



Occurrence and risks of antibiotics in the coastal aquatic environment of the Yellow Sea, North China

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HIGHLIGHTS

- Some antibiotics were ubiquitous in coastal seawater of the Yellow Sea, North China.
- Higher concentrations and spatial variations were presented in the semi-enclosed bay.
- Lower concentrations and spatial variations were presented in the open bays.
- Source of the antibiotics in the different bays varied largely.
- Parts of the antibiotics in one bay posed high ecological risks to some organisms.

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ABSTRACT

Eleven antibiotics in three different categories were investigated in two types of coastal bays (a semi-enclosed bay and an open bay) of the Yellow Sea and in fresh water (rivers and sewage treatment plants [STP] effluents) that discharged into the bays. The results revealed the presence of three predominant antibiotics: dehydration erythromycin, sulfamethoxazole and trimethoprim. These antibiotics were detected in the seawater and fresh water with concentrations of <0.23 – 50.4 ng L⁻¹ and <0.25 – 663.1 ng L⁻¹, respectively. In terms of the regional distribution of the compounds within the two types of bays, higher concentrations (<0.23 – 50.4 ng L⁻¹) and higher spatial variations (coefficients of variation: 98%–124%) were found in the semi-enclosed Jiaozhou Bay due to the poor water-exchange ability and to fresh-water inputs through rivers and/or STP effluents. In contrast, lower concentrations (<0.23 – 3.0 ng L⁻¹) and lower spatial variations (coefficients of variation: 36%–75%) were present in the open Yantai Bays due to the strong water-exchange with the open sea. The source apportionment suggested that 1) fresh-water inputs were the primary source of macrolides in the coastal water, and 2) mariculture affected the relative pollution levels of trimethoprim, sulfamethoxazole and sulfathiazole in the bays. In addition, a risk assessment based on the calculated risk quotient (RQ) showed that the dehydrated erythromycin, sulfamethoxazole and clarithromycin detected at most of the sampling sites in Jiaozhou Bay could pose high ($RQ > 1$) risks to the most sensitive aquatic microorganisms, such as *Synechococcus leopoliensis* and *Pseudokirchneriella subcapitata*, whilst in the Yantai Bays, the compounds could pose medium risks ($1 \geq RQ > 0.1$) to the same aquatic microorganisms.

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1. Introduction

Antibiotic residues are new pollutants in the environment. These compounds can cause ecological harm to organisms and promote antibiotic-resistance genes in bacterial populations (Eguchi et al., 2004; Kummerer, 2004). Antibiotic residues are widely present in faeces, medical waste, soil and all types of aquatic environments due to the extensive and long-term use of antibiotics in human

therapy and veterinary medicine as well as in promoting the growth of animals in livestock production (Kummerer, 2009). Terrestrial antibiotic residues could be transported to marine environments via riverine inputs (Jia et al., 2011; Zhang et al., 2012b; Zou et al., 2011) and sewage treatment plant (STP) effluents (Gulkowska et al., 2007; Minh et al., 2009). These antibiotics and/or the residues used in mariculture (Jia et al., 2011) cause various degrees of antibiotic pollution in the coastal environment. For example, as shown in Table 1, the maximum concentration (c_{\max}) of roxithromycin (RTM) was up to 630 ng L⁻¹ in Bohai Bay (Zou et al., 2011) of the Bohai Sea (BS), China, the c_{\max} of trimethoprim (TMP) was up to 330 ng L⁻¹ in Laizhou Bay (Zhang et al., 2012b) of the BS, the c_{\max}

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Table 1Global comparison of eight antibiotics in the marine aquatic environment (concentrations given in ng L⁻¹).

Study area	Sampling time	TMP		SMX		SDM		SDZ		ETM		RTM		AZM		CTM		Reference
		Max	Mean/ median	Max	Mean/ median	Max	Mean/ median	Max	Mean/ median	Max	Mean/ median	Max	Mean/ median	Max	Mean/ median	Max	Mean/ median	
Jiaozhou Bay of the Yellow Sea	04/2010	14.1	4.7/2.7	50.4	9.6/5.3	0.35	0.04/ nd	nd	nd	25.2	4.5/2.2	6.9	1.4/0.4	2.5	0.53/ <0.26	2.6	0.58/ <0.25	This study
Yantai Bays of the Yellow Sea	04/2010	2.6	1.4/1.3	3.0	1.4/1.2	<0.24	0.02/ nd	0.24	0.01/ nd	2.6	0.82/ 0.66	0.96	0.34/ <0.62	0.32	0.09/ <0.26	0.34	0.03/ <0.25	This study
Laizhou Bay of the Bohai Sea	09/2009	330	53/18	82	19/13	1.5	0.13/ nd	0.43	0.02/ nd	8.5	2.6/2.4	1.5	0.38/ <0.62	1.2	0.14/ nd	0.82	0.19/ <0.25	Zhang et al. (2012b)
Bohai Bay of the Bohai Sea	05/2009	120	5.6/	140	35	130	6.4/	41	3.4/	150	25/	630	27/	na		na		Zhang (2011)
Liaodong Bay of the Bohai Sea	05/2009	18.2	/3.6	76.9	/25.2	1.1	/0.3	9.1	/2.4	8.8	/7.9	3.4	/0.4	0.8	/0.5	0.9	/0.1	Jia (2011) and Jia et al. (2011)
Victoria Harbour, Hong Kong	12/2004	na		nd		nd		nd		5.2	/3.3	21.1	/6.1	na		na		Xu et al. (2007b)
Victoria Harbour, Hong Kong	06–08/2008	216	52/	47	13/	na		na		1730	213/	47	19/	na		na		Minh et al. (2009)
Victoria Harbour & Hong Kong coasts	12/2006	21.8	7.9/	na		na		na		486	91/	na	na	na		na		Gulkowska et al. (2007)
Shenzhen Bay, South China Sea	11/2005	na		880	^a	469	/	292	/	1340		206	/	na		na		Xu (2007)
Belgian coastal harbours	05,12/2007 04/2008 06/2009	29	/	43	/	na		na		Na		na		na		na		Wille et al. (2010)

nd: not detected.

na: not analysed.

