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Baseline

Occurrence, distribution, and bioaccumulation of antibiotics in coastal environment of Dalian, China

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ABSTRACT

Seawater, sediment, and aquatic organism samples were collected from 20 sampling sites in coastal environment of Dalian in August, 2011. The occurrence, distribution, and bioaccumulation of 20 antibiotics categorizing into three groups, including 14 sulfonamides (SAs), two chloramphenicols (CAPs) and four tetracyclines (TCs), were investigated. The results suggested that tetracyclines were the predominant antibiotics in the seawater (range: 2.11–9.23 ng L^{-1}), while sulfonamides were the dominant antibiotics in both sediments (range: 1.42–71.32 μg kg^{-1}) and aquatic organisms (range: 2.18–63.87 μg kg^{-1}). The sorption coefficient $K_{d,s}$ values revealed that sulfameter, sulfadiazine, sulfamethoxypyridazine, sulfamonomethoxine, chloramphenicol, and doxycycline presented higher sorption capacities than the other antibiotics. The average BAFs suggested that sulfamethazine, sulfamethiazole, sulfamonomethoxine, and doxycycline were potentially bioaccumulative, while sulfadiazine, sulfameter, sulfamethoxypyridazine, and chloramphenicol were bioaccumulative.

As very effective pharmaceuticals for preventing/treating diseases and promoting growth, antibiotics are extensively consumed in the human and veterinary medicine practice (Kümmerer, 2009; Martinez, 2009). Industrial aquaculture, usually spread over the coast in the developed and developing countries, leads to heavy use of antibiotics that to be the main resource of coastal contamination. In China, the annual usage of antibiotics has been approximately one quarter to that used in all of global countries (Jiang et al., 2011; Kümmerer, 2009). Occurrence of antibiotics in most areas of China has been found (Fang et al., 2012; Gao et al., 2012; Li et al., 2012; Zheng et al., 2012; Zhou et al., 2011). However, to our knowledge, antibiotic pollution in the coast of Dalian has not been investigated. The objective of this study is to investigate the occurrence and distribution of 20 antibiotics in seawater, sediments, and organisms for filling up data gap in this area. We also studied the adsorption capacities and bioaccumulation of these antibiotics.

In August of 2011, a total of 20 seawater, 20 sediment, and 13 biota samples were collected in coastal environment of Dalian (Fig. 1). As the difference of aquaculture environment, the sampling sites were divided into Area 1 (S1–S6), Area 2 (S7–S12) and Area 3 (S13–S20), respectively. The organism samples collected from S1, S7, S8, S13, S14, S16 and S19, respectively, covered species including *Crassostrea gigas* (from S1 and S16), *Patinopecten yessoensis* (from S1, S4, S7, S11, S12, S17, and S18), and *Chlamys farreri* (from S2, S8, S17, and S19). Seawater samples were collected using a stainless steel bucket and were immediately transferred to a 5-l pre-cleaned brown amber glass bottle capped with aluminum foil additionally. The bottle was rinsed with sample prior to sampling. Sediment samples were freeze-dried as soon as returning to the laboratory. Organism samples were lyophilized by tissue homogenizer and stored in 250 mL brown glass bottle. Seawater and organism samples were kept at 4 °C and −20 °C, respectively, and sediment samples were kept at room temperature for further treatment and analysis.

The extraction methods of SAs, CAPs, and TCs in seawater were performed following the previous study (Na et al., 2006, 2011; Ye et al., 2008, 2007a). Sediment sample (dry weight, 5.0 g) and aquatic organism sample (wet weight, 5.0 g) were loaded into 50 mL glass centrifuge tube. After blending the surrogate standards (100 ng) and samples, 20 mL of acetonitrile, acetic ether, and EDTA-Mcllvaine buffer (0.1 mol/L, pH = 4.0) was added for SAs, CAPs, and TCs extraction, respectively. The mixture was oscillated for 2 min of fully contact and centrifuged with high speed of 4000 r/min. The upper layer was separated to condense bottle. Repeat the extraction procedure for once more and combinate the two extractions. The subsequent procedures for antibiotics follows: **(CAPs)** The extraction solution of CAPs was concentrated to

