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OCCURRENCE AND SOURCE APPORTIONMENT OF SULFONAMIDES AND THEIR METABOLITES IN LIAODONG BAY AND THE ADJACENT LIAO RIVER BASIN, NORTH CHINA

Ai Jia, Jianying Hu,* Xiaoqin Wu, Hui Peng, Shimin Wu, and Zhaomin Dong
Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing, China

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Abstract—The presence of antibiotics in the environment is of great concern because of their potential for resistance selection among pathogens. In the present study we investigated the occurrence of 19 sulfonamides, five *N*-acetylated sulfonamide metabolites, and trimethoprim in the Liao River basin and adjacent Liaodong Bay, China, as well as 10 human/agricultural source samples. Within the 35 river samples, 12 sulfonamides, four acetylated sulfonamides, and trimethoprim were detected, with the dominant being sulfamethoxazole (66.6 ng/L), *N*-acetylsulfamethoxazole (63.1 ng/L), trimethoprim (29.0 ng/L), sulfadiazine (14.0 ng/L), and sulfamonomethoxine (8.4 ng/L); within the 36 marine samples, 10 chemicals were detected, with the main contributions from sulfamethoxazole (25.2 ng/L) and *N*-acetylsulfamethoxazole (28.6 ng/L). Sulfamethoxazole (25.9%), *N*-acetylsulfamethoxazole (46.6%), trimethoprim (22.9%), and sulfapyridine (1.4%) were the main chemicals from human sources, while sulfamonomethoxine, sulfamethazine, sulfaquinoxaline, sulfaguanidine, sulfadiazine, sulfanilamide, and sulfamethoxypyridazine were dominant in the animal husbandry sources, specifically, swine and poultry farms, and sulfamethoxazole (91%) was dominant in the mariculture source. A principal component analysis with multiple linear regression was performed to evaluate the source apportionment of total sulfonamides in Liaodong Bay. It was found that animal husbandry contributed 15.2% of total sulfonamides, while human sources contributed 28.5%, and combined human and mariculture sources contributed 56.3%. In addition, the mariculture contribution was 24.1% of total sulfonamides into the sea based on mass flux estimation. The present study is the first report that the environmental levels of sulfonamide metabolites were comparable to the corresponding parents; therefore, we should pay attention to their environmental occurrence. Source apportionment showed human discharge (60.7%) significantly contributed to these antibiotics in Liaodong Bay, which provides important information for environmental management. Environ. Toxicol. Chem. 2011;30:1252–1260. © 2011 SETAC

Keywords—Sulfonamides Occurrence River water Seawater Source apportionment

INTRODUCTION

A number of studies have highlighted the environmental occurrence of antibiotics after their huge usage throughout the world, and scientists are increasingly concerned about the induction and spread of antibiotic resistance and their resistant genes in response to an increased selective pressure [1,2]. Sulfonamides are a group of synthetic antibiotics that function as competitive antagonists to *p*-aminobenzoate in the bacterial enzymatic synthesis of dihydrofolic acid [3] and are often administered with trimethoprim to potentiate bactericidal effects [4]. However, some studies had found that continuous treatment with sulfonamides such as sulfamerazine and sulfadimethoxine could induce carcinomas [5–7]. In addition, sulfonamides resistance genes such as *sul1*, *sul2*, and *sul3* have been found in soils and natural rivers [8,9]. Therefore, a gene exchange process known as *horizontal transfer*, proven in the laboratory [10–12], may also happen in the environment [8], especially under continuous exposure of sulfonamides [10,13].

