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Occurrence and risks of antibiotics in the Laizhou Bay, China: Impacts of river discharge

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ABSTRACT

The presence of thirteen antibiotics categorized into four different groups (fluoroquinolones, macrolides, sulfonamides and trimethoprim) was investigated in the rivers discharging to the Laizhou Bay and the seawater of the bay, and the impacts of river discharge on the marine environment were assessed. The results revealed that the same antibiotics predominated in both the river water and the seawater. Additionally, the detected antibiotics in the river water were generally higher than those in the inner bay and in the open bay, reflecting the importance of the riverine inputs as a source of antibiotics. Risk assessment based on the calculated risk quotients (RQ) showed that enoxacin, ciprofloxacin, and sulfamethoxazole in the two aquatic environments both posed high ecological risks (RQ > 1) to the most sensitive aquatic organisms *Vibrio fischeri*, Microcystis *aeruginosa* and *Synechococcus leopoliensis*, respectively.

1. Introduction

The residues of antibiotics are widely present in aquatic environments when they are used extensively in human and veterinary medicine and in aquaculture (Kummerer, 2009). These antibiotics can cause ecological harm in organisms and promote antibiotic resistance genes (ARGs), a newly emerging contaminant, in bacterial populations (Eguchi et al., 2004; Kummerer, 2004). Past studies have focused on antibiotics in the terrestrial aquatic environment, such as sewage treatment plants (STPs), rivers and groundwater (Kummerer, 2009). However, it is well known that the ocean is an important sink of many terrestrial contaminants. Many of these contaminants (particularly hydrophilic compounds) are poured into marine environment by riverine inputs (Duan et al., 2008; Guan et al., 2009a,b; Kaly, 2004). Therefore, a few researchers have begun to study antibiotics in the marine environment (Martinez Bueno et al., 2009; Jia et al., 2011; Minh et al., 2009; Wille et al., 2010; Xu et al., 2007; Zou et al., 2011) and have found that riverine inputs, STP effluents and mariculture are the main sources of antibiotics in the marine environment (Holmstrom et al., 2003; Jia et al., 2011; Minh et al., 2009; Zou et al., 2011). Among these scarce studies, only a few have involved quantificational assessment of impacts of land-source inputs (riverine inputs or STP effluents) on the marine environment by flux (Minh et al., 2009; Zou et al., 2011), the dilution factor (Minh et al., 2009) or principal component analysis followed by multiple linear regression (PCA–MLR) (Jia et al., 2011). But to our knowledge, limited studies assessed the ecological risks of antibiotics in the marine environment by now (Backhaus et al., 2000).

Laizhou Bay, a typically semi-enclosed inner sea, is one of the three main bays of the Bohai Sea, North China, making up 10 percent of the total area. The bay is an important production base of fishery in China, with developed aquaculture and fishing industry providing large amounts of aquatic products to humans. Industrial and urban developments have been booming around the bay in recent years. This area is part of Bohai Economic Circle—an important economic zone in China. Besides the dense industries and population, there is developed animal husbandry and aquaculture in the area. Therefore, large amounts of industrial wastewaters, domestic sewage and wastewater from animal raising industries are produced and poured into the aquatic environment. In addition, there are more than 23 continental rivers (including the Yellow River—the second largest river in China) with a length of more than 10 km flowing into the Laizhou

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Bay. As a result, the residues of antibiotics in the domestic sewage and animal breeding discharge inevitably enter the rivers and further enter the bay via these riverine inputs. These antibiotics in the aquatic environment also pose ecological risk to the environment.

The target antibiotics in present study belong to four groups, fluoroquinolones (FQs), macrolides (MLs), sulfonamides (SAs) and trimethoprim. They are the most frequently prescribed antibiotics for human treatment and veterinary medicine in China. The most antibiotics selected in this study were also widely present in surface waters in Europe, the USA, and in China (Hirsch et al., 1999; Kolpin et al., 2002; McArdell et al., 2003; Xu et al., 2007; Zou et al., 2011).

The objectives of the present study were not only to fill up the data gap of the occurrence of antibiotics in the Laizhou Bay by analyzing the selected antibiotics in the sea water of the bay and the main rivers flowing into the sea, but also to look at the impact from river discharge on the coastal water by calculating their fluxes and the dilution factor of the antibiotics as they pass from the rivers into the sea and assess the ecological risk of the antibiotics in the marine environment using calculated risk quotients (RQs) (Hernando et al., 2006).

