Marine Environmental Research 78 (2012) 26–33

Contents lists available at SciVerse ScienceDirect

Marine Environmental Research

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

Occurrence and distribution of antibiotics in the Beibu Gulf, China: Impacts of river discharge and aquaculture activities

Qian Zheng [a,b], Ruijie Zhang [a], Yinghui Wang [c], Xiaohui Pan [d], Jianhui Tang [d], Gan Zhang [a,*]

[a] State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, No. 511 Kehua Street, Tianhe District, Guangzhou 510640, Guangdong Province, China
[b] Graduate University of the Chinese Academy of Sciences, Beijing 100039, China
[c] School of the Environment, Guangxi University, Guangxi 530004, China
[d] Key Laboratory of Coastal Environmental Process, CAS, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

ARTICLE INFO

Article history:
Received 3 December 2011
Received in revised form
23 March 2012
Accepted 23 March 2012

Keywords:
Chemical pollution
Antibiotics
Aquaculture
River discharge
Risk assessment
Coastal zone
Beibu Gulf
China

ABSTRACT

The occurrence and distribution of eleven selected antibiotics belonging to three groups were investigated in the Beibu Gulf. In addition, the potential effects of water discharged from four rivers and aquaculture activities were analyzed. Erythromycin–H_2O, sulfamethoxazole and trimethoprim were the most frequently detected compounds, with mean concentrations ranging from 0.51 to 6.30 ng L^{-1}. The concentrations of the rivers were generally higher than those of the gulf, implying that river discharge has an important effect on the Beibu Gulf. The concentrations of erythromycin–H_2O, sulfamethoxazole and sulfadimidine in the vicinity of aquaculture activities were higher, suggesting that a higher intensity of aquaculture activities could contribute to increasing levels of antibiotics in the environment. According to MEC (measured environmental concentration)/PNEC (predicted no-effect concentration), erythromycin, sulfamethoxazole and clarithromycin may present possible environmental risk to *Pseudokirchneriella subcapitata*, *Synechococcus leopoliensis* and *P. subcapitata*, respectively; therefore, attention should be given to the long-term ecological effects caused by the continuous discharge of antibiotics in the Beibu Gulf.

1. Introduction

Since the initial step in antibiotic therapy made by Paul Ehrlich at the beginning of the 20th century, much effort has been made to develop new antibiotic compounds to treat infectious diseases. In addition to protecting human and animal health (Schauss et al., 2009), antibiotics are also used as growth promoters in animals(Kemper, 2008). In China, it has been estimated that the annual usage of raw antibiotic materials is about 180,000 tons (including health and agricultural use). This means that each person consumes 138 g of antibiotics every year, which is ten times higher than in America (Tian, 2010).

With the rising usage of antibiotics, the occurrence of antibacterial agents in the environment has led to increasing concerns about potential environmental risks and the maintenance and spread of antibacterial resistance among microorganisms (Duong et al., 2008). Although antibiotics can be extensively metabolized by humans and animals (Kummerer, 2009), they may be disseminated into the environment from both human and agricultural sources, as excretions, flushed, out-of-date prescriptions, medical waste, discharge from wastewater treatment facilities and leaks from septic systems and agricultural waste-storage structures among others. Meanwhile other dissemination pathways occur via land applications of human and agricultural waste, surface runoff and unsaturated zone transport (Sarmah et al., 2006). These compounds are partially removed by wastewater treatment plants (WWTPs) (Tamtam et al., 2008). If they are not eliminated during the purification process, they pass through the sewage system and may accumulate in the environment (Kummerer, 2009; McArdell et al., 2003). At present, many studies on antibiotic exposure in different environmental matrices have shown that traces of antibiotics are present in surface water, groundwater, sediment and soil (Duong et al., 2008). Resistance appears to have emerged and spread rapidly in many regions (Duong et al., 2008), especially in aquatic environments.

Coinciding with rapid population growth during the 20th century, there has been a sharp increase in demand for seafood products (Sapkota et al., 2008). As a result, aquaculture systems

* Corresponding author. Tel.: +86 20 8529 0805; fax: +86 20 8529 0130.
E-mail address: zhanggan@gig.ac.cn (G. Zhang).

0141-1136/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marenvres.2012.03.007

have undergone unprecedented growth, more so than any other animal food-producing sector in the world (Munoz et al., 2010). These systems are evolving to become a significant contributor to meet the demands for seafood (Sapkota et al., 2008). Moreover, hygienic shortcomings in raising methods, including increased stocking densities, the crowding of farming sites in coastal waters, a lack of sanitary barriers and a failure to isolate aquaculture base units with infected animals (Cabello, 2006; Reboucas et al., 2011; Taylor et al., 2011), have increased the possibility of rapid spreading of infection. This possibility also results in an augmented use of prophylactic antibiotics, often with the misguided goal of compensating for these sanitary shortcomings (Cabello, 2006). Given the knowledge of resistance development, it is possible that similar problems may exist in connection with the use of antibiotics in aquaculture (Holmstrom et al., 2003).

The Beibu Gulf, located in the northwest of South China Sea, ranges from Leizhou Peninsula, Qiongzhou Strait and Hainan Island to Vietnam, and extends to the Guangxi coast in the north (Ma et al., 2010), covering an area of approximately 12.8×10^4 km^2. It is a semi-closed gulf, with the widest part reaching 180 nautical miles (nm); it has an average depth of approximately 38 m and maximum depth of less than 100 m. The climate around this gulf is subtropical and monsoonal (Chen et al., 2009). An important geographical feature is its large number of estuaries, from which the rivers can discharge nutrients into the gulf. Abundant natural food is necessary for fish, shrimp and shellfish growth.

The Beibu Gulf has traditionally played an important role in the economies of China and Vietnam, by providing a highly productive and diverse marine products resources. Marine culture has been the main industry, providing sea products to the adjacent regions, and highly efficient, low pollution, large-scale marine culture has been encouraged by the government. Given that antibiotics are widely used in not only human medicine but also in aquaculture and animal husbandry, it is important to examine the current status and sources of antibiotic pollution. In addition to assessing their potential impact on the Beibu Gulf.