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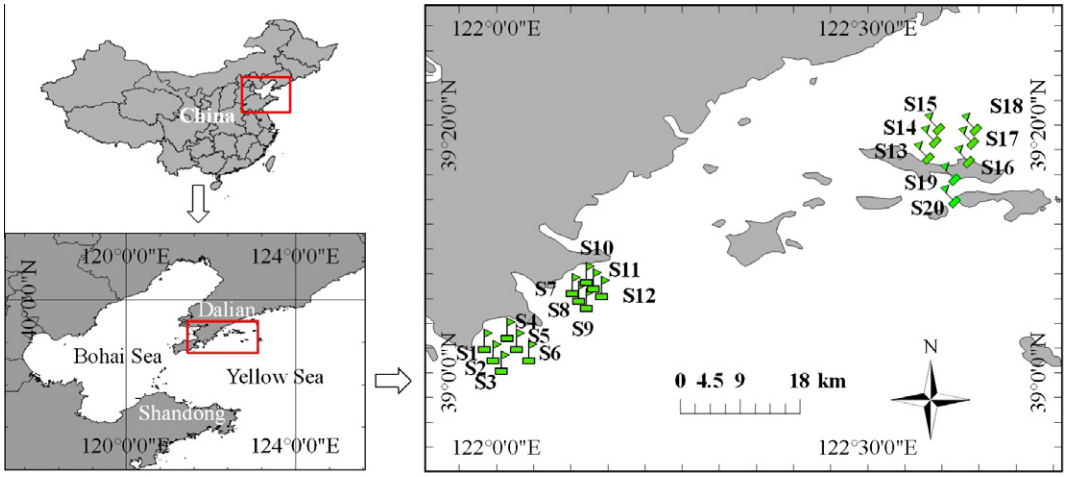

Fig. 1. Sampling sites (S1–S20) in the coast of Dalian.

Table 1
Correlation coefficients (r^2), recoveries (%), and limits of detection (LODs, $S/N = 3$) of 20 antibiotics.

Antibiotics	Recovery (%)				LODs		
	r^2	Seawater	Sediment	Organism	Seawater (ng L^{-1})	Sediment (μg kg^{-1})	Organism (μg kg^{-1})
Sulfacetamide (SAAM)	0.9903	85.23 ± 10.32	74.54 ± 14.29	82.94 ± 17.34	1.02	1.04	1.21
Sulfadiazine (SDZ)	0.9911	73.29 ± 5.98	82.48 ± 9.84	93.48 ± 13.48	0.47	0.96	1.08
Sulfathiazole (STZ)	0.9932	91.74 ± 9.45	72.87 ± 11.58	89.37 ± 10.55	0.89	1.87	2.12
Sulfamerazine (SMR)	0.9968	100.90 ± 7.34	91.90 ± 7.35	83.24 ± 9.83	2.40	2.45	2.54
Sulfamethazine (SM_2)	0.9929	94.02 ± 7.98	72.64 ± 2.37	78.06 ± 13.38	0.98	1.13	2.09
Sulfameter (SM)	0.9930	86.66 ± 11.93	86.05 ± 10.82	59.08 ± 13.41	1.05	5.78	1.92
Sulfamethiazole (SMTZ)	0.9945	98.81 ± 4.01	87.44 ± 7.62	73.22 ± 14.30	0.92	3.23	1.20
Sulfamonomethoxine (SMM)	0.9908	112.33 ± 8.23	67.29 ± 16.27	61.92 ± 3.87	0.66	2.49	1.43
Sulfachloropyridazine (SCP)	0.9964	71.57 ± 1.34	96.67 ± 13.90	63.28 ± 8.70	0.86	1.30	2.19
Sulfamethoxazole (SMZ)	0.9963	89.20 ± 3.22	90.33 ± 16.24	66.47 ± 12.22	0.83	1.82	1.17
Sulfadimethoxine (SDM)	0.9926	103.21 ± 6.28	96.72 ± 13.89	79.21 ± 16.98	0.51	3.17	3.78
Sulfamethoxypyridazine (SMP)	0.9932	88.45 ± 4.20	89.66 ± 5.48	71.98 ± 9.51	1.02	3.01	0.96
Sulfisoxazole (SIX)	0.9907	91.27 ± 9.23	88.30 ± 16.60	70.06 ± 6.35	0.53	2.56	4.60
Sulfadoxine (SDX)	0.9934	81.84 ± 9.45	90.62 ± 5.21	91.21 ± 7.21	0.34	2.12	2.73
Chloramphenicol (CAP)	0.9978	94.07 ± 3.43	96.83 ± 17.93	75.20 ± 10.83	0.04	0.73	0.62
Florophenicol (FF)	0.9991	80.79 ± 10.36	69.04 ± 16.43	67.56 ± 12.07	0.07	0.81	0.81
Oxytetracycline (OTC)	0.9934	99.91 ± 2.77	70.25 ± 4.28	80.78 ± 16.43	1.02	2.31	0.94
Doxycycline (DC)	0.9976	88.50 ± 5.98	98.34 ± 9.33	89.04 ± 18.05	0.29	1.10	1.02
Tetracycline (TC)	0.9992	95.03 ± 6.87	60.77 ± 8.25	60.79 ± 6.79	0.63	1.09	1.23
Chlortetracycline (CTC)	0.9923	80.26 ± 7.99	93.54 ± 10.90	67.42 ± 7.44	0.43	1.87	3.27