2. Materials and methods

2.1. Chemicals and standards

Thirteen target standards from the groups of fluoroquinolones (FQs), macrolides (MLs), sulfonamides (SAs) and trimethoprim were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The standards were norfloxacin (NOR), ofloxacin (OFL), ciprofloxacin (CPFX), enoxacin (ENO), enrofloxacin (ENRO), erythromycin (ETM), azithromycin (AZM), clarithromycin (CTM), roxitromycin (RTM), sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfamethazine (SMZ) and trimethoprim (TMP). A $^{13}C_3$-caffeine solution used as a surrogate standard was obtained from Cambridge Isotope Labs (1 mg mL^{-1} in methanol, USA). All the antibiotic compounds were dissolved in methanol and stored in a freezer. Erythromycin-H_2O (ETM-H_2O), the major degradation of erythromycin, was prepared by acidification from erythromycin using the method described by McArdell et al. (2003). The physicochemical properties and primary usage of the target compounds are given in Supplementary Table S1.

Methanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Formic acid was purchased from CNW (Germany). Disodium edetate dihydrate (Na_2EDTA) was analytical grade and 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 above.

2.2. Sample collection

The study area and sampling sites are shown in Fig. 1. Ten rivers of different sizes flowing into the Laizhou Bay were selected: the Yellow River (also named Huanghe River, HH), Guangli River (GL), Zimai River (ZM), Xiaoqing River (XQ), Bailang River (BL), Dihe River (DH), Yuhe River (YH), Weihe River (WH), Jiaolai River (JL) and the Dajiawa drainage system (DJW). HH is the largest river with the largest drainage area and discharge followed by XQ, WH and JLH. The river water samples are classified here into three categories according to the surrounding environment that predominantly impacts them: (1) residential, (2) agricultural and (3) industrial. Category one (CG1) includes six rivers: GL, ZM, XQ, BL, YH and DH. All these rivers flow across urban areas, and domestic sewage and industrial wastewater are discharged into these rivers. Category two (CG2) includes HH, WH and JLH, which mainly flow through agricultural areas within the study area. Category three (CG3) only includes DJW, which is influenced mostly by chemical or petrochemical industries.

A total of 27 seawater samples in the Laizhou Bay and 23 river water samples in the ten rivers were collected in September 2009 (Fig. 1). Detailed information about the sampling sites were shown in Supplementary Tables S2 and S3. All the samples were collected in a fishing boat except for parts of the river water samples taken from bridges at the centroid of flow. All the samples were collected (approximately 0–50 cm below the surface) using a stainless steel bucket and were immediately transferred to a 5-liter pre-cleaned amber glass bottle. The bottle was rinsed with 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

The antibiotics in the water were concentrated through a solid-phase extraction (SPE) by an Oasis HLB cartridge using the method described by Xu et al., 2007. In summary, a 2 L water sample was filtered through 0.45 μm glass fiber filters and 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.

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 instrumental analysis method was also optimized based on our previous method (Tang, 2009; Tang et al., 2009; Xu et al., 2007) for the four antibiotic classes. The separation of the FQs compounds was performed with Agilent 1200 series (Agilent, Palo Alto, USA) on an ODS-P (Dikma, USA) (4.6 mm × 250 mm, 3.5 μm) chromatograph column and the other antibiotics on an Agilent Zorbax XDB-C18 column (2.5 mm × 50 mm, 1.8 μm) with an guard column SecurityGuard^{TM} C18(4.0 mm × 3.0 mm). For mass spectrometric analysis, Sciex API 3200 (for FQs) or Agilent 6460 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, USA; Agilent, Palo Alto, USA) equipped with an electrospray ionization source in the positive mode (ESI+) was used to analyze the antibiotics. More conditions of liquid chromatography and mass spectrometry were described in Supplementary Table S1.

2.4. Quality analysis and quality control

A quantitative analysis of each compound was performed using HPLC–ESI-MS–MS in the MRM mode using one or 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. The $^{13}C_3$-caffeine was added as a surrogate standard to all samples prior to enrichment to avoid possible losses during the analytical procedure.

The limits of quantification (LOQ) for each compound in seawater and river water, obtained using the method described in Tang (2009) and Xu et al. (2007), were from 0.23 to 4.4 ng L^{-1} and from 0.26 to 5.0 ng L^{-1}, respectively (Supplementary Table S6).

For the recovery experiments, the thirteen target compounds were determined for seawater and river water using the standards addition method, i.e., 2l of filtered seawater or river water fortified with 100 ng of target analytes was treated in the same procedure as the field samples. The recovery rates of for these spiked antibiotics in seawater and river water were 68–88 percent and 70–82 percent, respectively (Supplementary Table S6). 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