^a No data.

of sulfamethoxazole (SMX) was up to 77 ng L⁻¹ in Liaodong Bay (Jia et al., 2011) of the BS, the c_{\max} of dehydrated erythromycin (ETM-H₂O) was up to 1730 ng L⁻¹ in Victoria Harbour of Hong Kong, China (Minh et al., 2009), the c_{\max} of SMX was up to 43 ng L⁻¹ in Belgian coastal harbours (Wille et al., 2010), and so on. However, the most recent studies mainly pay attention to semi-enclosed areas with poor water-exchange ability, such as the coastal areas of the three main bays (Bohai Bay, Laizhou Bay and Liaodong Bay) of the BS (Jia et al., 2011; Zhang et al., 2012b; Zou et al., 2011) and Victoria Harbour of Hong Kong (Gulkowska et al., 2007; Minh et al., 2009; Xu et al., 2007b). Limited information is available regarding the presence of antibiotics in open coastal areas, which usually have stronger water-exchange ability and less serious pollution than semi-enclosed areas. The detection of antibiotics in the open sea would confirm their ubiquitous character and could lead to new insights into their persistence. In this work, emphasis is placed upon the antibiotics in some parts of selected open bays in the Yellow Sea (YS), an open sea of China (Fig. 1A–2) near the BS, which is the only semi-enclosed sea in China. The open bays include Taozi Bay, Zifu Bay and Sishili Bay near Yantai City (Fig. 1A–3). For convenience, in the present study, the bays are named together as the “Yantai Bays” (YTBs). Because the YTBs are close to Bohai Strait, they are affected by the current from the BS. As an interesting comparison, one semi-enclosed bay (Jiaozhou Bay [JZB]) (Fig. 1A–4) in the open YS was also studied concurrently. The objectives of the present study are as follows: 1) to address the data gap regarding the occurrence of antibiotics in the open coastal environment; 2) to analyse the different levels of antibiotics in the open bay and the semi-enclosed bay; 3) to assess the sources of the antibiotics in these bays based on the antibiotic usage, distributions

and correlations between antibiotic concentrations; and 4) to assess the ecological risk of the antibiotics in the two different coastal environments using calculated risk quotients (RQs) (Hernando et al., 2006).

Eleven antibiotics belonging to three groups, macrolides, sulfonamides and diaminopyrimidines, were selected as the target compounds in this study. Information regarding the target compounds, including the physicochemical properties and primary usage, are given in Supplementary Table S1. Most of the target antibiotics are frequently prescribed for human treatment and veterinary medicine in China. These antibiotics are also present in surface waters in many countries in Europe, in the USA, and in China (Hirsch et al., 1999; Kolpin et al., 2002; McArdell et al., 2003; Xu et al., 2007b; Zou et al., 2011).

2. Materials and methods

2.1. Standards and chemicals

The eleven target compounds belong to three different antibacterial families: 1) macrolides, including erythromycin (ETM), spiramycin (SRM), azithromycin (AZM), clarithromycin (CTM) and roxithromycin (RTM); 2) sulfonamides, consisting of sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfadimidine (SDM), sulfathiazole (STZ) and sulfacetamide (SAAM); and 3) diaminopyrimidines, only including trimethoprim (TMP). The synergist TMP is often prescribed in combination with sulfonamides and has similar properties to sulfonamides; therefore, TMP is usually grouped with the sulfonamides when discussing the results. All of the target compounds were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The ¹³C₃-caffeine solution was obtained from