Sulfonamides and trimethoprim are the most frequently prescribed antibiotics used in human and veterinary medicine [14] to treat a variety of bacteria, virus, and protozoan infections [3,15,16], or as animal growth promoters to improve feed efficiency [17]. Of these sulfonamides, sulfamethoxazole, sulfapyridine, and sulfamerazine are primarily used in human treatment, while sulfadiazine, sulfadimethoxine, sulfamethazine, sulfathiazole, and sulfachloropyridazine are frequently used in veterinary medicine [17]. After oral application, sulfonamides are excreted via urine and are ultimately released into the aquatic environment through sewage treatment plant effluent or agricultural runoff. The removal efficiency of these compounds during wastewater treatment has proven to be poor [18], and relatively high concentrations of sulfonamides have been ubiquitously detected in surface water samples [19–24]. While most of these studies often focused on a narrow range of sulfonamides (mainly sulfamethoxazole, sulfamethazine, and occasionally sulfapyridine and sulfadiazine), 20 to 30 sulfonamides are commercially available and prevalently used around the world [25], which is worth noting because of their potential abuse.

In addition to the environmental occurrence of sulfonamides, their metabolites should get more attention. The main metabolic pathway for sulfonamides is supposed to be *N*-acetylation [26] as exemplified by sulfamethoxazole (SMX), of which only 15 to 25% is present in unchanged parent form and 43% is present as *N*-acetylsulfamethoxazole (NAcSMX) in the urine [27]. The retransformation of *N*-acetylated metabolites into sulfonamides can occur in the environment via microbial activities [1,28]. This phenomenon has been demonstrated by the transformation of NAcSMX to SMX in a water-sediment coexisting test system [29] and *N*-acetylsulfamethazine to its active parent sulfamethazine during the storage of manure [30]. Thus, sulfonamides metabolites are likely to be an extra source of active sulfonamides within the environment. To our best knowledge, however, only a few studies have focused on the detection of NAcSMX in sewage treatment plants [18], while the

All Supplemental Data may be found in the online version of this article.

* To whom correspondence may be addressed (hujy@urban.pku.edu.cn).

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metabolites of a broad range of sulfonamides in environmental matrices have not been reported.

In the present study we developed a liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS) method for simultaneously analyzing 19 sulfonamides, five *N*-acetylated metabolites, and trimethoprim in sewage treatment plant (STP) influent, effluent, and environmental river samples, by improving the solid-phase extraction (SPE) cleanup method. The method was then applied to investigate the occurrence of target analytes in the Liao River basin, the adjacent Liaodong Bay, as well as human and agricultural influenced source samples in northern China. The parent sulfonamides included in the present study were chosen because they have potential for use in human medical treatment and animal husbandry in the Liaodong Bay region. Finally, source apportionment analyses were carried out using principal component analyses with multiple linear regression based on the profiles of all target compounds to interpret the contribution from human and agricultural sources to total sulfonamides in Liaodong Bay.

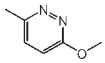
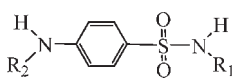
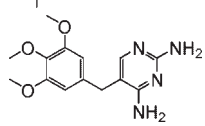
MATERIALS AND METHODS

Chemicals and materials

Sulfaguanidine (SGD), sulfanilamide (SA), sulfathiazole (STZ), sulfisomidine (SIM), sulfamonomethoxine (SMM), sulfisoxazole (SIA), sulfachloropyridazine (SCP), sulfapyridine (SPD), sulfadiazine (SDZ), sulfamethazine (SMA), sulfamethoxazole (SMX), sulfamerazine (SMR), sulfaquinoxaline (SQX), sulfameter (SME), sulfamethizole (SMT), sulfamoxol (SMO), sulfadimethoxine (SDM), sulfamethoxypyridazine (SMP), sulfanitran (SNT), and trimethoprim (TMP) were all obtained from Sigma-Aldrich. $^{13}C_6$-sulfamethazine ($^{13}C_6$-SMA) was obtained from Cambridge Isotope Laboratories. *N*-acetylsulfapyridine (NAcSPD), *N*-acetylsulfadiazine (NAcSDZ), *N*-acetylsulfamethazine (NAcSMA), *N*-acetylsulfamethoxazole (NAcSMX), *N*-acetylsulfamerazine (NAcSMR), and *N*-acetylsulfamethoxazole-d_5 (NAcSMX-d_5) were purchased from Toronto Research Chemicals (Table 1).

