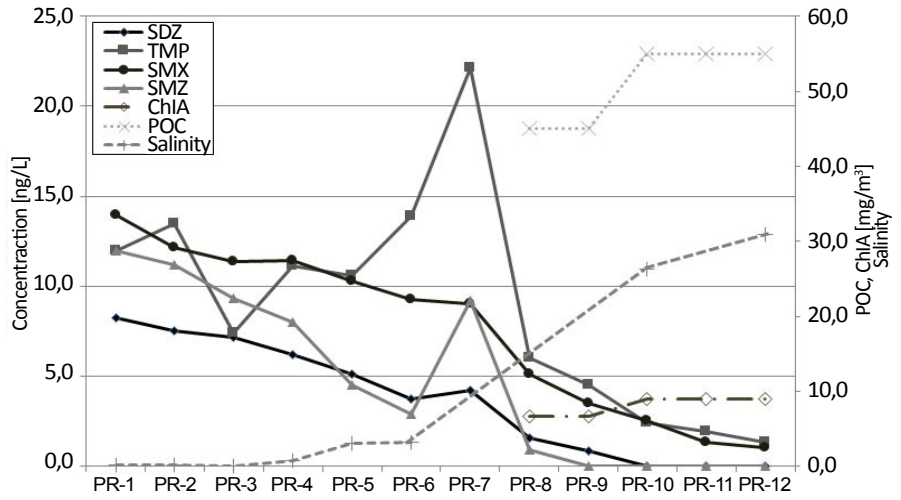

Figure 4 Pearl River estuary surface water concentrations of sulfadiazine (SDZ), sulfamerazine (SMZ), sulfamethoxazole (SMX) and trimethoprim (TMP) with POC, ChlA and salinity.

ng/L, respectively). As mentioned above SMZ is an unauthorized antibiotic and only used in veterinary applications [22,50]. A possible source for SMZ and TMP could be intensive aquacultural activities in the area. SMZ is the only SA which was not detected at every station, possibly reflecting a lower input due to its limited usage. SDZ and SMX in combination with TMP are not only used in veterinary applications but also in human applications to treat bacterial infections [12,51]. Like in the East China Sea, all SAs and TMP display an inverse correlation with salinity (∑r=-0.96). In the Pearl River Estuary the dilution effect is much more dominant than in the Huangpu River. On one hand the dilution is driven by the tidal stage of the river and the riverine input of the other tributaries [32,52]. On the other hand Xu et al. suggested that the occurrence and dilution may more depend on the daily waste water production cycle and its discharge than on the tidal stage [36]. The waste water treatment rate for SAs and TMP in the Pearl River Estuary region varies between 52-85% [8,53]. The region is heavily influenced by urban industry and agricultural activities and has high contribution to China's annual poultry, pig and aquaculture production [54]. Until now China has no requirements for livestock waste treatments, basically meaning it is directly discharged into the river [8]. As a consequence the discharge of treated and untreated waste water increases the concentration of SAs and other antibiotics in general in the aquatic environment [32]. A comparison with former studies shows that, higher concentrations are detected upstream than downstream. Nevertheless the concentrations downstream did not change significantly over time (2005-2016). Due to that it can be assumed that the Pearl River Estuary has been exposed to these antibiotics in low ng/L range for a long time. Leaving it up for discussion which long-term effect they might have on the biota and how significant the occurrence of antibiotic resistant genes (sulI and sulII) [55] **(Figures 1b and 4 and Tables 1 and 3)**.

Mass load into analyzed systems

An approximation of the mass load of SAs and TMP into the rivers was performed using the annual discharge volume (Huangpu River: 10 billion m^3, Pearl River Estuary: 336 billion m^3) and the average measured concentration (Huangpu River: 23.2 ng/L, Pearl River Estuary: 25.1 ng/L) [24,27]. Huangpu River discharges an estimated total amount of 232 kg per year into the Changjiang River. In comparison with the Pearl River Estuary, the Huangpu River is a more static system and mostly influenced by the discharge of the Tai Lake and some additional unidentified waste water. The sampled area is mostly influenced by industrial and urban activities. Due to the waste water policy of Shanghai, it is possible that the influence of the domestic waste water has declined in the urban area. For the Pearl River Estuary a mass load of approximately 8.4 t per year was estimated. The Pearl River Estuary is much more influenced by industrial and agricultural activities and waste water (treated and untreated). Moreover, its annual discharge volume is higher than the Huangpu River, which justifies the higher mass load.

Conclusion

Water samples from three different Chinese water systems (Huangpu River, East China Sea and Pearl River Estuary) were analysed for SAs and TMP. Antibiotics were detected in all three systems in low ng/L range. It is the first study reporting antibiotics in the East China Sea. In the Huangpu River SDZ, SMX and TMP occur at a stable concentration. The same three antibiotics were detected in the East China Sea, at slightly lower concentration than in the Huangpu River. The concentrations slightly decrease from coastal stations towards the open sea. Their occurrence in the open sea points out the long distance they can be transported and their possible stability in the marine environment. All four antibiotics were detected in the Pearl River Estuary. The concentrations of the Pearl River are in similar range as those in the Huangpu River. The mass load, however, is much higher because of the greater water volume. Furthermore, the concentrations in the Pearl River Estuary are stronger diluted than in the Huangpu River. Both cities pursue different water policies and waste water treatment management. However, the widespread co-existence of these antibiotics in the aquatic

environment indicates that the common sources are the discharge of treated and untreated water as well as the direct input via aquaculture [48]. Even though SAs are hydrophilic and mostly occur in the water phase, a future research focus should also be put on their occurrence in marine sediments [56-58].

Acknowledgement

This study was partly financed by the German Academic Exchange Service "DAAD-Doktorandenstipendium" (57212311) and the project of NSFC: 91228201. We thank Klaus Bohn (LPL) for logistical help. We also thank the working group of oceanography at Shanghai Jiao Tong University and especially Xi Ying and Yan Liu for their help and Li Bo (Zhejiang Ocean University) for the possibility to participate in the cruise with R/V ZheHai Ke 1. We are grateful for the help of Wang Yufeng and the team at Guangzhou Marine Geological Survey.

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