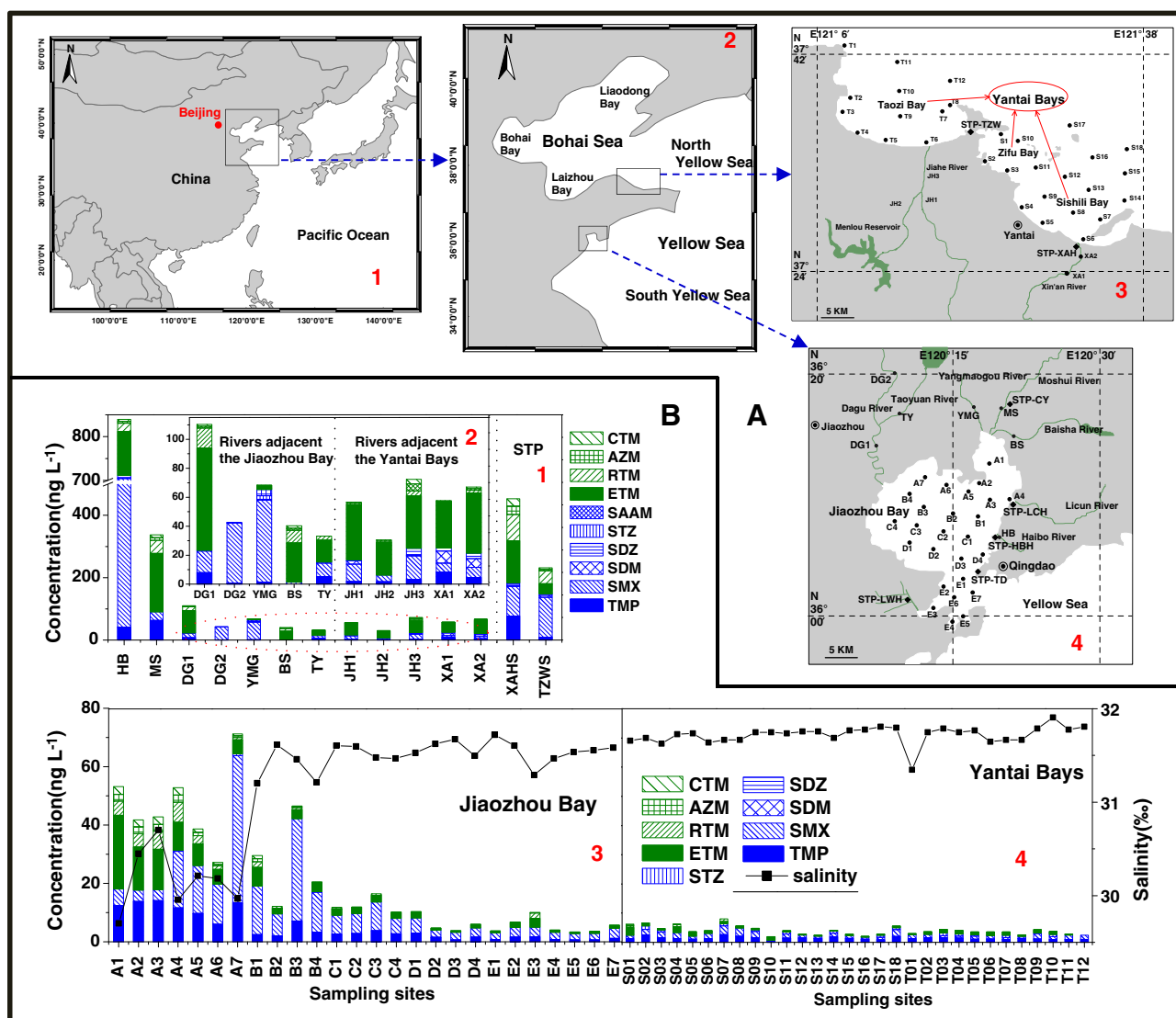


Fig. 1. A: Sampling sites in Jiaozhou Bay (JZB), the Yantai Bays (YTBs) and their adjacent rivers and sewage-plant treatments (STP); Fig. B-1, 2: Antibiotic concentrations in the river-water samples and STP effluents around JZB and the YTBs; Fig. B-3, 4: Antibiotic concentrations and salinity profiles in the seawater of JZB and the YTBs.

Cambridge Isotope Labs (1 mg mL⁻¹ in methanol; USA) and used as a surrogate standard. Individual stock solutions of antibiotics were prepared at concentrations of 200 mg L⁻¹ by dissolving appropriate amounts of the antibiotics in methanol and storing in the dark at -20 °C. Erythromycin-H₂O (ETM-H₂O), a major degradation product of erythromycin, was obtained by acidifying erythromycin using the method described by McArdell et al. (2003).

Methanol (HPLC grade) was obtained from Merck (Darmstadt, Germany). Formic acid and ammonium acetate were purchased from CNW (Germany). Disodium edetate dihydrate (Na₂EDTA) was analytical grade and obtained from Tianjin Chemical (Tianjin, China). Ultra-pure water was prepared with a Milli-Q water-purification system (Millipore, Bedford, Massachusetts, USA). Unless otherwise indicated, the chemicals used in the analysis were analytical grade or above.

2.2. Study areas and sample collection

The study areas and sampling sites are shown in Fig. 1-A. JZB is a semi-enclosed bay located in the South Yellow Sea near Qingdao City. YTBs, including Taozi Bay, Zifu Bay and Sishili Bay, are all open bays located in the North Yellow Sea near Yantai City. Qingdao and Yantai are two economically developed cities in Shangdong Province of

China. The GDPs of these cities are the top two in Shangdong Province, and the GDP of Shangdong Province was ranked the second in China in 2010. Detailed information on the four bays and the two cities (e.g., their area, coastline, industrial sewage, population, aquatic products, livestock, poultry and the area of aquatic farming) is provided in Supplementary Table 2.

Seventy samples were collected in April 2010. Fifty-six samples were coastal seawater samples: thirty samples were collected in the YTBs, and twenty-six samples were collected in JZB. Fourteen freshwater samples were collected from seven rivers and two STPs: five river-water samples were from the Jiahe River (JH) and the Xin'an River (XA), which both discharge into the YTBs (Fig. 1-A, 3); seven river-water samples were from the Dagou River (DG), Taoyuan River (TY), Yangmaogou River (YMG), Moshui River (MS), Baisha River (BS) and Haibo River (HB), which are adjacent to JZB (Fig. 1-A, 4); two STP effluents were collected from the Taoziwan STP (STP-TZW) and Xinanhe STP (STP-XAH) near the coastline of the YTBs (Fig. 1-A, 3). It should be noted that the river-water samples in the MS and HB were close to the Chengyang STP (STP-CY) and Haibohe STP (STP-HBH) (Fig. 1-A, 4), respectively. More information on the STPs is given in Supplementary Table S3. All of the seawater samples were collected from 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 amber glass bottle, which was pre-cleaned successively with detergent, tap water, distilled water and methanol and finally dried. The bottle was rinsed with sample water 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 samples were concentrated by pre-conditioned solid-phase extraction (SPE) using the Oasis HLB cartridge (500 mg, 6 mL, Waters Corporation, Milford, MA, USA). Before the extraction, a specific volume of a water sample (2 L seawater, 1 L river water or 0.5 L sewage water) was filtered through 0.7 µm glass-fibre filters (GF/F, Whatman, Mainstone, England) and then acidified to pH = 3.0 with 3.0 mol L⁻¹ H₂SO₄, followed by the addition of 0.2 g Na₂EDTA as the chelating agent and 100 ng of ¹³C₃-caffeine as the surrogate to monitor the recovery. Each water sample was passed through the SPE cartridge. The analytes were eluted with 2 mL of methanol 3 times. The volume of each of the analytes was reduced to approximately 20 µL, and then, the analyte was dissolved in 40% aqueous methanol to the final volume of 1.0 mL. The details of the SPE process are shown in Supplementary Text S1.

The extracted antibiotics were analysed using high-performance liquid chromatography–electrospray-ionisation tandem mass spectrometry (HPLC–ESI–MS–MS) with multiple-reaction monitoring (MRM). The instrumental analysis method was also optimised based on our previous method (Zhang et al., 2012b) for the three antibiotic classes. The compounds were separated with an Agilent 1200 series (Agilent, Palo Alto, USA) on an Agilent Zorbax XRD-C18 column (2.1 mm × 50 mm, 1.8 µm) with a guard column SecurityGuard™ C18 (4.0 mm × 3.0 mm). For the mass spectrometric analysis, an Agilent 6460 triple quadrupole mass spectrometer (Agilent, Palo Alto, USA) equipped with an electrospray-ionisation source in the positive mode (ESI+) was used to analyse the antibiotics. More details of the conditions of the liquid chromatography and mass spectrometry are described in Supplementary Text S2.

2.4. Quality analysis and quality control

A quantitative analysis of each compound was performed by HPLC–ESI–MS–MS in the MRM mode using two of the highest characteristic precursor ion/product ion transitions (Supplementary Table S4). Together with the retention times, the characteristic ions were used to ensure correct peak assignment and peak purity. The ¹³C₃-caffeine was added as a surrogate standard to all of the

samples prior to analysis to monitor possible losses during the analytical procedure.