The high-performance liquid chromatography (HPLC)-grade methanol, acetone, dichloromethane, ethyl acetate, and

Table 1. Chemical structures and electrospray tandem mass spectrometry (ESI-MS/MS) spectra features of target sulfonamides, their metabolites, and trimethoprim

Compound[a]	R_1	R_2	Mass spectra[b]	Compound	R_1	R_2	Mass spectra
SGD		H	173 (92,156)	SQX		H	301 (92,156)
SA	H	H	215 (92,156)	SME		H	281 (92,156)
STZ		H	256 (92,156)	SMT		H	271(92,156)
SIM		H	279 (92,124)	SMO		H	268 (92,156)
SMM		H	281 (92,156)	SDM		H	311 (92,156)
SIA		H	268 (92,156)	SCP		H	285 (92,156)
SMP		H	281 (92,156)	SNT			336 (64,156)
SDZ		H	251 (92,156)	NAcSDZ			293 (134,198)
SPD		H	250 (92,156)	NAcSPD			292 (134,198)
SMA		H	279 (92,186)	NAcSMA			281 (92,156)
SMX		H	254 (92,156)	NAcSMX			321 (134,186)
SMR		H	265 (92,110)	NAcSMR			307 (134,172)
SAs				TMP			291 (110, 123)

[a] SAs = sulfonamide antibiotics; SGD = sulfaguanidine; SA = sulfanilamide; STZ = sulfathiazole; SIM = sulfisomidine; SMM = sulfamonomethoxine; SIA = sulfisoxazole; SMP = sulfamethoxypyridazine; SQX = sulfaquinoxaline; SME = sulfameter; SMT = sulfamethizole; SMO = sulfamoxol; SDM = sulfadimethoxine; SCP = sulfachloropyridazine; SNT = sulfanitran; SDZ = sulfadiazine; NAcSDZ = *N*-acetylsulfadiazine; SPD = sulfapyridine; NAcSPD = *N*-acetylsulfapyridine; SMA = sulfamethazine; NAcSMA = *N*-acetylsulfamethazine; SMX = sulfamethoxazole; NAcSMX = *N*-acetylsulfamethoxazole; SMR = sulfamerazine; NAcSMR = *N*-acetylsulfamerazine; TMP = trimethoprim.

[b] Multi-selected reaction monitoring transition in ESI-MS/MS: precursor ion (product ions).

hexane were purchased from Fisher Chemicals and HPLC grade formic acid was purchased from Dima Technology. Ethylenediamine tetraacetic acid disodium (Na_2EDTA) of analytical reagent grade was obtained from Sinopharm Chemical Reagent. Distilled water was purified by a Milli-Q Synthesis water purification system (Millipore). The SPE cartridges, including Oasis hydrophilic-lipophilic balance (HLB) (500 mg/6 ml) and Sep-Pak Silica (500 mg/3 ml), were from Waters. Stock solutions (1000 mg/L in methanol) for all standard substances were prepared and stored at −20°C.

Sample sites and collection

The Xiaoling River, Daling River, Shuangtaizi River, and Daliao River are the four main rivers flowing into Liaodong Bay, Bohai Sea. Thirty-five water samples from these four rivers and their tributaries and 36 seawater samples from Liaodong Bay were collected in May 2009 from the subsurface zone (30–50 cm depth) (Fig. 1).