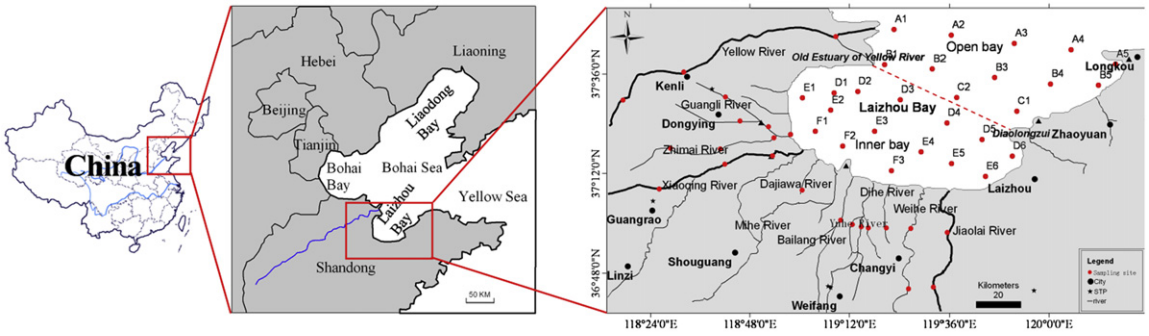

Fig. 1. Map of sampling sites in the Laizhou Bay and its adjacent rivers.

Table 1
Summary of results for the selected antibiotics in the rivers discharging to the Laizhou Bay (n=23).

	Fluroquinolones					TMP	Sulfonamides			Macrolides			
	ENO	NOR	CPFX	ENRO	OFL		SMX	SMZ	SDZ	ETM	RTM	AZM	CTM
Mean[a] (ng L^{-1})	116	118	101	10.6	9.9	1133	62.8	12.8	1.7	62.1	37.9	22.3	5.0
SD[b] (ng L^{-1})	107	131	83.3	8.6	13.8	3052	112	23.6	4.3	85.6	56.1	29.2	7.4
Median (ng L^{-1})	89.1	81.0	81.0	11.2	1.2	5.9	32.2	3.0	< LOQ[d]	12.9	6.8	3.3	1.6
Min (ng L^{-1})	nd[c]	nd	nd	nd	nd	nd	0.36	< LOQ	nd	0.38	< LOQ	nd	nd
Max (ng L^{-1})	508	572	346	24.6	45.4	13,600	527	108	18.7	282	227	88.0	32.9
Detection rate (percent)	91	83	91	57	39	96	100	96	48	100	83	91	87

[a] Mean values were calculated using the measured values if it was above the LOQ, the 1/2 LOQ if it < LOQ or 0 if not detected.
[b] SD: standard deviation.
[c] nd: not detected.
[d] LOQ: limits of quantification.

contamination. More detailed information about quality analysis and quality control were shown in Supplementary Tables S2 and S6.

3. Results

3.1. Levels and distributions of antibiotics in rivers around the Laizhou Bay

All thirteen antibiotics were detected in the rivers around the Laizhou Bay. It should be noted that ETM was detected as its dehydration product erythromycin-H_2O (ETM-H_2O), which was the predominant form of ETM in aquatic environment (McArdell et al., 2003).

Table 1 summarizes the statistics of the detected antibiotic concentrations in the rivers. All raw data are available in Supplementary Table S7. In general, the detected antibiotics concentrations ranged from 0.36 ng L^{-1} to 13.6 μg L^{-1}. Among all the antibiotics, 77 percent of them had detection frequencies of more than 80 percent, with even the lowest near 40 percent, indicating the widespread occurrence of these drugs in the rivers. The maximum concentration of TMP was up to 13.6 μg L^{-1}, and its mean concentration was much higher than its median concentration, indicating that point source pollution of TMP occurred in this area.

Based on the predominant pollutants among all four groups, the median concentrations of FQs were generally higher than those of SAs and MLs. Among the FQs group, NOR, CPFX and ENO were the predominant compounds, with similar median concentrations of approximately 100 ng L^{-1}. However, the compounds ENRO and OFL presented relatively lower concentrations. The differences of their concentrations may be related to their consumption or environmental behavior. For example, the medical consumption of ENO, NOR and CPFX is relatively high in China (Wang and Cheng, 2009; Zhang and Liu, 2010), and NOR and CPFX are also used extensively in animal husbandry and aquaculture. Therefore, the three antibiotics may be widely present in the environment. ENRO is only used as veterinary drug, and OFL is mainly used as human medicine, so their consumption is limited. In addition, ENRO can be partly metabolized to CPFX in the body of animals and can be easily photodegraded in the natural environment (Wu et al., 2006). As a result, ENRO was detected at low frequency and low concentrations. All the SAs, TMP and MLs presented low or moderate median concentrations from ng L^{-1} to tens of ng L^{-1}. Among the SAs and TMP which is a synergist often prescribed in combination with SAs, SMX and TMP were the predominant compounds, which presented higher mean concentrations than SDM and SMZ. This result may be due to SDM and SMZ being mainly applied to animals, but SMX and TMP are applied to humans in addition to animals. Among the MLs group, there were also two predominant compounds: ETM and RTM, with mean concentrations of 61 ng L^{-1} and 43 ng L^{-1}, respectively.