The limits of quantification (LOQ) for each compound in seawater, river-water and sewage-water samples, obtained using the method described by Tang (2009) and Xu et al. (2007b), ranged from 0.23 to 6.6 ng L⁻¹, from 0.26 to 7.4 ng L⁻¹ and from 1.43 to 22 ng L⁻¹, respectively (Supplementary Table S5).

For the recovery experiments, the eleven target compounds were determined in seawater, river water and sewage water using the standard addition method, i.e., 2 L of filtered seawater, 1 L of filtered river water or 0.5 L of filtered sewage water fortified with 100 ng of the target analytes was treated in the same procedure as the field samples. The recovery rates of these spiked antibiotics in seawater, river water and sewage water were 78–88%, 71–82% and 45–70%, respectively (Supplementary Table S5). Field and procedural blanks were treated as the control sample to check for any possible contamination in the field sampling and in the laboratory. The analysis of these blanks demonstrated that the extraction and sampling procedures were free of contamination. More detailed information about the quality of the analysis and quality control is shown in Supplementary Text S3.

2.5. Risk-assessment method

The potential environmental risk of the detected antibiotics was assessed on the basis of the risk quotient (RQ), according to the European technical guidance document on risk assessment (TGD) (EC, 2003). The RQ value can be calculated using the following formula:

$$RQ = (PEC \text{ or } MEC) / PNEC \quad (1)$$

where PEC, MEC and PNEC are abbreviations of the predicted environmental concentration, measured environmental concentration and predicted no-effect concentration, respectively. 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 a fixed assessment factor of 1000. Once long-term/chronic NOEC values for one, two or three trophic levels are available, the assessment factors of 100, 50 or 10, respectively, are used (EC, 2003). In some studies, an assessment factor of 1000 was used for long-term/chronic EC₅₀/LC₅₀ values, although this factor value 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 our past publications, a substantial amount of acute or chronic toxicity data for the selected antibiotics for non-target organisms were collected from the literature and were shown in the supplementary material (Zhang et al., 2012a, 2012b). PNEC values were also calculated based on the toxicity data and are shown in Table 2. The RQ for each detected antibiotic should be calculated using the MEC in the surface water of the

Table 2
Aquatic toxicity data of eight antibiotics for the most sensitive aquatic species.

Compound	Non-target organism	Toxicity data (mg L ⁻¹)	Toxicity	AF ^a	PNEC ^b (ng L ⁻¹)	Reference
TMP	<i>R. salina</i>	EC ₅₀ = 16	Acute	1000	16,000	Lutzhof et al. (1999)
SMX	<i>S. leopoliensis</i>	EC ₅₀ = 0.027	Acute	1000	27	Ferrari et al. (2004)
SDM	<i>S. vacuolatus</i>	EC ₅₀ = 19.52	Chronic	1000	19,520	Bialk-Bielinska et al. (2011)
SDZ	<i>S. capricornutum</i>	EC ₅₀ = 2.2	Acute	1000	2200	Eguchi et al. (2004)
ETM-H ₂ O	<i>P. subcapitata</i>	EC ₅₀ = 0.02	Chronic	1000	20	Isidori et al. (2005)
RTM	<i>P. subcapitata</i>	NOEC = 0.01	Chronic	100	100	Yang et al. (2008)
CTM	<i>P. subcapitata</i>	EC ₅₀ = 0.002	Chronic	1000	2	Isidori et al. (2005)
AZM	<i>Daphnia</i> sp	EC ₅₀ > 120	Acute	1000	> 120,000	FDA-CDER (1996)

NOEC: no observable effect concentration.

EC₅₀: half maximal effective concentration.

^a AF: assessment factor.

^b PNEC: predicted no-effect concentration.

studied area and the PNEC. For a better elucidation of the risk levels, the ratios are always classified into three risk levels: 0.01–0.1, low risk; 0.1–1, medium risk; > 1 high risk (Hernando et al., 2006).

3. Results and discussion

3.1. Occurrence of the target antibiotics in the seawater and freshwater

Fig. 1-B shows the antibiotic profiles in the seawater samples from the coastal bays of the Yellow Sea, river water and STP effluents adjacent to these bays. All of the raw data are available in Supplementary Table S6–S7. As shown in Fig. 1-B, nine antibiotics of the eleven target compounds, excluding SRM and SAAM, were detected in the seawater, and ten antibiotics, excluding SRM, were detected in the freshwater. Among the detected antibiotics, ETM-H₂O, SMX and TMP were the predominant compounds in the seawater and freshwater, with detection frequencies of 98%–100% and 82%–100%, respectively. Except for the three predominant antibiotics aforementioned, the other antibiotics were detected sporadically in the seawater, with detection frequencies of less than 30%.

The concentrations of the detected antibiotics ranged from <0.23 ng L⁻¹ to 50.4 ng L⁻¹ in the seawater and from <0.24 ng L⁻¹ to 663 ng L⁻¹ in the freshwater. Among the detected antibiotics, the three predominant antibiotics, ETM-H₂O, SMX and TMP, also presented the highest concentrations both in the seawater and the freshwater. The individual mean concentrations of these three compounds ranged from 2.5 to 5.2 ng L⁻¹ in the seawater and from 15.9 to 77.4 ng L⁻¹ in the freshwater. Compared with the three predominant antibiotics, the other antibiotics showed lower concentrations. The mean concentrations of the remaining antibiotics were all less than 0.85 ng L⁻¹ in the seawater and less than 11 ng L⁻¹ in the freshwater.

The higher detection frequencies and concentrations of the three predominant antibiotics compared to the other antibiotics may be due to several factors, such as the compounds' consumption, fate and behavioural processes, and so on. For example, SMX and TMP are administered to both humans and animals; however, SDM and SDZ are mainly administered to animals. In contrast, the prescription of STZ to animals has been forbidden due to the antibiotic's serious side effects. Therefore, larger quantities of SMX and TMP may be consumed than those of SDM, SDZ and STZ in China. Among macrolides, ETM is used more frequently than RTM, AZM and CTM because of its lower price. Moreover, the higher sorption ability of RTM and CTM to particles (Yang et al., 2010; Zhang, 2011) may reduce their dissolved concentrations in the aquatic environment.