The river locations cover both urban and agricultural areas. Only one sewage treatment plant, which processes approximately two-thirds (100,000 m^3/d) of the municipal wastewater of the Xinglongtai area, is in the urban district of Panjin City. All untreated sewage is discharged directly into local rivers. Animal husbandries include approximately 452,000 swine, 26,000 cattle, and 14 million poultry and are widespread throughout the Panjin area [31]. To identify the human and agricultural source apportionment of the target chemicals, various source samples were collected. In detail, untreated urban sewage from one large canal in the Shuangtaizi area, the outflow from one drainage station, the 24-h composite influents and effluents from the STP, and the discharge from one local hospital were collected and identified as the human-used sulfonamides source (HS1 to HS5 in Fig. 1). Discharge from field streams of intensive swine, duck, chicken, and cattle farms were collected and identified as the animal husbandry source samples (AS1 to AS4 in Fig. 1), and one mariculture sample from a shrimp pond was also collected (MQ in Fig. 1). All water samples were collected in amber glass bottles and filtered with a glass microfiber filter (GF/C, 1.2 μm) (Whatman) before being extracted by HLB cartridges on the same day of collection. These samples were then extracted and prepared for determination of the target 25 analytes, including sulfonamides, their metabolites, and trimethoprim. Detailed sample preparation and LC-MS/MS conditions are provided in the Supplemental Data.

Quantitation

The LC-MS/MS identification was accomplished by comparing the retention time (within 2%) and the signal ratio (within 20%) of two selected product ions in the environmental samples with standards. To compensate for both variations in the SPE process and instrument response, $^{13}C_6$-SMA and NAcSMX-d_5 were used as surrogate standards for parent sulfonamides and *N*-acetylated metabolites, respectively. Instrument detection limits (IDLs) were estimated using a signal-to-noise approach of the standard dilutions reaching a ratio of three, and method detection limits (MDLs) were determined by calculating the lowest concentration of the target chemicals in various water matrices that yielded an ion signal-to-noise of three. Recoveries of all target compounds and surrogates were analyzed by spiking standard solution and the surrogates to the influent, effluent, river, and sea samples ($n = 3$), and quality control was identified by distilled water blanks and duplicate samples in every 10 samples.

Source apportionment method

Source apportionment analysis was conducted using principal component analysis followed by multiple linear regression (PCA-MLR) with SPSS software. Concentrations of variables can be regarded as linear combinations of a number of factors (or sources), and the purpose of PCA is to reproduce the correlation matrix in a minimum number of factors [32,33]. Each factor is orthogonal to all others, which results in the smallest possible covariance. The first factor represents the

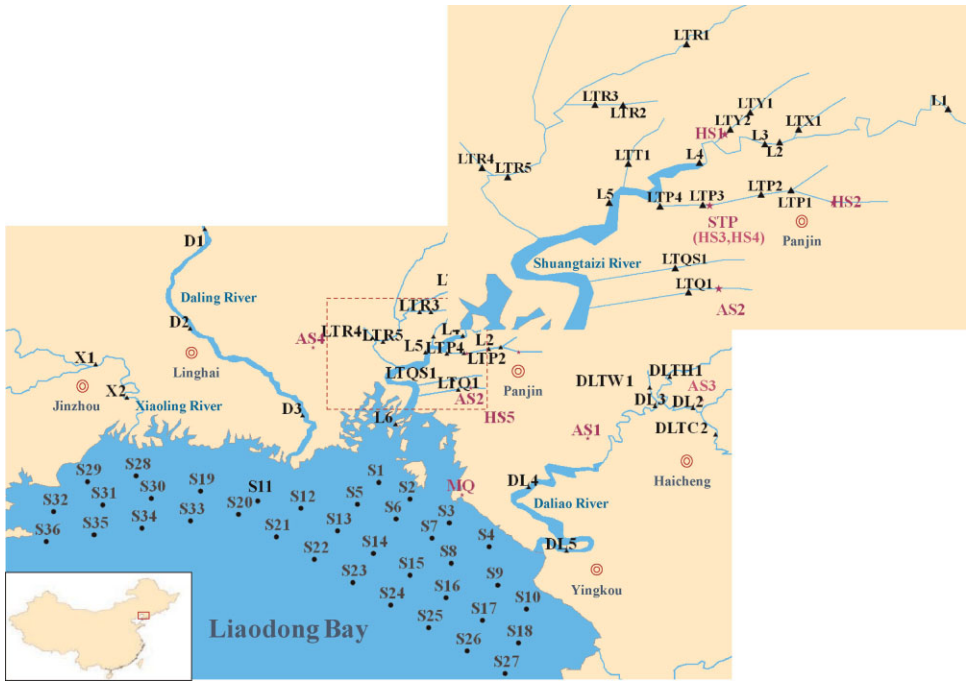


Fig. 1. Study area and sampling locations, China.