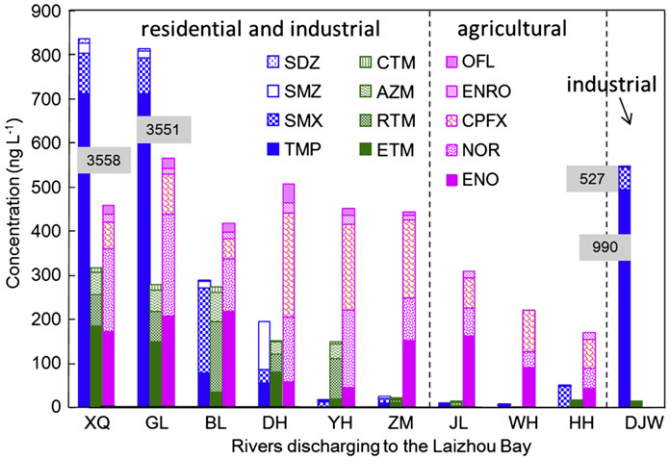

Fig. 2. Antibiotic concentrations (mean) profile in the ten rivers.

Fig. 2 and Supplementary Table S8 show the antibiotics profile in each river. It should be noted that there is some limitation using only one sample to reflect a snapshot for some rivers. The riverine antibiotics in this area presented different spatial distributions. Apparent differences of the antibiotics profile were evident among the residential, agricultural and industrial areas. For example, as shown in Fig. 2, in the river of the chemical industrial area (DJW), only SAs, TMP and MLs were detected, and TMP and SMX contributed 99 percent of the total antibiotics pollution in this river. The concentrations (990 ng L^{-1} and 527 ng L^{-1}, respectively) of the two antibiotics were at a very high level among all the investigated rivers, while the levels of the other antibiotics except TMP and SMX in this river were very low. These levels may be due to point pollution from certain pharmaceutical industries located on the area (Larsson et al., 2007; Li et al., 2008a,b). All the four antibiotic groups were detected in the other two environments. However, the mean concentration of each target compound (in particular, SAs+TMP (P=0.004) and MLs (P=0.024)) in the rivers of the mixed-zone of residential and industrial area (GL, ZM, XQ, BL, YH and DH) was noticeably higher than that in the rivers of the agricultural area (HH, WH and JLH) (Fig. 2), which may be related to the domestic effluents from cities or animal breeding discharge in the suburbs (Jiang et al., 2008, 2011) and the wastewater from some pharmaceutical industries (Larsson et al., 2007; Li et al., 2008a,b). Among all the rivers, XQ and GL were the two most contaminated, with a total concentration of approximately 4.5 μg L^{-1}. The TMP concentrations in the two rivers were both as high as 3.6 μg L^{-1} and in one sample was up to 13.6 μg L^{-1}. The high level is noticeably higher than that in the other rivers of the study area, many other global surface

waters, such as the Huangpu River in Shanghai, China (Jiang et al., 2011), the Haihe River and its tributaries in North China (Zou et al., 2011), the Seine River in France (Tamtam et al., 2008), the Elbe River in Germany (Wiegel et al., 2004) and others (Kim and Carlson, 2007; Kolpin et al., 2002; Managaki et al., 2007), and even some STP effluents (Hirsch et al., 1999). Therefore, the high level of TMP in the river may be related to the discharge of some pharmaceutical production wastewater (Larsson et al., 2007; Li et al., 2008a,b). For the Yellow River, although it flows across some large cities before it flows into this area, all the antibiotics in the river were at tens of ng L^{-1} or below the 10 ng L^{-1} level. The possible reasons for the low levels are that the enormous flow of the river would dilute the pollutants and that few new pollutants are discharged into the river in the rural areas.

3.2. Levels and distributions of the selected antibiotics in the Laizhou Bay

Similarly to the rivers flowing into the Laizhou Bay, all the thirteen antibiotic compounds were detected in the bay. However, their detection frequencies and concentrations in the bay were obviously lower than those in the rivers. Table 2 summarizes the statistics of the detected antibiotic concentrations and all raw data are available in Supplementary Table S9. In general, the detected antibiotic concentrations ranged from 0.25 to 330 ng L^{-1}. Among all the antibiotics, TMP, SMX, ETM and NOR were the most frequently detected compounds, with frequencies of 100 percent. CPFX and ENO also exhibited high detection frequencies of about 90 percent.