In this study, a monitoring program was conducted to determine the levels of target antibiotics and distribution characteristics in the Beibu Gulf and its tributary rivers; the effects of aquaculture activities in this area on the Beibu Gulf are discussed, and an environmental risk assessment of several antibiotics according to the MEC/PNEC is performed. The main monitoring locations of the Beibu Gulf are the Maowei Sea, Qinzhou Bay, Sanniang Bay, Maoling River, Qin River, Jingu River and Dafeng River.

2. Materials and methods

2.1. Chemicals and standards

Target compounds were selected according to information found in the literature on their occurrence and ubiquity in aquatic environment, as well as according to their human use and consumption worldwide (Ginebreda et al., 2010). Macrolides (MLs) and Sulfonamides (SAs) constitute an important group of pharmaceuticals in today's human and veterinary medicine practice. Therefore, the compounds selected for this study belong to three different antibacterial families (Feitosa-Felizzola and Chiron, 2009): macrolides, including clarithromycin (CTM), azithromycin (AZM), erythromycin–H_2O (ETM–H_2O), roxithromycin (RTM) and spiramycin (SRM); sulfonamides consist of sulfamethoxazole (SMX), sulfadiazine (SDZ), sulfadimidine (SMZ), sulfacetamide (SAAM) and sulfathiazole (STZ); and trimethoprim (TMP). $^{13}C_3$-caffeine solution used as surrogate standard was obtained from Cambridge Isotope Labs (1 mg mL^{-1} in methanol, USA). All antibiotic compounds were dissolved in methanol and stored in a freezer. Many reports have shown that ETM–H_2O is the predominant form of ETM in aquatic environment (Xu et al., 2007b).

Methanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Formic acid was purchased from CNW (Germany). Disodium edetate dihydrate (Na_2EDTA) of analytical grade was obtained from Tianjin Chemical (Tianjin, China). Ultra-pure water was prepared with a Milli-Q water purification system (Millipore, Bedford, MA, USA). Unless otherwise indicated, the chemicals used in the analysis were analytical grade or higher.

2.2. Sample collection

A total of thirty-five seawater samples from the Beibu Gulf and seventeen river water samples from four rivers were collected in October, 2010 (Fig. 1). All of the seawater samples were collected on a fishing boat, and the river water samples were taken from bridges at the centroid of the flow. All of the samples were collected (approximately 0–50 cm below the surface) using a stainless steel bucket and were immediately transferred to a 5-L pre-cleaned amber glass bottle. The bottle was rinsed with a sample prior to sampling. The samples were kept at 4 °C in a cold storage room before further treatment and analysis in the laboratory.

2.3. Sample extraction and analysis

Antibiotics in water were concentrated through solid-phase extraction (SPE) using an Oasis HLB cartridge, following the method described by Xu et al. (2007b) (Xu et al., 2007a). In summary, 1.2 L of water sample was filtered through 0.45-μm glass fiber filters and was then acidified to pH = 3.0. The HLB column was then rinsed with 10 mL of ultra-water (pH 3.0) and dried under nitrogen gas for 1 h. After drying, the cartridge was eluted with methanol.

For the recovery experiments, 1.2 L of filtered seawater or river water fortified with 100 ng of target analytes was treated using the same procedure used for the field samples. The extracted antibiotics were analyzed using high-performance liquid chromatography–electrospray ionization tandem mass spectrometry (HPLC-ESI–MS-MS) with multiple reaction monitoring (MRM). The separation of the target compounds were performed with Agilent 1200 series (Agilent, Palo Alto, USA) on an Agilent ZorbaxXDB-C18 column (2.1 mm × 50 mm, 1.8 μm) with a guard column Security Guard^{TM}C18(4.0 mm × 3.0 mm). For mass

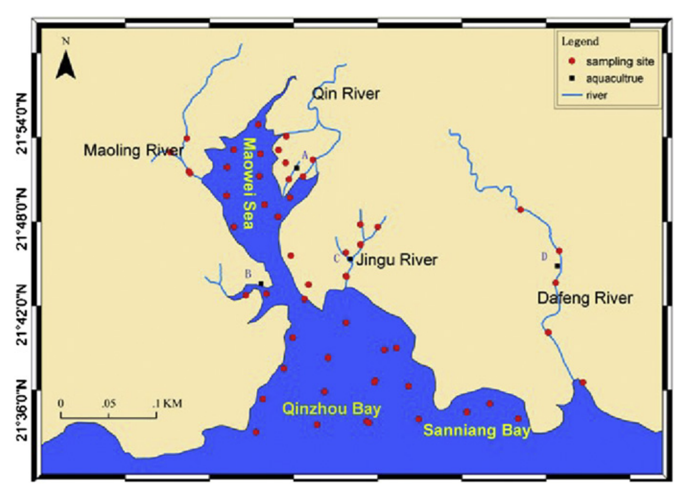

Fig. 1. Water sampling locations from the Beibu Gulf and the four rivers.

spectrometric analysis, Agilent 6460triple quadrupole mass spectrometer (Agilent, Palo Alto, USA) equipped with an electrospray ionization source in the positive mode (ESI+) was used to analyze the antibiotics. The conditions for the liquid chromatography and mass spectrometry have been described in previous studies (Tang, 2009; Tang et al., 2009; Zhang, 2011; Zhang et al., 2012a).

2.4. Quality analysis and quality control

Quantitative analysis of each compound was performed using HPLC-ESI–MS-MS in the MRM mode, employing two of the highest characteristic precursor ion/product ion transitions. Together with the retention times, the characteristic ions were used to ensure correct peak assignment and peak purity. $^{13}C_3$-caffeine was chosen to be a surrogate standard in the antibiotics experiment in many papers (Xu et al., 2007b; Zou et al., 2011). Therefore, we also use this substance as the surrogate standard to all samples prior to enrichment to avoid possible losses during the analytical procedure. The recovery rates of for these spiked antibiotics in seawater and river water were higher than 68% and 70%, respectively.

The limits of detection (LOD) were defined as signal-to-noise (S/N) ratios of 3 and the limits of quantification (LOQ) were defined as a signal-to-noise (S/N) ratios of 10. The results indicated that the LOQs for each compound in seawater and river water were from 0.15 to 4.4 ng L^{-1} and from 0.18 to 5.0 ng L^{-1}, respectively.