weighted loading of the original variables that account for the greatest variability, and each subsequent factor accounts for less variability than the previous. Multiple linear regression was than performed on the significant factors to determine the mass apportionment of each source to total concentration. Stepwise modeling was used to allow each independent factor to enter into the regression equation if it could significantly increase the correlation, and a default significance level of 0.05 was used here. After normalization, the MLR equation can finally be expressed as:

$$\hat{Z}_{sum} = \sum B_k FS_k \quad (1)$$

where $\hat{Z}_{sum}$ is the standard normalized deviate of the sum of the chemical concentrations (i.e., $\hat{Z}_{sum} = (Z_{sum} - mean[Z_{sum}])/\sigma$, and σ is the standard deviation of Z_{sum}), B_k represents the regression coefficients, and FS_k are factor scores calculated by the PCA analysis.

Thus, the mean percentage contribution can be calculated by $B_k/\sum B_k$, and the contribution of each source (C_k) was estimated as:

$$C_k(\text{ng/L}) = \text{mean}[Z_{sum}] \times (B_k/\sum B_k) + B_k \sigma FS_k \quad (2)$$

The PCA-MLR methodology had been used in many cases as exemplified by the source identification of PAHs in atmosphere [32] and steroid hormones in urban rivers [34].

RESULTS AND DISCUSSION

Quantitation and quality control

All analytes showed maximum sensitivity in the positive ionization mode. The protonated molecular ion ($[M + H]^+$) was chosen as the precursor ion, and the most abundant product ions selected for the transitions are listed in Table 1. For *N*-acetylated sulfonamides, the product ions, $[C_8H_8NO]^+$ (m/z 134) and $[C_8H_8NO_3S]^+$ (m/z 198), previously reported in NAcSMX [18], were also observed as the most abundant ions in the collision-induced dissociation spectra for NAcSMX, NAcSPD, and NAcSDZ. While the fragment of $[C_8H_8NO]^+$ (m/z 134) was also abundant, $[C_6H_8N_3O_2S]^+$ (m/z 186) and $[C_5H_6N_3O_2S]^+$ (m/z 172) were observed in the mass spectra of NAcSMA and NAcSMR, respectively. Those fragmentations were consistent with their parent sulfonamides [35]. The $[M + H]^+$ to $[C_8H_8NO]^+$ (m/z 134) transition was selected for quantitation of all *N*-acetylated sulfonamides due to its higher response, while the other transition was used for confirmation.

No contamination of blanks was detected throughout the whole determination time, and the standard deviations of the field duplicates were all within $\pm 10\%$. In the recovery experiments ($n = 3$), the mean absolute recoveries of target chemicals and surrogates in sea samples ranged from 65 to 119%, with a relative standard deviation (RSD) lower than 10.7% (Supplemental Data, Table S2). For the river and source samples, to simultaneously extract all sulfonamides their *N*-acetylated metabolites, and trimethoprim, a cleanup procedure using the Silica cartridge was optimized (Supplemental Data), and the absolute recoveries from spiked STP influent, effluent, and river water were 67 to 91%, 73 to 117%, and 62 to 120% for all analytes, respectively. The MDLs for all chemicals were in the range of 0.3 to 7 ng/L for influent, 0.2 to 3 ng/L for effluent, 0.2 to 1.4 ng/L for river, and 0.04 to 1.2 ng/L for sea samples, respectively. Detailed recoveries, IDLs, and MDLs for all analytes are listed in the Supplemental Data (Table S2).