Based on the predominant pollutants assessed, the concentrations of FQs were comparable to the level of TMP and higher than those of SAs, while MLs presented the lowest levels in the four groups. ENO, NOR and CPFX were the predominant compounds among the FQs group, with the highest concentrations up to 209 ng L^{-1}, 103 ng L^{-1} and 66 ng L^{-1}, respectively, and their mean concentrations ranged from 31 ng L^{-1} to 60 ng L^{-1}. Similar to the antibiotics in the rivers discharging to the Laizhou Bay, TMP, SMX and ETM were the predominant compounds in their own groups. TMP was the compound with the highest concentration among all the analytes, and its mean concentration was 53 ng L^{-1}. Next to TMP, SMX was detected in the seawater with a mean concentration of 19 ng L^{-1}, which reflects the extensive usage of TMP and SMX in this area. MLs were detected at low concentrations. ETM exhibited the highest concentration among the MLs, but its maximum concentration was only 8.5 ng L^{-1}. Meanwhile, RTM, CTM and AZM were observed at significantly lower maximum concentrations, all being merely approximately 1 ng L^{-1}.

Compared with the other marine aquatic environments (Supplementary Table S2), the concentration of TMP in the Laizhou Bay was at the highest level, which was higher than the levels of Bohai Bay (max: 120 ng L^{-1}) (Zou et al., 2011), Liaodong Bay (max: 18.2 ng L^{-1}) and Belgian coastal harbors (max: 29 ng L^{-1}, Wille et al., 2010), while the concentrations of OFL and RTM in the bay were at a low level, which was significantly lower than the levels of Bohai Bay (max: 5100 and 630, respectively) (Zou et al., 2011) and Victoria Harbor (max: 16.4 and 21.1, respectively) (Xu et al., 2007). The concentrations of ETM, SMX, CPFX and NOR were at a medium level. Therefore, the results indicated that the pollution of TMP in the Laizhou Bay was more serious than the other marine environment.

The Laizhou Bay can be divided into two parts by drawing a line from the old estuary of the Yellow River to Diaolongzui (Fig. 1). Fig. 3 shows the spatial distributions of the antibiotics in the Laizhou Bay. In general, the detection frequencies of the antibiotics in the inner bay were higher than those in the open bay, and the concentrations of each antibiotic compound in the inner bay were significantly higher than those in the open bay. The spatial distribution of SAs and TMP was obvious, i.e., the samples near the southwest shore presented significantly higher concentrations than the samples far from shore. This trend was reasonable and could be explained as follows: (1) there is highly dense urbanization and extensive animal cultivation industry in the southwest area adjacent Laizhou Bay, and (2) many main rivers flow through the area and into the inner bay. Especially, the high level of TMP in the southwest coastal water should be caused by the discharge of Xiaoqing River and Guangli River.

Table 2
Summary results for the selected antibiotics in the Laizhou Bay (n=27).

Laizhou Bay	Fluroquinolones					TMP	Sulfonamides			Macrolides			
	ENO	NOR	CPFX	ENRO	OFL		SMX	SMZ	SDZ	ETM	RTM	AZM	CTM
Mean[a] (ng L^{-1})	62	40	31	1.8	0.24	53	19	0.13	0.02	2.6	0.38	0.14	0.19
SD[b] (ng L^{-1})	41	25	19	2.7	1.2	82	20	0.29	0.08	1.8	0.25	0.26	0.19
Median (ng L^{-1})	59	38	25	nd[c]	nd	18	13	nd	nd	2.4	< LOQ[d]	nd	< LOQ
Min (ng L^{-1})	nd	7.5	nd	nd	nd	1.3	1.5	nd	nd	0.9	< LOQ	nd	nd
Max (ng L^{-1})	209	103	66	7.6	6.5	330	82	1.5	0.43	8.5	1.5	1.2	0.82
Detection rate (percent)	89	100	93	30	3.7	100	100	15	3.7	100	7.4	22	37

[a] Mean values were calculated using the measured values if it above the LOQ, the 1/2 LOQ if it < LOQ or 0 if not detected.
[b] SD: standard deviation.
[c] nd: not detected.
[d] LOQ: limits of quantification.

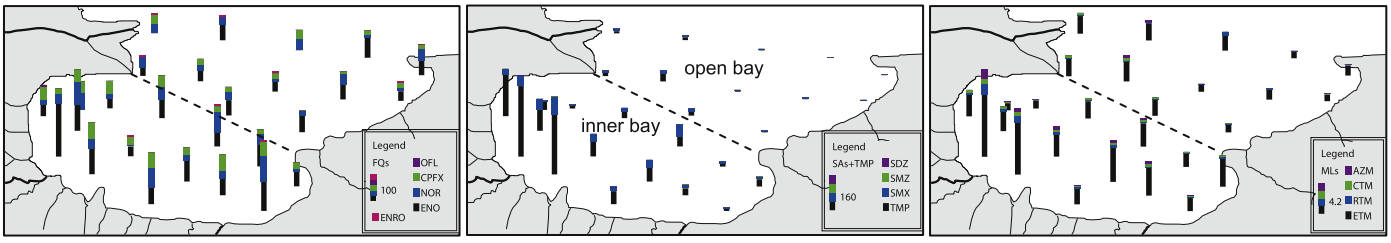

Fig. 3. Distribution map of the antibiotic concentrations (ng L^{-1}) in the surface water of the Laizhou Bay.