Recoveries of the eleven target compounds were determined for seawater and river water using the standards addition method. The standards addition was employed to compensate for matrix effects. The concentrations of all target antibiotics were then determined in the seawater and river water spiked with antibiotics as well as in the same seawater and river water without the antibiotic addition. The high recoveries ((78 ± 6) %) of the added antibiotics suggest that matrix effects were relatively minor. Field and procedural blanks were treated as controls for possible contamination in the laboratory and in field sampling. Analysis of these blanks demonstrated that the extraction and sampling procedures were free of contamination.

3. Results and discussions

3.1. Occurrence of the selected antibiotics in the Beibu Gulf

A total of eleven antibiotic compounds grouped into three classes were detected in the Beibu Gulf, and the levels of all the selected compounds were generally found to be in the ng L^{-1} range. Except for SAAM and SRM, all of the selected antibiotics were detected in at least one sample. ETM–H_2O was the predominant antibiotic and was detected with the highest frequency (100%) and at the highest concentrations (1.10–50.9 ng L^{-1}); this is most likely because it is the most commonly prescribed drugs for human and animal and must be widely used around the Beibu Gulf. A relatively high frequency (97.1%) and high concentrations (up to 10.4 ng L^{-1}) of SMX were detected in this gulf; SMX is the most frequently detected sulfonamide in groundwater due to the fact that it does not degrade easily and is hydrophilic enough to move into the aquatic environments (Kolpin et al., 2002; Tang, 2009; Tang et al., 2009; Xu et al., 2007a, 2007b). Perhaps because TMP is used as a synergist of sulfonamides, not only in combination with SMX in human medicine but also with SDZ in aquaculture (Graslund and Bengtsson, 2001), it showed the third highest detection frequency of 88.6%, with concentrations up to 3.77 ng L^{-1}. It could be seen from Table 1 that the concentrations of the above three antibiotics are comparable to those measured in the Elbe River, Germany (Wiegel et al., 2004), Tyne River, United Kingdom (Roberts and Thomas, 2006) and the Mekong Delta, Vietnam (Managaki et al., 2007), but are much lower than those found in the Pearl River, South China, even in the low-water season (Xu et al., 2007b) and in 139 streams studied in America (Kolpin et al., 2002).

The remaining six compounds were detected at levels higher than the LOQ in the seawater, with detection frequencies of 10%~50%. The low treatment effect of sulfonamides has led to a gradual replacement by macrolides and other antibiotics in China over the past 10 years, they are still used in poultry and aquaculture due to their low cost (Zou et al., 2011). Owing to the aquaculture source in this gulf, the frequencies of SMZ and SDZ were both 42.9%, with concentrations up to 3.41 ng L^{-1} and 3.39 ng L^{-1}, respectively.

Table 1
Global concentration comparison of eight antibiotics in surface water.

Sampling locations	Concentrations (ng L^{-1})								References
	ETM–H_2O	RTM	AZM	CTM	SDZ	SMX	SMZ	TMP	
Beibu Gulf, China	1.10–50.9	nd[a]–0.53	nd–0.64	nd–0.72	nd–3.41	nd–10.4	nd–3.39	nd–3.77	This study
Bohai Bay, China	nd–150(30)	nd–630(113)	na[b]	na	nd–41(17)	nd–140(19)	nd–130(37)	nd–120(54)	(Zou et al., 2011)
Leizhou Bay, China	0.9–8.5	nd–1.5	nd–1.2	nd–0.82	nd–0.43	1.5–82	nd–1.5	1.3–330	(Zhang et al., 2012b)
Pearl River, South China	13–423(63) [c]	nd–105(20)	na	na	3–141(46)	2–165(52)	4–179(59)	na	(Xu et al., 2007b)
	423–636(489)[d]	13–169(70)	na	na	135–336(218)	111–193(143)	107–323(210)	na	
Mekong Delta, Vietnam	9–12	nd	nd	nd	nd	20–33	<50	5–20	(Managaki et al., 2007)
Tamagawa River, Japan	21–448	<60	<500	<300	<150	4–23	nd	<100	
Victoria Harbour, Hong Kong, China	4.7–1900(213)	nd–47 (19)	na	na	na	nd–47.5 (13)	nd–8.6	2.6–216(52)	(Minh et al., 2009)
Huangpu River, China	na	0.13–9.93	na	na	1.4–40.6	4.9–55.2	2.1–623.3	2.2–62.4	(Jiang et al., 2011)
Seine River, France	na	na	na	na	na	40–140	<10,4.4%	nd–36(16)	(Tamtam et al., 2008)
Elbe River, Germany	30–40	<30–40	na	<30–40	na	30–70	<30	<30–40	(Wiegel et al., 2004)
139 streams, America	1700(21.5%) [e]	180(4.8%)	na	na	nd	520 (19%)	120(4.8%)	300(27.4%)	(Kolpin et al., 2002)
Youngsan River, South Korea	0–450(120)	nd	na	na	10–20	0–110	10–20	10–20	(Kim and Carlson, 2007)
Tyne River, United Kingdom	4–70	na	na	na	na	<20	na	4–19	(Roberts and Thomas, 2006)
Five estuaries, United Kingdom	<4	na	na	na	na	<20	na	<4–569	(Thomas and Hilton, 2004)

[a] Not detected(below the LOQ).
[b] Not analyzed.
[c] Range (mean concentration) in high-water season.
[d] Range (mean concentration) in low-water season.
[e] Max concentration (detection frequency).

Table 2
Antibiotic concentrations in water samples from the Maoling, Qin, Jingu and Dafeng Rivers (ng L^{-1})

Sampling Location		Sulfonamides				Macrolides				Trimethoprim
		SDZ	STZ	SMZ	SMX	ETM	CTM	AZM	RTM	TMP
Maoling River ($n = 3$)	Range	nd–0.24	nd–0.22	nd–0.49	nd–6.83	2.59–4.28	nd	nd	nd–0.24	0.39–0.80
	Detection Rate (%)	33	33	67	67	100	–	–	33	100
Qin River ($n = 4$)	Range	0.65–4.80	nd–0.24	0.62–6.57	2.63–15.9	7.62–47.6	0.26–0.34	0.28–0.76	0.27–0.48	2.27–3.51
	Detection Rate (%)	100	50	100	100	100	100	100	100	100
Jingu River ($n = 5$)	Range	nd–0.35	nd–0.76	0.22–0.52	4.44–10.5	5.39–18.2	nd	Nd	nd–0.43	0.41–4.11
	Detection Rate (%)	40	40	100	100	100	–	–	40	100
Dafeng River ($n = 5$)	Range	nd–1.10	nd–0.22	nd–0.35	0.65–1.81	3.37–19.2	nd	nd–0.21	nd–0.35	nd–1.30
	Detection Rate (%)	60	20	40	100	100	–	20	20	80
Total Detection Rate (%)($n = 17$)		59	35	76	94	100	24	29	47	94

Although macrolides are widely used in human drugs, the corresponding figures for RTM, CTM and AZM were 31.4%, 25.7% and 25.7%, respectively, and their concentrations were all below 1 ng L^{-1}. It may be that these pharmaceuticals, which come from the industrial and municipal wastewater of rivers, were degraded and diluted as they travel over long distances. STZ had the minimal detection frequency, 11.4%, with concentrations lower than 0.5 ng L^{-1}, most likely because it has severe side effects and is not as commonly used now.