Concentration levels

River. Of the 25 target chemicals, 12 sulfonamides (SMX, SMM, SDZ, SMA, SPD, SGD, SA, STZ, SCP, SQX, SIM, and SDM), four *N*-acetylated metabolites (NAcSMX, NAcSPD, NAcSDZ, and NAcSMA), and TMP were detected in the river samples collected from the Liao River basin (Fig. 2; Table 2). The typical MRM LC-MS/MS chromatograms obtained from one river sample are shown in Figure S1 (Supplemental Data). Sulfamethoxazole, TMP, SMM, SDZ, and SMA were detected in 97 to 100% of the 35 river samples. The median concentrations of SMX and TMP were 66.6 ng/L and 29.0 ng/L, with a range of 6.7 to 173.2 ng/L and 5.3 to 121.1 ng/L, respectively. These results are comparable to those reported in surface water in the UK [20], Germany [22], Pearl River, China [23], and Hong Kong [24], but lower than those in US streams [19,21]. The concentration levels of SMA (<MDL-26.4 ng/L, median 4.1 ng/L) and SDZ (1.0–30.5 ng/L, median 14.0 ng/L) were lower than those obtained from the Pearl River, China (67 ng/L and 38 ng/L) [23]. SMM was first detected in surface water with concentrations (1.2–35.1 ng/L) comparable to SDZ and SMA, indicating that it should not be neglected in future investigations. The detection frequencies for SPD, SGD, SA, STZ, SCP, and SQX were 77, 77, 77, 54, 51, and 37%, respectively, and their median concentrations were between 2.1 ng/L (SCP) and 4.3 ng/L (SA). Of these chemicals, only the presence of SPD had been reported previously in Tamagawa River, Japan [14], with a maximum concentration (132 ng/L) much higher than the present study (15.7 ng/L). Sulfisomidine (0.4 ng/L) and SDM (1.0 ng/L) were detected in one or two of the 35 river sites, while SMR, SIA, SME, SMT, SMO, SMP, and SNT were not found in any of the Liao River basin samples.

The detection frequencies of *N*-acetylated sulfonamides metabolites were 100, 60, 26, and 23% for NAcSMX, NAcSMA, NAcSPD, and NAcSDZ, respectively, and the concentrations were generally comparable to their parent sulfonamides. *N*-acetylsulfamethoxazole (11.8–268.5 ng/L, median 63.1 ng/L) had the highest concentration in the four detected metabolites, followed by NAcSPD (<MDL-13.7 ng/L, median 4.4 ng/L), NAcSMA (<MDL-11.5 ng/L, median 2.7 ng/L), and NAcSDZ (<MDL-3.3 ng/L, median 2.4 ng/L). The ratio of *N*-acetylated sulfonamide to its parent compound was in the range of 0.24 to 2.65 for NAcSMX/SMX, and the concentration of NAcSMX was larger than SMX in 16 of the 35 river samples. The ratios for NAcSMA/SMA, NAcSPD/SPD, and NAcSDZ/SDZ were 0.15 to 2.09, 0.40 to 1.34, and 0.09 to 0.21, respectively, indicating that *N*-acetylated sulfonamide metabolism should not be underestimated in future environmental studies.

The median concentration of the 17 detected compounds for the 35 river samples was 192.1 ng/L, with a range from 27.1 (site X1) to 627.4 ng/L (site DL2). The distributions of target compounds were different along the rivers. Seven chemicals, including SMX, SMM, SDZ, SPD, SMA, TMP, and NAcSMX, were detected in all four rivers, but the concentrations of SMM, SDZ, and SMA in Xiaoling River and Daling River were lower than those in Shuangtaizi River and Daliao River. Results showed that SGD, SA, SCP, SQX, and metabolites including NAcSMA, NAcSPD, and NAcSDZ were all distributed in Shuangtaizi River and Daliao River, while STZ was only detected in Shuangtaizi River and its tributaries. Because SMM, SDZ, SMA, SGD, SA, SCP, SQX, and STZ were mostly assumed to be animal-used antibiotics [28,36], the results