4. Discussion

4.1. Effects of river discharge on the Laizhou Bay

In this study, the effects of river discharge on the Laizhou Bay were evaluated by comparison of the antibiotic contents in the rivers and the bay. The antibiotics with higher detection frequencies and concentrations were found in both the rivers and the bay. In addition, the average concentrations of each selected compound in the rivers were higher than those in the Laizhou Bay. The decreased concentration may mainly result from the seawater dilution when river water was discharged into the Laizhou Bay (Zou et al., 2011). In general, the dilution factors observed for pharmaceuticals from point source discharges to receiving surface waters such as rivers and streams range from approximately ten to hundreds (Minh et al., 2009). Because of the large differences of antibiotic contamination levels in different rivers, the dilution factors of antibiotics in the rivers and the sea had a wide range. Table 3 shows the concentration radio of antibiotics in rivers and the seawaters near the river. In general, mean dilution factors of each antibiotic ranged from 1.6 to 122 (Table 3), which are gerenally lower than those of STPs effluents and the receiving seawater (10–400) reported in Victoria Harbor (Minh et al., 2009). But some extraordinarily high ratios were encountered for RTM and AZM at Bailang River and its receiving water (about 500; Table 3). Different dilution factors were observed for the different antibiotics and different rivers. Generally, MLs presented higher dilution factors than the other three groups for the antibiotics. Xiaoqing River and Guangli River presented higher dilution factors of SAs and TMP than the other rivers. The higher dilution factors of MLs may be explained by the following reasons: (1) the MLs in the sea mainly come from the discharge of the rivers, (2) higher attenuation rates caused by the strong adsorption (Li and Zhang, 2010; Tolls, 2001) and low half-lives (Xu et al., 2009). And the lower factors of the other antibiotics may be caused by the following reasons: (1) these drugs were used in mariculture, (2) some of these antibiotics, such as SMX and TMP, showed lower attenuation rates caused by the weak adsorption or long half-lives (Benotti and Brownawell, 2009; Xu et al., 2009).

As described above, flux is a key parameter to assess the effect of river discharge on the marine environment and is equal to the concentrations of pollutants in the river multiplied by the flow. As shown in Table 4, the annual fluxes of antibiotics discharged into the sea by each river were estimated. According to incomplete statistics, more than 7.6 metric tons of antibiotics were transported into the Laizhou Bay via rivers. Among all the rivers, the Yellow River contributed the most (nearly 4.5 metric tons) because of its enormous annual flow. Xiaoqing River also discharged large amounts of antibiotics (approximately 2.6 metric tons of thirteen target compounds) into the bay because of its high antibiotic contents. But it should be pointed that calculating a flux for a river based on a couple of samples and one season has the statistical limitations of the sampling design when reaching these conclusions. Although this estimate has some limitations, it does reflect the effect of river discharge on the Laizhou Bay in degree.

4.2. Risk assessment

The potential environmental risk of the detected antibiotics was assessed on the basis of the risk quotients (RQ), according to the European technical guidance document on risk assessment (TGD) (EC, 2003). The RQ value can be calculated through the predicted environmental concentration (PEC) or measured environmental concentration (MEC) divided by predicted no-effect concentration (PNEC). According to the TGD, when only short-term/acute toxicity data EC50/LC50 are available, the calculation of PNEC is obtained from EC50/LC50 divided by an assessment factor of 1000. Once long-term/chronic NOEC values for one, two

Table 4
Estimated annual amount of thirteen antibiotics flowing into the Laizjou Bay from rivers.

River	Concentration ($ng\ L^{-1}$)	Flow ($\times 10^8\ m^3\ year^{-1}$)	Amount ($kg\ year^{-1}$)
Guangli River	4491	–[a]	–
Zhimai River	480	2.82	135
Xiaoqing River	4450	5.82	2590
Bailang River	979	0.113	11
Yuhe River	461	–	–
Dihe River	851	–	–
Jiaolai River	325	2.53	82
Weihe River	231	13.1	303
Yellow River	225	189	4253
Dajiawa	1533	–	–

[a] No data.

Table 3
Dilution factors of the antibiotic in each river and the seawater near the river.