Regarding its spatial distribution, the Beibu Gulf can be divided into two parts: a semi-closed sea, the Maowei Sea, and open waters, including the Qinzhou Bay and Sanniang Bay. According to our data, the antibiotic concentrations gradually decreased from the semi-closed sea to the open waters; this trend is reasonable and can be explained by three factors. First, the Qin River is the mother river of Qinzhou city and its wastewater is discharged into the Maowei Sea. Second, there are two aquaculture bases in this semi-closed sea; it is known that the pollution of the Maowei Sea mainly results from the combined effects of river discharge and aquaculture activities. Third, there are no centralized, large-scale sources in the open bays, and the open bays are large enough that the exchange of marine water between the bays and the outside sea is highly efficient for the dispersion of pollutants.

3.2. Occurrence of the selected antibiotics in rivers

Similar to the Beibu Gulf, eleven target compounds were investigated in four rivers, the Qin River, the Maoling River, the Jingu River and the Dafeng River. Table 2 shows a summary of the antibiotic concentrations in the four rivers.

Of the detected antibiotics, ETM–H_2O, SMX and TMP had higher detection frequencies and were the main compounds found in these rivers; this finding reflects, to some extent, the importance of river discharge to the Beibu Gulf. ETM–H_2O was detected in all 17 samples, with concentrations ranging from 2.59 to 47.6 ng L^{-1}. SMX and TMP were found at the same frequency of 94%, with concentrations up to 15.9 ng L^{-1} and 4.11 ng L^{-1}, respectively. Although the frequencies of CTM, AZM, RTM and STZ were all greater than 20%, their concentrations were lower than 1 ng L^{-1}, which may reflect rather low consumption in this region. Obviously, the concentrations of most of the antibiotics studied are much lower than those found in the Haihe River and its tributaries in China (Zou et al., 2011), and similar to those observed in the Elbe River, Germany (Wiegel et al., 2004) and the Tyne River, United Kingdom (Roberts and Thomas, 2006).

The Maoling River and the Qin River are located in the Maowei Sea estuary, as the Qin River is the longest river and flows through highly populated areas of Qinzhou city, it is inevitable that sewage from the city would be discharged into it. Except for SAAM, SRM and STZ, all of the antibiotics studied were detected in the four samples. However, likely due to its lower usage and the greater self-purification caused by its large flow and drainage area, the concentrations in the Maoling River were very low, the highest concentration was only 6.83 ng L^{-1}. The Jingu River and the Dafeng River both discharged into open bays. The Jingu River is upstream of the Qinzhou Bay and intermittently transports water into the bay; however, the highest concentration among all of the antibiotics studied was only 18.2 ng L^{-1}. Furthermore, the concentrations of seven antibiotics were near the LOQ. Due to tidal effects, the water of the downstream estuarine area in the Dafeng River is expected to mix with seawater. As a consequence, the pollutants from the Dafeng River should enter Sanniang Bay and affect its environmental quality. Nevertheless, except for ETM–H_2O, the concentrations and frequencies of the compounds were very low.

In general, the dilution factors from point source discharges to receiving surface waters such as rivers and streams are on the order of tens to hundreds (Minh et al., 2009). However, the average concentrations of the Qin River, Jingu River and Dafeng River were

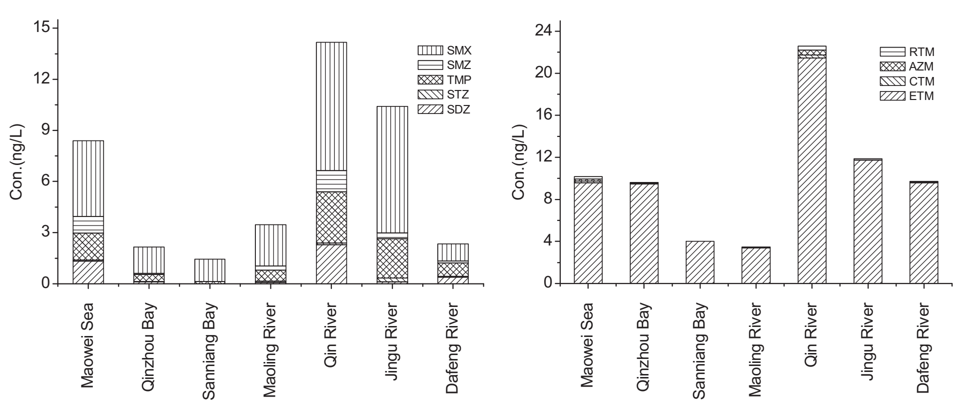

Fig. 2. Antibiotic concentration (mean) profiles for the Beibu Gulf and the four rivers.