3.2. Environmental distributions of the target antibiotics in the freshwater and seawater

Among the different freshwater environments, high levels of antibiotic pollution were detected in the STP effluents and the rivers close to STPs, indicating that the STP effluents were an important source of antibiotics in the surface water. The total concentrations of the selected antibiotics in the STP effluents and rivers close to STPs in the present study ranged from 232 to 839 ng L⁻¹, whilst the concentrations in all of the other rivers were much lower (31–110 ng L⁻¹) (Fig. 1B-1). The sampling site HB in Haibo River is close to the effluent of STP-HBH (Fig. 1A-3) and presents the most serious antibiotic pollution reported in this study, with the total concentration and SMX level of 839 and more than 660 ng L⁻¹, respectively. The concentration of SMX in this study was even higher than that in the STP effluent from Guangzhou (78 ng L⁻¹) (Xu et al., 2007a) and was similar to those detected in the STPs from Sweden (Lindberg et al., 2005), Switzerland (McArdell et al., 2003) and Canada (Miao et al., 2004). It can therefore be assumed that the STP-HBH effluent has a higher concentration than the river water from HB. Similarly, STP-CY effluent may contain a higher concentration than the sample MS from the Moshui river, with the total concentration

of 338 ng L⁻¹. The total antibiotic concentrations from STP-TZW and STP-XAH near the YTBs were 454 ng L⁻¹ and 232 ng L⁻¹ (Fig. 1B-1), respectively, presenting similar pollution levels to STP-CY but lower than STP-HBH, both of which were adjacent to JZB.

Except for the rivers close to STPs, the overall concentrations of the antibiotics in all of the other rivers discharged into JZB and the YTBs were similar (Fig. 1B-2), i.e., the total concentrations ranged from 34 to 110 ng L⁻¹ and from 31 to 67 ng L⁻¹ in the rivers adjacent to JZB and the YTBs, respectively.

The results presented in Fig. 1B-3, 4 show that the antibiotic pollution was more serious in JZB than in the YTBs. For example, the mean concentrations of the three predominant antibiotics TMP, SMX and ETM-H₂O in JZB (4.5–9.6 ng L⁻¹) were all significantly higher than those in the YTBs (0.82–1.4 ng L⁻¹) (Student's t-test, $p < 0.01$), and the levels of the other macrolides RTM, AZM and CTM in JZB (0.53–1.4 ng L⁻¹) were also obviously higher than those in the YTBs (0.03–0.34 ng L⁻¹) (Student's t-test, $p < 0.01$). The large spatial variations among these bays could be affected by several factors, such as source discharge loads, degradation ability and dilution ability. Because of the limited data regarding the total fluxes of the river and sewage discharges, definite source discharge loads could not be determined in the present study. However, it is clear that higher dilution potential occurred at the open YTBs than the semi-enclosed JZB because of the strong water-exchange ability of the YTBs with the open sea. Higher salinity of the YTBs (range: 31.36‰–31.92‰; mean: $31.73 \pm 0.09\%$) compared with JZB (range: 29.69‰–31.75‰; mean: $31.17 \pm 0.65\%$) (Supplementary Table S7) could support the statement above, i.e., open seawater increased salinity and diluted pollutants, and freshwater runoff decreased salinity but brought in antibiotics.

Compared with other coastal seawater, the antibiotics in the coastal bays of the Yellow Sea were at medium or low pollution levels (Table 1). All of the antibiotic concentrations in the open YTBs were much lower than those in the three main semi-enclosed bays of the BS (Jia, 2011; Jia et al., 2011; Zhang et al., 2012b; Zou et al., 2011). The concentrations of the macrolide antibiotics in JZB were higher than those in Laizhou Bay (Zhang et al., 2012b) and Liaodong Bay (Jia, 2011) but lower than those in Bohai Bay (Zou et al., 2011), whilst the concentrations of sulfonamides and TMP in JZB were obviously lower than those in Bohai Bay (Zou et al., 2011), Laizhou Bay (Zhang et al., 2012b) and Shenzhen Bay (Xu, 2007) but similar to those in Liaodong Bay (Jia et al., 2011) and Belgian coastal harbours (Wille et al., 2010).

As shown in Fig. 1B-3 and B-4, there are much more detailed distribution features in these bays. One significant feature is that the concentrations of antibiotics in the semi-enclosed JZB exhibit much larger spatial variations than those in the open YTBs. The coefficients of variation (CV) ($CV = [\text{standard deviation}/\text{mean}] \times 100\%$) of the three predominant compounds TMP, SMX and ETM-H₂O in JZB ranged from 98% to 124%, whilst those in the YTBs ranged from 36% to 75%. In JZB, all of the samples were divided into five profiles named A, B, C, D and E from the north bank to the southern mouth of the bay. The antibiotic concentrations decreased sharply from profile A near the north coast to profile E in the southern mouth of the bay (Supplementary Fig. S2) due to adsorption, dilution, photolysis, hydrolysis and/or biodegradation. This phenomenon may clearly show the transport process of the antibiotics from the rivers to the mouth of the bay with the river inputs, sea currents and/or diffusion. The attenuation can even be described roughly using the exponential attenuation model $C = C_0 e^{-kL}$ mentioned in our previous study (Zhang et al., 2012a) (Supplementary Table S8). The attenuation-rate coefficients (k values) indicated that ETM-H₂O (k : 0.22 km⁻¹) was most prone to attenuation, followed by TMP (k : 0.19 km⁻¹) and SMX (k : 0.16 km⁻¹). The same trend was also observed in Bohai Bay (Zhang, 2011). The high sorption to particles and sediments (Li and Zhang, 2010; Tolls, 2001; Xu et al., 2009) and relatively shorter half-life (Xu et al., 2009) are two

possibly important mechanisms contributing to the relatively high attenuation capacity for ETM-H₂O. For example, the normalised distribution coefficient (K_{oc}) of ETM-H₂O between seawater and sediments is 50,550 L kg⁻¹, but the K_{oc} of SMX is 13,350 L kg⁻¹ (Xu et al., 2009); the half-life of ETM-H₂O is 7.3 d in the seawater of a flume system, but the half-life of SMX is 14.7 d in the same environment (Xu et al., 2009). The lower k values of TMP and SMX indicate that these two antibiotics are more stable than ETM-H₂O. Additionally, the use of TMP and SMX in mariculture may be another reason for their lower k values. In the YTBs, none of the antibiotics present significant spatial variations except the concentrations of ETM-H₂O in the sampling sites near the coast (S1, S2, S4–S6 and T2–T10), which were higher than those in the area away from the coast. That pattern may indicate that the ETM-H₂O in the sea was mainly impacted by the emission of terrestrial pollutants, whilst TMP and SMX occurred in the sea extensively because of their use in mariculture.