Dilution factor	Fluoroquinolones					TMP	Sulfonamides			Macrolides			
	ENO	NOR	CPFX	ENRO	OFL		SMX	SMZ	SDZ	ETM	RTM	AZM	CTM
HH[a]/A1[b]	21.4	1.2	1.9	0.75	/[d]	0.08	3.4	7.2	/	4.9	1.5	/	13.6
GL/mean(F1,F2) [c]	1.3	5.9	2.1	/	/	11.7	1.3	33.8	16.6	19.0	55.9	54.0	19.4
ZM/mean(F1,F2)	1.0	2.4	4.0	/	/	0.03	0.15	12.9	0.0	1.5	3.7	3.3	2.0
XQ/mean(F1,F2)	1.1	4.8	1.4	/	/	11.7	1.4	52.1	30.7	23.4	58.1	60.0	14.4
JL/mean(F3,E6)	1.8	0.71	1.2	3.3	/	0.07	0.27	/	/	1.8	23.9	13.8	6.4
WH/mean(F3,E6)	1.0	0.40	1.6	0.9	/	0.15	0.05	/	/	0.36	1.1	0	0
DH/F3	0.90	1.8	3.8	/	/	0.89	1.5	/	/	35.6	137	211	21.4
YH/F3	0.46	1.5	2.2	/	/	0.02	0.51	/	/	8.8	294	248	35.8
BL/F3	3.4	1.5	0.7	/	/	1.3	9.5	/	/	15.7	511	509	105
DJW/F2	0	0	0	/	/	3.5	6.4	2.6	/	1.3	3.1	3.3	1.3
Mean	3.2	2.0	1.9	1.6	/	2.9	2.5	21.7	15.8	11.2	109	122	21.9

[a] Boldface type means the abbreviation of the river name.
[b] **HH**/A1: concentration radios of antibiotics in Huanghe River and seawater from A1.
[c] **GL**/mean(F1,F2): concentration radios of antibiotics in Guangli River and seawater from F1 and F2.
[d] Antibiotic was not detected in the seawater.

or three trophic levels are available, an assessment factor of 100, 50 or ten is used (EC, 2003). While in some studies, an assessment factor of 1000 was used long-term/chronic EC50/LC50 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 and shown in Supplementary Tables S11–S13. PNEC values were also calculated based on the toxicity data and shown in Table 5. And the RQ for each detected antibiotic was calculated using MEC in the surface water of the studied area and the PNEC shown in Table 5 (Fig. 4).

To better elucidate the risk levels, the ratios were classified into three risk levels: 0.01–0.1, low risk; 0.1–1, medium risk; >1 high risk (Hernando et al., 2006). As shown in Fig. 4, nine antibiotics posed at least low risk to aquatic organisms both in the seawater and river water of the studied area. Among the nine antibiotics, only ENO, CPFX and SMX posed high risk to the relevant sensitive aquatic organisms(*Vibrio fischeri*, *Microcystis aeruginosa* and *Synechococcus leopoliensis*, respectively) both in the bay and the rivers. The proportions of the samples causing the high risk by the ENO and CPFX were both about 90 percent in the two aquatic environments. And the maximum RQ of CPFX in the river water was up to 69. Except the three antibiotics above, NOR, OFL, ETM, RTM and CTM also can pose high risk to the relevant sensitive species (Table 5) in different proportions (13–48 percent) of river water samples (Fig. 4). And the proportions causing more than medium risk by them were 48–87 percent in the riverine samples and 4–96 percent in the seawater samples of the bay. For TMP, more than 22 percent and 13 percent of RQ exceeded 0.01 and 0.1 in the riverine samples, respectively, while only 11 percent of RQ exceeded 0.01 in the seawater samples. Except the nine antibiotics described above, the RQ values for SMZ, SDZ and AZM were all less than 0.01 both in the two aquatic environments, suggesting that theses antibiotics are unlikely to cause adverse toxic effects on the selected aquatic organisms. Summarily, most of antibiotics in this study area showed relatively higher ecological risk to the relevant aquatic organisms. Similar results were reported by Kummerer and Henninger (2003) and Lee et al. (2008) in different countries. Thus, it is worthwhile to pay attention to the detection of antibiotics in our surface waters and the control of their abuse.

However, it should be noted that the risk assessment above has some limitations because we could not collected all the toxicity data and the mixture toxicity of the compounds was not considered.