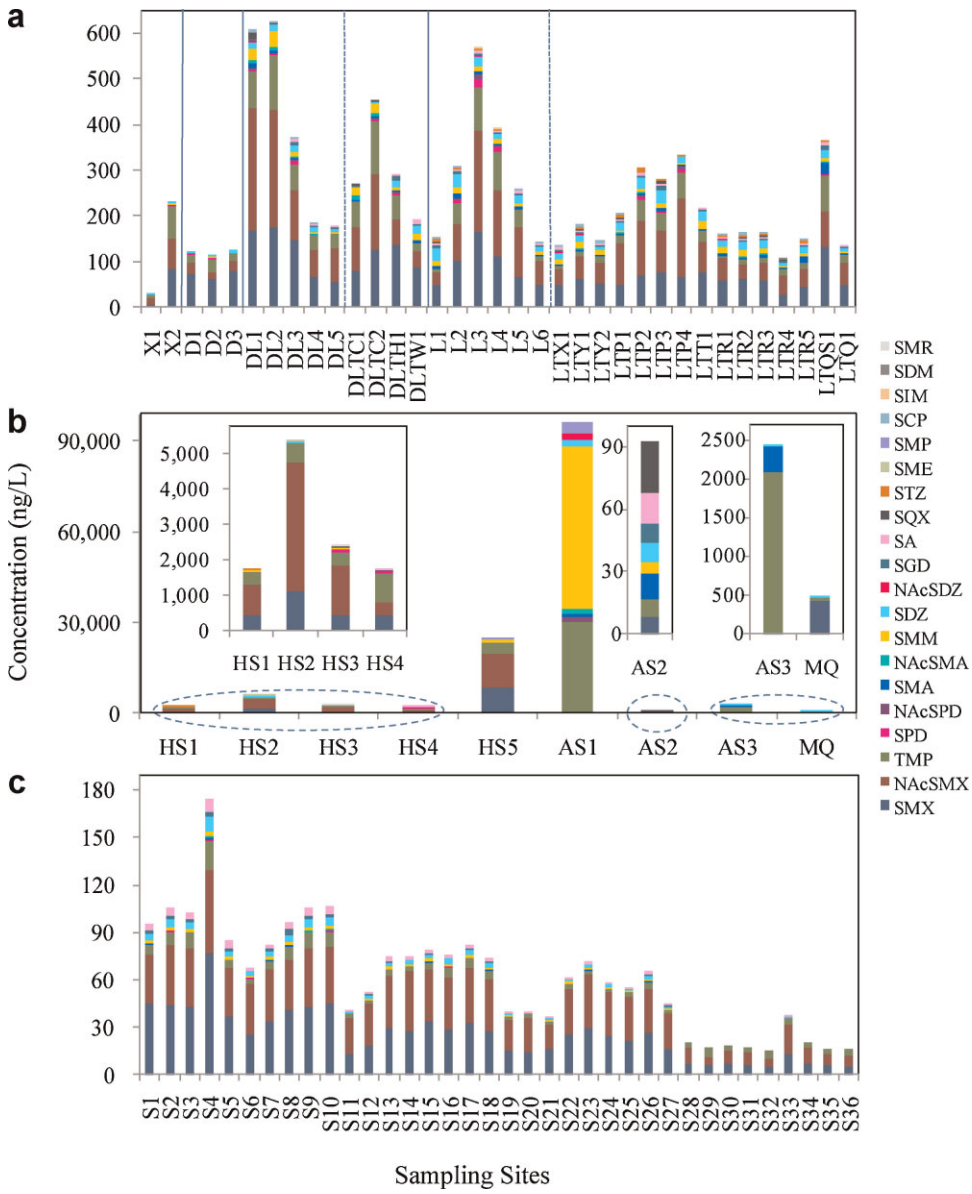


Fig. 2. Distribution of all detected chemicals in river (**a**), source (**b**), and sea (**c**) samples. SMX = sulfamethoxazole; NAcSMX= *N*-acetylsulfamethoxazole; TMP= trimethoprim; SPD= sulfapyridine; NAcSPD= *N*-acetylsulfapyridine; SMA= sulfamethazine; NAcSMA= *N*-acetylsulfamethazine; SMM= sulfamonomethoxine; SDZ= sulfadiazine; NAcSDZ= *N*-acetylsulfadiazine; SGD= sulfaguanidine; SA= sulfanilamide; SQX= sulfaquinoxaline; STZ= sulfathiazole; SME= sulfameter; SMP= sulfamethoxypyridazine; SCP= sulfachloropyridazine; SIM= sulfisomidine; SDM= sulfadimethoxine; SMR= sulfamerazine.