1 mL without further treatment; **(SAs)** SAs were cleaned up by Oasis® MCX cartridge (3 mL, 60 mg). The extraction was added 10 mL of isopropanol for reducing boiling point of acetonitrile under vacuum rotary evaporation. The mixture was following solvent displacement to 5 mL of 10% methanol aqueous solution. The cartridges were conditioned with 6 mL methanol, followed by 6 mL ultra-pure water, and the concentration was passed through the cartridges at a rate of about 6 mL/min. After the isolation procedure, cartridges were rinsed with 10 mL ultra-pure water and dried for 20 min under vacuum. The analytes were eluted with 6 mL methanol–ammonia (95:5, v:v) into a test tube; **(TCs)** TCs was further pretreated by Oasis® HLB cartridge (3 mL, 60 mg). The activating method was the same as Oasis® MCX cartridge for SAs. The extraction was passed through the cartridges at a rate of about 6 mL/min. Then, the cartridge was rinsed by 6 mL of 5% methanol aqueous solution. The analytes were eluted with 6 mL methanol into a test tube. Additional 0.5 mL of *n*-hexane was needed for biota sample preparation to eliminate lipid when the elution was concentrated to 1 mL. After full contact, the sublayer was introduced into HPLC–MS/MS system for analyzing.

The instrumental analysis methods were also optimized based on our studies in 2007 and 2009 (Na et al., 2009; Ye et al., 2007a, 2007b). Extracted samples were analyzed by TSQ Quantum high performance liquid chromatography–mass spectrometry (HPLC/MS, Thermo Fisher Scientific, USA). Antibiotics were separated by C18 reverse phase columns. Mass spectrometer detections of antibiotics were operated in selected reaction monitoring mode. The quantitation curves of antibiotics were performed with wide liner ranges (0.2–1000 ng L^{-1}) and good correlation coefficients ($r^2 > 0.990$). The limit of detection (LOD) for each antibiotic was defined as the concentrations corresponding to the signal-to-noise (S/N) of 3. Recoveries of target antibiotics ranged from 59.1% to 112.3%. All samples were analyzed in triplicates, and the relative standard deviation ($n = 3$) was less than 18.2%. Analysis of reagent blanks demonstrated that the analytical system and glassware were free of contamination (Table 1).

The presence of antibiotics in the seawater samples from the study areas was summarized in Table 2. A total of 18 out of the 20 test antibiotics were detected in seawater samples. Concentrations of ∑SAs, ∑CAPs, and ∑TCs were ranged from *nd* to