Moreover, the spatial distribution of the antibiotic concentrations in the bays can also be proven by the homochronous salinity distribution, i.e., the salinity decreases from the mouth of JZB to the north coast (Fig. 1B–3) with the river inputs, whilst the salinity in the YTBs presents small spatial variation (Fig. 1B–4). In view of the correlations between salinity and antibiotic concentrations in the bays, a significant inverse correlation can be found between the salinity and the total antibiotic concentrations in JZB ($r^2 = 0.74$, $n = 26$, $p < 0.001$), but there is no significant correlation in the YTBs ($r^2 = 0.02$, $n = 30$, $p = 0.48$), suggesting that the inputs of fresh water significantly enrich the antibiotics in JZB but not in the YTBs.

3.3. Source apportionment of the antibiotics in the coastal seawater of the JZB and YTBs

Antibiotic inputs from freshwater discharge and antibiotic residues from mariculture are the two main sources of the antibiotics in coastal water. Occasionally, the antibiotics in one coastal area originate from another coastal area due to sea currents. As discussed previously, freshwater inputs and poor water-exchange ability caused high antibiotic concentrations in JZB, especially in its north coast area. Among the detected antibiotics, the macrolide pollution levels in the sampling sites A1–A4 of the northeast coast were the most serious in the whole bay. This pattern may be due to the following factors: (1) many more rivers (such as Moshui River, Baisha River and Lichun River) and STP effluents (such as Chenyang STP and Lichun STP) (Fig. 1–A3) discharge into the bay on the northeast bank; (2) these rivers flow through the urban area of Qingdao City, which is densely populated, and the city discharges parts of its domestic sewage treated and/or untreated (Yu, 2010); and (3) macrolides are mainly used as human medicine, although some of the ETM is used as veterinary medicine. In contrast

to the macrolides, high concentrations of SMX were present in the area around A7 and B3 in the northwest coast of the bay, and their concentrations in the north-central area of the bay did not decrease significantly. For TMP, its highest concentration was present on both the northeast and northwest coasts, and the levels decreased sharply in the south-central area of the bay. In view of the fact that TMP has been prescribed in combination with sulfonamides as human and veterinary medicine and based on the antibiotic distribution features, we may deduce that the pollution of SMX in the bay is impacted largely by the mariculture in the north coast area and that of TMP is impacted by both freshwater inputs and mariculture. This deduction can also be supported by the correlations of these antibiotics shown in Table 3, i.e., the concentrations of four macrolide compounds and salinity in the bay were significantly correlated at the 0.01 level; the concentrations of SMX were significantly correlated at the 0.05 level with salinity but showed no significant correlation with the macrolides in the bay; the concentrations of TMP presented a significant correlation at the 0.01 level with salinity and a significant correlation at the 0.05 level with concentrations of SMX.

Although a large amount of fresh water was discharged into the YTBs, low antibiotic pollution occurred in the YTBs. This pattern may be due to the following processes: 1) strong water exchange with the open sea causing strong dilution and quick transport of the pollutants; 2) the STPs near the YTBs discharging their effluents into the deep sea; and (3) fast degradation of some of the antibiotics in seawater with high salinity because of the combination with cations (Christian et al., 2003), fast photolysis due to high transmittance, and so on. Among these reasons, we believe that the first reason is the main cause of the observed pattern. As mentioned in the introduction, parts of the seawater of the YTBs were from the BS due to the coastal current. Some studies also reported that high levels of antibiotic pollution occurred in the semi-enclosed coastal area of the BS (Jia et al., 2011; Zhang et al., 2012b; Zou et al., 2011). However, our homochronous study showed that the antibiotics in the BS attenuated sharply during the transport process from the coast to the Bohai strait. The antibiotic concentrations in the strait were lower than the levels in the YTBs. Therefore, the antibiotic pollution in the YTBs was mainly from local sources.

Although the veterinary medicine STZ has been forbidden for use in animals, it was still detected in 43% of the sampling sites in the YTBs at a low concentration (max: 1.3 ng L⁻¹; mean: 0.32 ng L⁻¹) but not in the adjacent rivers, suggesting that STZ may be used illegally in mariculture. Table 3 suggests that the concentrations of STZ were significantly correlated at the 0.05 level with the concentrations of TMP, and the concentrations of TMP also present a significant correlation at the 0.01 level (Table 3) with the concentrations of SMX in these bays. In addition, the concentrations of the three antibiotics were generally higher than the concentrations of the macrolides. Therefore, we may deduce that mariculture affects the pollution of the sulfonamides and TMP in the YTBs.

Table 3
Correlations among the main antibiotics and salinity in Jiaozhou Bay and the Yantai Bays.

Pearson correlation		Jiaozhou Bay (n = 26)							
		TMP	SMX	STZ ^a	ETM-H ₂ O	RTM	AZM	CTM	Salinity
Yantai Bays (n = 30)	TMP	1	0.491*	/	0.805**	0.804**	0.857**	0.861**	−0.868**
	SMX	0.675**	1	/	0.084	0.080	0.152	0.122	−0.467*
	STZ	0.460*	0.126	1	/	/	/	/	/
	ETM-H ₂ O	−0.149	−0.055	−0.060	1	0.826**	0.904**	0.916**	−0.781**
	RTM ^a	/	/	/	/	1	0.963**	0.967**	−0.732**
	AZM ^a	/	/	/	/	/	1	0.996**	−0.799**
	CTM ^a	/	/	/	/	/	/	1	−0.794**
	Salinity	−0.169	−0.204	0.164	−0.035	−0.136	/	/	1

^a The antibiotics detected in fewer than 5 sampling sites were not analysed for their correlation.