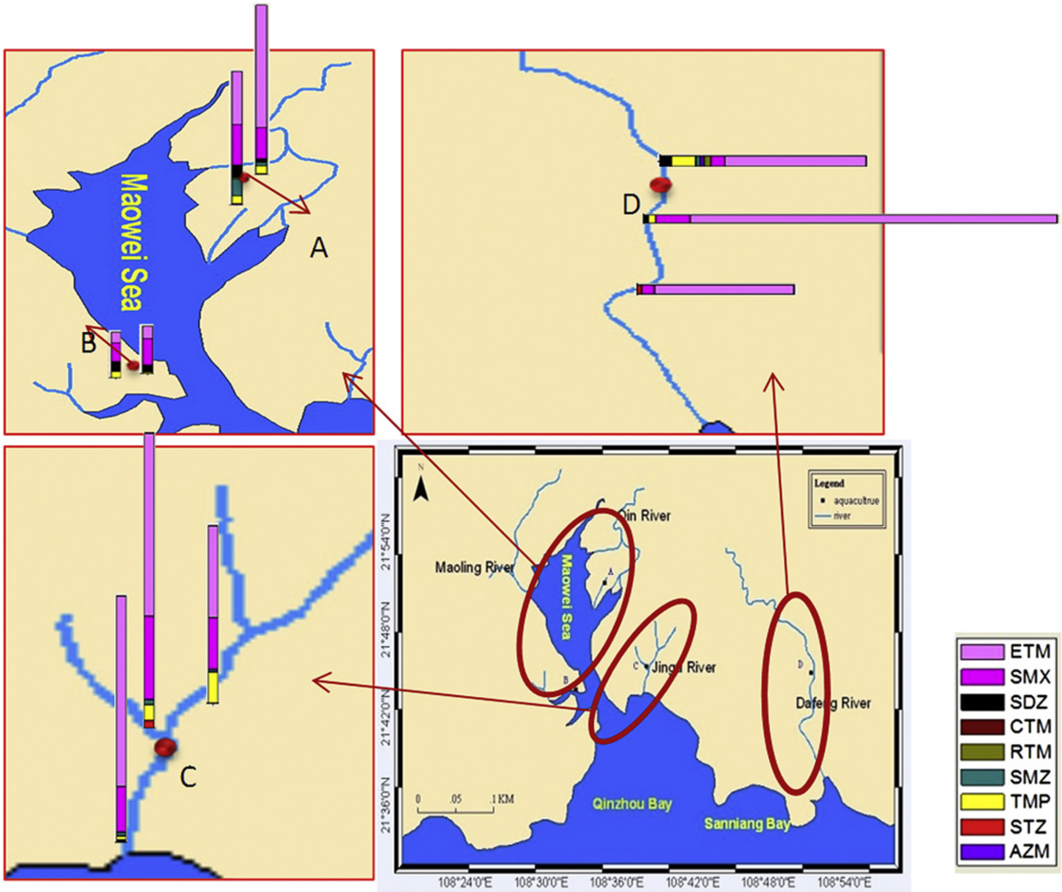

Fig. 3. Antibiotic concentrations in the aquaculture bases.

only twice those in the gulf; even the concentration in the Maoling River was lower than that of the Maowei Sea (Fig. 2), which indicates that except sewage, wastewater, there are other pollution sources in the Beibu Gulf, such as, aquaculture. Based on the average concentrations, the evidence here shows that the four rivers all contribute to the antibiotic content of the gulf and that the Qin River has the greatest influence on the gulf compared to the other three rivers, which emphasizes the effects of human activities on antibiotic exposure in the environment. However, on the whole, the impacts are not significant.

3.3. Impacts of aquaculture activities

The Beibu Gulf is famous for its many kinds of fish, shrimp and shellfish, especially in the Maowei Sea. The gulf has the largest natural oyster seedling bases in China with an annual production reaching one billion ton. The seedlings not only use in this locality, but also support Guangdong, Fujian, Hainan and other provinces. It is known that antibiotics are widely used in human and veterinary drugs for preventing or treating infections, as well as for promoting growth (Holmstrom et al., 2003; Smith et al., 2002). However, aquatic products can easily become infected due to large stocking densities; to avoid disease and reduce losses, antibiotic over-use frequently occurs in artificial breeding.

The concentrations of eleven antibiotics were analyzed in the vicinity of oyster bases and shrimp farms in the Beibu Gulf. Fig. 3 exhibits antibiotic concentration profiles for the oyster bases and shrimp farms. In general, three compounds, SMX, SDZ and ETM–H_2O were found in the water samples, with concentrations ranging from 2.3 to 19.2 ng L^{-1}. Although the concentrations were not high and were much lower than those observed in other aquatic environments, the kinds of antibiotics mainly used in aquaculture could be determined according to concentration changes in the sampling sites. There are several potential reasons why antibiotic concentrations are low. Firstly, the purification of the Beibu Gulf promotes the diffusion of antibiotics; secondly, the consumption of antibiotics is most likely very low and is effectively controlled by the government.

ETM–H_2O, SMX and SDZ were the predominant compounds around the oyster bases. Although ETM and SMX are usually used in human medicines, their use as veterinary drugs introduces

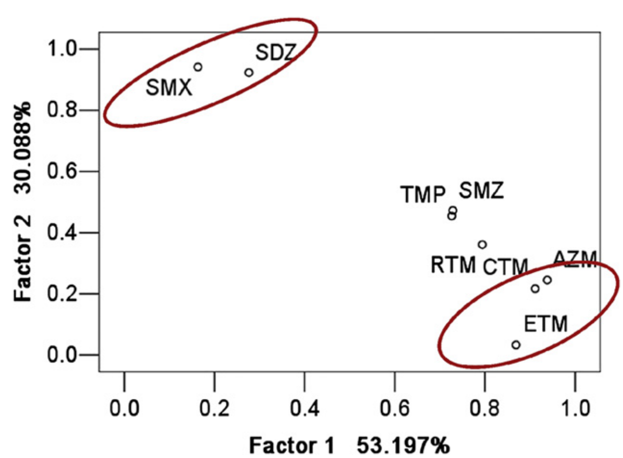

Fig. 4. Principal components analysis for the Maowei Sea.

Table 3
Aquatic toxicity data of twelve antibiotics to the most sensitive aquatic species.