Table 5
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
ENO	*V. fischeri*	NOEC[c]=0.00288	Chronic	100	28.8	Backhaus et al. (2000)
NOR	*V. fischeri*	NOEC=0.01038	Chronic	100	103.8	Backhaus et al. (2000)
CPFX	*M. aeruginosa*	EC50[d]=0.005	Acute	1000	5	Halling-Sorensen et al. (2000)
OFL	*P. subcapitata*	NOEC=0.00113	Chronic	100	11.3	Backhaus et al. (2000)
TMP	*R. salina*	EC50=16	Acute	1000	16,000	Lutzhoft et al. (1999)
SMX	*S. leopoliensis*	EC50=0.027	Acute	1000	27	Ferrari et al. (2004)
SMZ	*S. vacuolatus*	EC50=19.52	Chronic	1000	19,520	Bialk-Bielinska et al. (2011)
SDZ	*S. capricornutum*	EC50=2.2	Acute	1000	2200	Eguchi et al. (2004)
ETM	*P. subcapitata*	EC50=0.02	Chronic	1000	20	Isidori et al. (2005)
RTM	*P. subcapitata*	NOEC=0.01	Chronic	100	100	Yang et al. (2008)
CTM	*P. subcapitata*	EC50=0.002	Chronic	1000	2	Isidori et al. (2005)
AZM	*Daphnia* sp.	*EC50 > 120*	Acute	1000	> 120,000	FDA–CDER (1996)

[a] AF: assessment factor.
[b] PNEC: predicted no effect concentration.
[c] NOEC: no observable effect concentration.
[d] EC50: half maximal effective concentration.

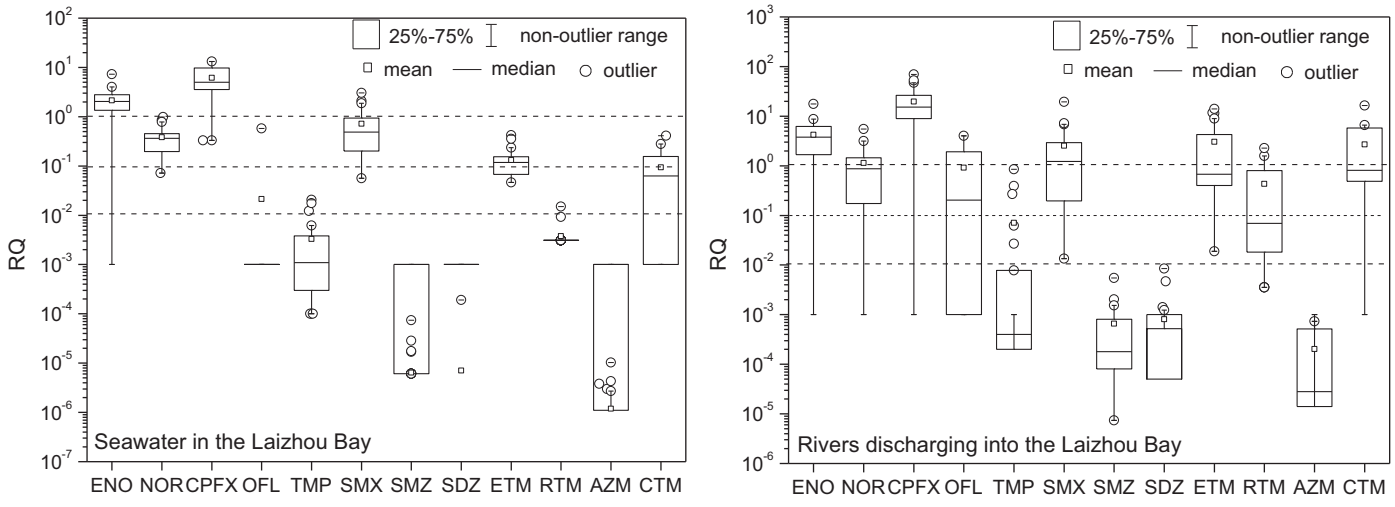

Fig. 4. Boxplots for the calculated risk quotients (RQ) for the twelve antibiotics detected in the Laizhou Bay and the rivers discharging to the bay.

5. Conclusions

This study investigated the occurrence and distribution of thirteen antibiotics in coastal water and the main rivers discharging to the sea in case of Laizhou Bay, a highly polluted sea area in China, and to assess the impacts of riverine inputs on the marine environments. The results showed that all thirteen antibiotics were found in the Laizhou Bay, with the highest concentrations up to hundreds of ng L^{-1}. ENO, NOR, CPFX, TMP, SMX, ETM and RTM were the dominant antibiotics in the bay. Spatially, the antibiotic concentrations in the inner bay were significantly higher than those in the open bay. In view of the high density of human activities and many main rivers pouring into the bay in Southwest Laizhou Bay, these antibiotic concentrations highlight the remarkable effects of human activities and river discharge on exposure to antibiotics in the marine environment. For the rivers flowing into the Laizhou Bay, all the detected antibiotic concentrations were higher than those in the bay, and the predominant antibiotics in the rivers were the same as those in the bay. These results indicate that river discharge may act as a main source of antibiotics to the Laizhou Bay. In addition, risk assessment showed that the ENO, CPFX and SMX posed a high risk to the relevant sensitive aquatic organisms in the Laizhou Bay.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2012.03.002.

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