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

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

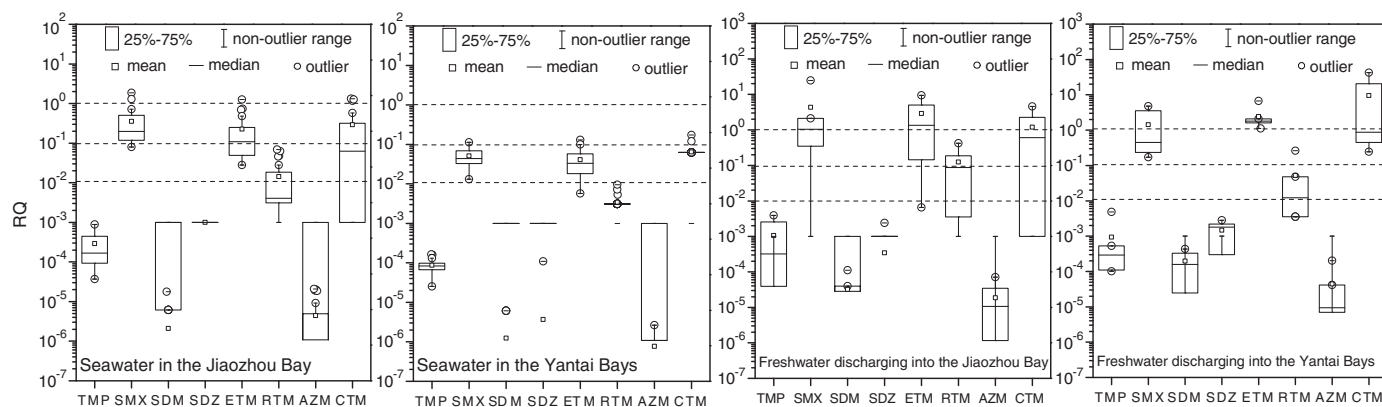


Fig. 2. Boxplots for the calculated risk quotients (RQ) of the eight antibiotics detected in Jiaozhou Bay, the Yantai Bays and the freshwater discharged into these bays. Freshwater includes river water and sewage treatment-plant effluents.

3.4. Risk assessment

As discussed above, some antibiotics occurred widely in the coastal seawater of the Yellow sea, in the freshwater of some of the rivers and in STPs at a wide range of concentrations. To quantify the risk from these compounds to the environment, their RQs were calculated based on the method in Section 2.5 and shown in Fig. 2. Four antibiotics, SMX, ETM-H₂O, RTM and CTM, could pose at least a low risk to aquatic organisms in both the seawater and the freshwater of the studied area. Three of these antibiotics, SMX, ETM-H₂O and CTM, could pose high risks to the most sensitive aquatic microorganisms (*Synechococcus leopoliensis*, *Pseudokirchneriella subcapitata* and *Pseudokirchneriella subcapitata*, respectively) due to the presence of the antibiotics in the STP effluents and the rivers close to STPs of the studied area. The compounds also could pose high or medium risks to the most sensitive aquatic microorganisms at most of the other riverine sampling sites. In JZB, these antibiotics could pose high or medium risks at most of the sampling sites. In the YTBs, these compounds could pose medium or low risks at most of the sampling sites. Except for the three antibiotics above, RTM could also pose medium or low risks to *Pseudokirchneriella subcapitata* in some of the freshwater and seawater sampling sites. In contrast to the four antibiotics mentioned above, the RQ values for TMP, SDM, SDZ and AZM were all less than 0.01 in the studied aquatic environments, suggesting that these antibiotics were unlikely to cause adverse toxic effects on the selected aquatic organisms. In summary, SMX, ETM-H₂O and CTM in this study area showed relatively high ecological risk to aquatic microorganisms, especially in freshwater. Similar results were reported in Laizhou Bay of the BS (Zhang et al., 2012b), surface water of Germany (Kummerer and Henninger, 2003) and Korea (Lee et al., 2008), i.e., the SMX, ETM-H₂O and CTM levels in those studies also showed relatively high ecological risk. Thus, this study shows that it is worthwhile to pay attention to the elimination of antibiotics in regional surface waters and the control of their potential hazard.

However, it should be noted that the risk assessment above contained some limitations due to the following factors: 1) the risk assessment performed for the coastal water environments was based on toxicity data mainly from freshwater species; 2) there was limited accessibility to total toxicity data; and 3) there was a lack of complex toxicity data for multiple compounds.

4. Conclusions

Nine or ten selected antibiotics belonging to three categories were detected in the coastal seawater from two types of bays (semi-enclosed and open) of the Yellow Sea or in the freshwater discharged into the sea. Three antibiotics, ETM-H₂O, SMX and TMP, occurred widely in the coastal water of the Yellow Sea and the freshwater with the concentrations of <0.23–50.4 ng L⁻¹ and <0.25–663.1 ng L⁻¹, respectively. In

terms of their regional distribution in the two types of bays, higher concentrations and spatial variations were present in the semi-enclosed JZB due to the poor water exchange and high freshwater inputs due to rivers and/or STP effluents. However, lower concentrations and lower spatial variations were presented in the open YTBs due to the strong water exchange with the open sea. The spatial distribution features and correlations of the detected antibiotics and the correlations between the antibiotics and the salinity both indicated that 1) freshwater inputs were the primary source of macrolides in the coastal water, and 2) mariculture affected the pollution levels of TMP, SMX and STZ in the bays. In addition, risk assessment based on the calculated RQ showed that the SMX, ETM-H₂O and CTM levels at most of the sampling sites in the JZB or YTBs could pose high (RQ > 1) or medium risks (1 ≥ RQ > 0.1) to the most sensitive aquatic microorganisms, such as *Synechococcus leopoliensis* and *Pseudokirchneriella subcapitata*.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2013.02.024>.