Compound	Non-target organism	Toxicity data (mg L^{-1})	Toxicity	AF[a]	PNEC[b] (ng L^{-1})	Reference
TMP	*R. salina*	EC_{50}[c]16	Acute	1000	16,000	(Lutzhoft et al., 1999)
SMX	*S. leopoliensis*	$EC_{50} = 0.027$	Acute	1000	27	(Ferrari et al., 2004)
SMZ	*S. vacuolatus*	$EC_{50} = 19.52$	Chronic	1000	19,520	(Bialk-Bielinska et al., 2011)
SDZ	*S. capricornutum*	$EC_{50} = 2.2$	Acute	1000	2200	(Eguchi et al., 2004)
ETM	*P. subcapitata*	$EC_{50} = 0.02$	Chronic	1000	20	(Isidori et al., 2005)
RTM	*P. subcapitata*	NOEC[d] = 0.01	Chronic	100	100	(Yang et al., 2008)
CTM	*P. subcapitata*	$EC_{50} = 0.002$	Chronic	1000	2	(Isidori et al., 2005)
AZM	*Daphnia* sp.	$EC_{50} > 120$	Acute	1000	>120,000	(FDA-CDER, 1996)

[a] AF: Assessment factor.
[b] PNEC: Predicted no-effect concentration.
[c] EC_{50}: half maximal effective concentration.
[d] NOEC: no observable effect concentration.

another pathway for these compounds to enter into the environment; for example, SMX is used in veterinary medicine in France (Tamtam et al., 2008), and ETM is used in shrimp hatcheries in southeast Asia (Gesamp, 1997). Using principal component analysis (PCA) (Fig. 4), SMX and SDZ were grouped together indicating that these two antibiotics may have similar sources and environmental occurrence rates, while SDZ is well known to be widely used in veterinary medicine, so SMX and SDZ had the same application, and were mainly applied to the oyster bases. Also based on PCA, ETM–H_2O is classified with CTM, AZM and RTM which are pharmaceuticals; hence, it can be deduced that, in spite of being widely used as veterinary and human medicine, the source of ETM–H_2O is dominated by human drugs in the Maowei Sea.

Near the shrimp farms, only ETM–H_2O was detected, with the highest concentrations in the wastewater samples; the other compounds were not found in any of the samples collected from the shrimp farms. This result reveals that ETM is probably the main antibiotic used in the shrimp farms.

The wastewater from these bases or farms will eventually discharge into rivers or the Beibu Gulf directly via sewage runoff lines. Therefore, aquaculture activities contribute to the presence of antibiotics in the Beibu Gulf; although due to limitations of analytical techniques, we did not know the exactly data of bioavailability of antibiotics in the oyster and shrimp, the low levels of antibiotics near the aquaculture bases reveal that the antibiotic residues caused by aquaculture activities are not significant, therefore, we can infer the aquatic products are not seriously contaminated with antibiotics.

3.4. Environmental risk assessment and significance of results

Due to the large amounts of these compounds released into the environment and their potential to exert biological effects, the risk they present to the environment cannot be ignored (Fent et al., 2006).

A risk quotient (RQ) is usually calculated from a predicted or measured environmental concentration (PEC or MEC, respectively) and a predicted no-effect concentration (PNEC). According to the TGD, when only short-term/acute toxicity data EC_{50}/LC_{50} are available, the calculation of PNEC is obtained from EC_{50}/LC_{50} divided by an assessment factor of 1000. Once long-term/chronic no observed effect concentration (NOEC) values for one, two or three trophic levels are available, an assessment factor of 100, 50 or 10 is used (EC, 2003). While in some studies, an assessment factor of 1000 was used long-term/chronic EC_{50}/LC_{50} values, although the assessment factor reduces the degree of uncertainty in the extrapolation from the test data on a limited number of species compared to the real environment (Isidori et al., 2005). In this study, many acute or chronic toxicity data of the selected antibiotics on non-target organisms were collected from literatures, PNEC values were calculated based on the toxicity data and shown in the Table 3. And the RQ values of the target antibiotics calculated according to the relational expression are listed in Table 4. The ratios found here were classified into three risk levels: low (values between 0.01 and 0.1), medium (values between 0.1 and 1) and high (values above 1)(de Souza et al., 2009).

Although the selected antibiotics in the Beibu Gulf and the four rivers studied were detected at low concentrations, the calculation results show that ETM might present a significant chronic environmental risk to the *Pseudokirchneriella subcapitata*; SMX and CTM have acute effects to *Synechococcus leopoliensis* and Chronic effects to *P. subcapitata*, respectively, and the RQs of TMP, SDZ, RTM and AZM are all below 0.01. In the Maoling River, Jingu River and Dafeng River, the RQ values of ETM and SMX are almost greater than 0.1, corresponding to moderate effects, while the other compounds have low environmental risks to the species. The results for the Qin River are similar to those of the Beibu Gulf. ETM, SMX, and CTM

Table 4
MECs and PNECs for 8 kinds of antibiotics.

Compounds	PNEC[a] (ng L^{-1})	Maximum MEC[b] (ng L^{-1})					RQ[c] (Maximum MEC/PNEC)				
		Beibu Gulf	Maoling River	Qin River	Jingu River	Dafeng River	Beibu Gulf	Maoling River	Qin River	Jingu River	Dafeng River
TMP	16,000	3.77	0.80	3.51	4.11	1.30	0.00024	0.00005	0.00022	0.00026	0.000081
SMX	27	10.4	6.83	15.9	10.5	1.81	0.38	0.25	0.59	0.39	0.067
SMZ	19,520	3.39	0.49	6.57	0.52	0.34	1.7×10^{-4}	2.5×10^{-5}	3.4×10^{-4}	2.7×10^{-5}	1.7×10^{-5}
SDZ	2200	3.41	0.24	4.80	0.35	1.10	0.0016	1.1×10^{-4}	0.0022	1.6×10^{-4}	0.0005
ETM	20	50.9	4.28	47.6	18.2	19.2	2.545	0.214	2.38	0.91	0.96
RTM	100	0.53	0.24	0.48	0.43	0.35	0.0053	0.0024	0.0048	0.00426	0.0035
CTM	2	0.72	nd[d]	0.34	nd	0.21	0.36	–	0.17	–	0.10
AZM	>120,000	0.64	nd	0.76	nd	nd			–		

[a] PNEC: Predicted no-effect concentration.
[b] MEC: Measured environmental concentration.
[c] RQ: Risk quotient.
[d] nd: not detected(below the LOQ).

have been found to be some of the most harmful compounds for aquatic environment with regard to environmental risk (Isidori et al., 2005). For example, in Italy, ETM in aquatic environments has been selected as one of a restricted group of priority pollutants identified by three conserved standards, thus, it is worthwhile to pay attention to its levels in surface waters (Lee et al., 2008). Apart from chemical pollution caused by the antibiotics themselves, the use of antibiotics may also accelerate the development of antibiotic resistance genes (ARGs) and bacteria, which could lead to health risks to humans and animals (Kummerer, 2004; Taylor et al., 2011; Zhang et al., 2009). One of the major factors causing the collapse of the Taiwan shrimp farming industry in 1988 was the indiscriminate use of antibiotics, which resulted in resistant pathogens (Lin, 1989). The development of bacterial antibiotic resistance in aquaculture environments could contribute to bacterial antibiotic resistance occurring among human populations (Sapkota et al., 2008).