References

- Bialk-Bielinska A, Stolte S, Arning J, Uebers U, Boeschen A, Stepnowski P, et al. Ecotoxicity evaluation of selected sulfonamides. *Chemosphere* 2011;85:928–33.
- Christian T, Schneider RJ, Farber HA, Skutlarek D, Meyer MT, Goldbach HE. Determination of antibiotic residues in manure, soil, and surface waters. *Acta Hydrochim Hydrobiol* 2003;31:36–44.
- EC. In: Commission E, editor. 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; 2003. p. 100–3.
- Eguchi K, Nagase H, Ozawa M, Endoh YS, Goto K, Hirata K, et al. Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. *Chemosphere* 2004;57:1733–8.
- FDA-CDER. Retrospective review of ecotoxicity data submitted in environmental assessments (docket no. 96N-0057). Rockville, MD, USA: FDA Center for Drug Evaluation and Research; 1996.
- Ferrari B, Mons R, Vولات B, Frayse B, Paxeus N, Lo Giudice R, et al. Environmental risk assessment of six human pharmaceuticals: are the current environmental risk

- assessment procedures sufficient for the protection of the aquatic environment? *Environ Toxicol Chem* 2004;23:1344–54.
- Gulkowska A, He YH, So MK, Yeung LWY, Leung HW, Giesy JP, et al. The occurrence of selected antibiotics in Hong Kong coastal waters. *Mar Pollut Bull* 2007;54:1287–93.
- Hernando MD, Mezcuca M, Fernandez-Alba AR, Barcelo D. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta* 2006;69:334–42.
- Hirsch R, Ternes T, Haberer K, Kratz KL. Occurrence of antibiotics in the aquatic environment. *Sci Total Environ* 1999;225:109–18.
- Isidori M, Lavorgna M, Nardelli A, Pascarella L, Parrella A. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Sci Total Environ* 2005;346:87–98.
- Jia A. Detection, occurrence and fate of antibiotics in the environment. Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences. Doctor. Peking University, Beijing, China, 2011.
- Jia A, Hu J, Wu X, Peng H, Wu S, Dong Z. Occurrence and source apportionment of sulfonamides and their metabolites in Liaodong Bay and the adjacent Liao River Basin, North China. *Environ Toxicol Chem* 2011;30:1252–60.
- Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, et al. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. *Environ Sci Technol* 2002;36:1202–11.
- Kummerer K. Resistance in the environment. *J Antimicrob Chemother* 2004;54:311–20.
- Kummerer K. Antibiotics in the aquatic environment – a review – part I. *Chemosphere* 2009;75:417–34.
- Kummerer K, Henninger A. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clin Microbiol Infect* 2003;9:1203–14.
- Lee YJ, Lee SE, Lee DS, Kim YH. Risk assessment of human antibiotics in Korean aquatic environment. *Environ Toxicol Pharmacol* 2008;26:216–21.
- Li B, Zhang T. Biodegradation and adsorption of antibiotics in the activated sludge process. *Environ Sci Technol* 2010;44:3468–73.
- Lindberg RH, Wennberg P, Johansson MI, Tysklind M, Andersson BAV. Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. *Environ Sci Technol* 2005;39:3421–9.
- Lutzhof HCH, Halling-Sorensen B, Jorgensen SE. Algal toxicity of antibacterial agents applied in Danish fish farming. *Arch Environ Contam Toxicol* 1999;36:1–6.
- McArdell CS, Molnar E, Suter MJF, Giger W. Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley Watershed, Switzerland. *Environ Sci Technol* 2003;37:5479–86.
- Miao XS, Bishay F, Chen M, Metcalfe CD. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. *Environ Sci Technol* 2004;38:3533–41.
- Minh TB, Leung HW, Loi IH, Chan WH, So MK, Mao JQ, et al. Antibiotics in the Hong Kong metropolitan area: ubiquitous distribution and fate in Victoria Harbour. *Mar Pollut Bull* 2009;58:1052–62.
- Tang CM. Occurrence and Behavior of Antibacterials in Aqueous Environment of the Pearl River Delta, South China State Key Laboratory of Organic Geochemistry. Master. Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, Guangdong, China, 2009.
- Tolls J. Sorption of veterinary pharmaceuticals in soils: a review. *Environ Sci Technol* 2001;35:3397–406.
- Wille K, Noppe H, Verheyden K, Vanden Bussche J, De Wulf E, Van Caeter P, et al. Validation and application of an LC–MS/MS method for the simultaneous quantification of 13 pharmaceuticals in seawater. *Anal Bioanal Chem* 2010;397:1797–808.
- Xu WH. Occurrence and environmental fate of selected antibiotics in the aquatic environment of the Pearl River Delta. State Key Laboratory of Organic Geochemistry. Doctor. Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, Guangdong, China, 2007.
- Xu WH, Zhang G, Li XD, Zou SC, Li P, Hu ZH, et al. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. *Water Res* 2007a;41:4526–34.
- Xu WH, Zhang G, Zou SC, Li XD, Liu YC. Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *Environ Pollut* 2007b;145:672–9.
- Xu WH, Zhang G, Wai OWH, Zou SC, Li XD. Transport and adsorption of antibiotics by marine sediments in a dynamic environment. *J Soils Sediments* 2009;9:364–73.
- Yang L-H, Ying G-G, Su H-C, Stauber JL, Adams MS, Binet MT. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. *Environ Toxicol Chem* 2008;27:1201–8.
- Yang JF, Ying GG, Zhao JL, Tao R, Su HC, Chen F. Simultaneous determination of four classes of antibiotics in sediments of the Pearl Rivers using RRLC–MS/MS. *Sci Total Environ* 2010;408:3424–32.
- Yu Y. Integrated Coastal Area and River Basin Management in Jiaozhou Bay (in Chinese). Doctor. Ocean University of China, Qingdao, 2010.
- Zhang R. Selected Antibiotics in North China Coastal Zones and Dongjiang River Catchment. Key Laboratory of Coastal Environmental Process, CAS. Doctor. Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, Shandong, China, 2011.
- Zhang R, Tang J, Li J, Zheng Q, Liu D, Chen Y, et al. Antibiotics in the offshore waters of the Bohai Sea and the Yellow Sea in China: occurrence, distribution and ecological risks. *Environ Pollut* 2012a;174:71–7.
- Zhang R, Zhang G, Zheng Q, Tang J, Chen Y, Xu W, et al. Occurrence and risks of antibiotics in the Laizhou Bay, China: impacts of river discharge. *Ecotoxicol Environ Saf* 2012b;80:208–15.
- Zou S, Xu W, Zhang R, Tang J, Chen Y, Zhang G. Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: impacts of river discharge and aquaculture activities. *Environ Pollut* 2011;159:2913–20.