4. Conclusions

The aim of this study was to examine the anthropogenic impact of target antibiotics in the Beibu Gulf. The most frequently detected contaminants were ETM–H_2O, SMX and TMP. Due to the strong hydrodynamic action of the gulf, which acts to deliver and diffuse these substances (Benmao and Manxin, 2004) and the government control that ensures low consumption levels, the concentrations of the compounds were all found in the ng L^{-1} level. Antibiotic pollution in this area is not serious, and while river discharge and aquaculture activities both introduce antibiotics into the Beibu Gulf, they do not have a significant effect. However, according to their RQs, ETM still presents a high aquatic environmental risk to *P. subcapitata*; SMX and CTM also have acute effects to *S. leopoliensis* and Chronic effects to *P. subcapitata*, respectively. Therefore, the monitoring of drug use in pharmaceutical and aquaculture activities should be encouraged to improve the management of antibiotics and promote their rational use to benefit public health and food safety (Reboucas et al., 2011). And with a growing number of antibiotics application to our lives, other structurally diverse and clinically relevant drugs of antibiotics are also used widely. So, the further research which are able to determine the extent of environmental pollution is also urgent needed.

Acknowledgment

This study was supported by the Knowledge Innovative Program of the Chinese Academy of Sciences (KZCX2-YW-Q02-01), Guangxi Natural Science Foundation(2010GXNSFE013006), and the CAS/SAFEA International Partnership Program for Creative Research Teams (KZCX2-YW-T001)

References

Benmao, H., Manxin, W., 2004. The characteristics of the bionomical environment in Qinzhou bay and the relationship between the characteristics and the self-purifying conditions of the water body. Marine Science Bulletin 24, 50–54.

Bialk-Bielinska, A., Stolte, S., Arning, J., Uebers, U., Boeschen, A., Stepnowski, P., Matzke, M., 2011. Ecotoxicity evaluation of selected sulfonamides. Chemosphere 85, 928–933.

Cabello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental Microbiology 8, 1137–1144.

Chen, Z., Xu, S., Qiu, Y., Lin, Z., Jia, X., 2009. Modeling the effects of fishery management and marine protected areas on the Beibu Gulf using spatial ecosystem simulation. Fisheries Research (Amsterdam) 100, 222–229.

de Souza, S.M.L., de Vasconcelos, E.C., Dziedzic, M., de Oliveira, C.M.R., 2009. Environmental risk assessment of antibiotics: an intensive care unit analysis. Chemosphere 77, 962–967.

Duong, H.A., Pham, N.H., Nguyen, H.T., Hoang, T.T., Pham, H.V., Pham, V.C., Berg, M., Giger, W., Alder, A.C., 2008. Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in Hanoi, Vietnam. Chemosphere 72, 968–973.

EC, 2003. European Commission Technical Guidance Document in Support of Commission Directive 93//67/EEC on Risk Assessment for New Notified Substances and Commission Regulation(EC) No 1488/94 on Risk Assessment for Existing Substance, Part II. In: Commission, E.(ED.), PP., pp. 100–103.

Eguchi, K., Nagase, H., Ozawa, M., Endoh, Y.S., Goto, K., Hirata, K., Miyamoto, K., Yoshimura, H., 2004. Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. Chemosphere 57, 1733–1738.

FDA-CDER, 1996. Retrospective Review of Ecotoxicity Data Submitted in Environmental Assessments (Docket No. 96N-0057). FDA Center for Drug Evaluation and Research, Rockville, MD, USA.

Feitosa-Felizzola, J., Chiron, S., 2009. Occurrence and distribution of selected antibiotics in a small Mediterranean stream (Arc River, Southern France). Journal of Hydrology 364, 50–57.

Fent, K., Weston, A.A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. Aquatic Toxicology 76, 122–159.

Ferrari, B., Mons, R., Vollat, B., Fraysse, B., Paxeus, N., Lo Giudice, R., Pollio, A., Garric, J., 2004. Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environmental Toxicology and Chemistry 23, 1344–1354.

Gesamp, 1997. Towards safe and effective use of chemicals in coastal aquaculture. GESAMP Reports and Studies 0 (I–VI), 1–40.

Ginebreda, A., Munoz, I., Lopez de Alda, M., Brix, R., Lopez-Doval, J., Barcelo, D., 2010. Environmental risk assessment of pharmaceuticals in rivers: relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). Environment International 36, 153–162.

Graslund, S., Bengtsson, B.E., 2001. Chemicals and biological products used in south-east Asian shrimp farming, and their potential impact on the environment - a review. Science of the Total Environment 280, 93–131.

Holmstrom, K., Graslund, S., Wahlstrom, A., Poungshompoo, S., Bengtsson, B.E., Kautsky, N., 2003. Antibiotic use in shrimp farming and implications for environmental impacts and human health. International Journal of Food Science and Technology 38, 255–266.

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L., Parrella, A., 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. Science of the Total Environment 346, 87–98.

Jiang, L., Hu, X.L., Yin, D.Q., Zhang, H.C., Yu, Z.Y., 2011. Occurrence, distribution and seasonal variation of antibiotics in the Huangpu River, Shanghai, China. Chemosphere 82, 822–828.

Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecological Indicators 8, 1–13.

Kim, S.C., Carlson, K., 2007. Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. Environmental Science & Technology 41, 50–57.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environmental Science & Technology 36, 1202–1211.

Kummerer, K., 2004. Resistance in the environment. Journal of Antimicrobial Chemotherapy 54, 311–320.

Kummerer, K., 2009. Antibiotics in the aquatic environment – a review – Part I. Chemosphere 75, 417–434.

Lee, Y.J., Lee, S.E., Lee, D.S., Kim, Y.H., 2008. Risk assessment of human antibiotics in Korean aquatic environment. Environmental Toxicology and Pharmacology 26, 216–221.

Lin, C.K., 1989. Shrimp culture in Taiwan: what went wrong? World Aquaculture 20, 19–20.

Lutzhoft, H.C.H., Halling-Sorensen, B., Jorgensen, S.E., 1999. Algal toxicity of antibacterial agents applied in Danish fish farming. Archives of Environmental Contamination and Toxicology 36, 1–6.

Ma, F., Wang, Y., Li, Y., Ye, C., Xu, Z., Zhang, F., 2010. The application of geostatistics in grain size trend analysis: a case study of eastern Beibu Gulf. Journal of Geographical Sciences 20, 77–90.

Managaki, S., Murata, A., Takada, H., Tuyen, B.C., Chiem, N.H., 2007. Distribution of macrolides, sulfonamides, and trimethoprim in tropical waters: ubiquitous occurrence of veterinary antibiotics in the Mekong Delta. Environmental Science & Technology 41, 8004–8010.

McArdell, C.S., Molnar, E., Suter, M.J.F., Giger, W., 2003. Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley Watershed, Switzerland. Environmental Science & Technology 37, 5479–5486.

Minh, T.B., Leung, H.W., Loi, I.H., Chan, W.H., So, M.K., Mao, J.Q., Choi, D., Lam, J.C.W., Zheng, G., Martin, M., Lee, J.H.W., Lam, P.K.S., Richardson, B.J., 2009. Antibiotics in the Hong Kong metropolitan area: ubiquitous distribution and fate in Victoria Harbour. Marine Pollution Bulletin 58, 1052–1062.

Munoz, I., Martinez Bueno, M.J., Agueera, A., Fernandez-Alba, A.R., 2010. Environmental and human health risk assessment of organic micro-pollutants occurring in a Spanish marine fish farm. Environmental Pollution 158, 1809–1816.

Reboucas, R.H., de Sousa, O.V., Lima, A.S., Vasconcelos, F.R., de Carvalho, P.B., Silva dos Fernandes Vieira, R.H., 2011. Antimicrobial resistance profile of Vibrio species isolated from marine shrimp farming environments (Litopenaeus vannamei) at Ceara, Brazil. Environmental Research 111, 21–24.

Roberts, P.H., Thomas, K.V., 2006. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Science of the Total Environment 356, 143–153.

Sapkota, A., Sapkota, A.R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., Lawrence, R., 2008. Aquaculture practices and potential human health risks: current knowledge and future priorities. Environment International 34, 1215–1226.

Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65, 725–759.

Schauss, K., Focks, A., Heuer, H., Kotzerke, A., Schmitt, H., Thiele-Bruhn, S., Smalla, K., Wilke, B.M., Matthies, M., Amelung, W., Klasmeier, J., Schloter, M., 2009. Analysis, fate and effects of the antibiotic sulfadiazine in soil ecosystems. Trac-Trends in Analytical Chemistry 28, 612–618.

Smith, D.L., Harris, A.D., Johnson, J.A., Silbergeld, E.K., Morris, J.G., 2002. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proceedings of the National Academy of Sciences of the United States of America 99, 6434–6439.

Tamtam, F., Mercier, F., Le Bot, B., Eurin, J., Dinh, Q.T., Clement, M., Chevreuil, M., 2008. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Science of the Total Environment 393, 84–95.

Tang, C.M., Huang, Q.X., Yu, Y.Y., Peng, X.Z., 2009. Multiresidue determination of sulfonamides, macrolides, trimethoprim, and chloramphenicol in sewage sludge and sediment using ultrasonic extraction coupled with solid phase extraction and liquid chromatography-tandem mass spectrometry. Chinese Journal of Analytical Chemistry 37, 1119–1124.

Tang, C.M., 2009. Occurrence and Behavior of Antibacterials in Aqueous Environment of the Pearl River Delta, South China State Key Laboratory of Organic Geochemistry. Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, Guangdong, China.

Taylor, N.G.H., Verner-Jeffreys, D.W., Baker-Austin, C., 2011. Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? Trends in Ecology & Evolution 26, 278–284.

Thomas, K.V., Hilton, M.J., 2004. The occurrence of selected human pharmaceutical compounds in UK estuaries. Marine Pollution Bulletin 49, 436–444.

Tian, Y., 2010. Annual Average Antibiotics Consumption Per Capita in China is Ten Times that in United States: Three Reasons Causing Abuse of Antibiotics.

Wiegel, S., Aulinger, A., Brockmeyer, R., Harms, H., Loffler, J., Reincke, H., Schmidt, R., Stachel, B., von Tumpling, W., Wanke, A., 2004. Pharmaceuticals in the river Elbe and its tributaries. Chemosphere 57, 107–126.

Xu, W.H., Zhang, G., Li, X.D., Zou, S.C., Li, P., Hu, Z.H., Li, J., 2007a. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. Water Research 41, 4526–4534.

Xu, W.H., Zhang, G., Zou, S.C., Li, X.D., Liu, Y.C., 2007b. Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. Environmental Pollution 145, 672–679.

Yang, L.H., Ying, G.G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga Pseudokirchneriella subcapitata. Environmental Toxicology and Chemistry 27, 1201–1208.

Zhang, X.X., Zhang, T., Fang, H., 2009. Antibiotic resistance genes in water environment. Applied Microbiology and Biotechnology 82, 397–414.

Zhang, R., Zhang, G., Tang, J., Xu, W., Li, J., Liu, X., Zou, Y., Chen, X., Li, X., 2012a. Levels, spatial distribution and sources of selected antibiotics in the East River (Dongjiang), South China. Aquatic Ecosystem Health & Management. Accepted in June 16, 2011.

Zhang, R., Zhang, G., Zheng, Q., Tang, J., Chen, Y., Xu, W., Zhou, Y., Chen, X., 2012b. Occurrence and risks of antibiotics in the Laizhou Bay, China: impacts of river discharge. Ecotoxicology and Environmental Safety. doi:10.1016/j.ecoenv.2012.03.002.

Zhang, R., 2011. Selected Antibiotics in North China Coastal Zones and Dongjiang River Catchment, Key Laboratory of Coastal Environmental Process, CAS. Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences. Yantai, Shandong, China.

Zou, S.C., Xu, W.H., Zhang, R.J., Tang, J.H., Chen, Y.J., Zhang, G., 2011. Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: impacts of river discharge and aquaculture activities. Estuarine, Coastal and Shelf Science.