suggest that the influence of livestock was more notable in the Shuangtaizi River and Daliao River region, while the Xiaoling River and Daling River were largely influenced by humans.

Sea. Of the 25 target compounds, SMX, SDZ, SA, SMM, SMA, SGD, SPD, NAcSMX, NAcSMA, and TMP were detected in the sea samples (Fig. 2; Table 2). The median concentrations of NAcSMX and SMX were 28.6 ng/L and 25.2 ng/L, respectively, followed by TMP (3.6 ng/L), SDZ (2.4 ng/L), SA (2.0 ng/L), SMM (1.6 ng/L), SGD (0.9 ng/L), NAcSMA (0.6 ng/L), SMA (0.3 ng/L), and SPD (0.2 ng/L). The median concentration of total chemicals in the sea samples (63.4 ng/L) was much lower than that of the river samples (192.1 ng/L), but the highest total concentration detected in site S4 (174.4 ng/L) was similar to its nearest estuarial site (DL5) in the Daliao River (176.4 ng/L). In all sea samples, NAcSMX, SMX, and TMP were detected, while SDZ, SA, SMM, SMA, SGD, SPD, and NAcSMA were mainly found in samples from the eastern area of Liaodong Bay, which is adjacent to Shuangtaizi River and Daliao River. The total content was also higher in the eastern area than the western area adjacent to Xiaoling River and Daling River, indicating that the occurrence of target chemicals in the sea was largely influenced by the adjacent river basins.

Source apportionment

Sulfonamides: human and agricultural sources. To further understand the presence of target chemicals in environmental samples, we also analyzed 10 source samples that possibly contribute to the occurrence of sulfonamide antibiotics in the Liao River basin and Liaodong Bay (Fig. 1). Two untreated urban sewage discharge samples (HS1, HS2), STP influent and effluent composite samples (HS3, HS4), and one hospital wastewater (HS5) were assumed to be the main source type of sulfonamides from human activities. The agricultural sources

Table 2. Concentrations (ng/L) and detection frequencies of sulfonamides and trimethoprim in Liao River basin and its adjacent Liaodong Bay, China

	River				Sea			
Compound[a]	n[b]	Frequency	Median	Range	n	Frequency	Median	Range
SMX	35	100%	66.6	6.7–173.2	36	100%	25.2	4.3–76.9
NAcSMX	35	100%	63.1	11.8–268.5	36	100%	28.6	5.9–52.8
TMP	35	100%	29.0	5.3–121.1	36	100%	3.6	1.4–18.2
SMM	35	100%	8.4	1.2–35.1	26	72%	1.6	<MDL–3.3
SDZ	35	100%	14.0	1.0–30.5	28	78%	2.4	<MDL–9.1
NAcSDZ	8	23%	2.4	<MDL–3.3	–	–	–	–
SPD	27	77%	2.3	<MDL–15.7	14	39%	0.2	<MDL–0.6
NAcSPD	9	26%	4.4	<MDL–13.7	–	–	–	–
SMA	34	97%	4.1	<MDL–26.4	24	67%	0.3	<MDL–1.1
NAcSMA	21	60%	2.7	<MDL–11.5	5	14%	0.6	<MDL–0.9
SCP	18	51%	2.1	<MDL–8.1	–	–	–	–
SQX	13	37%	2.9	<MDL–13.6	–	–	–	–
SGD	27	77%	3.6	<MDL–8.0	21	58%	0.9	<MDL–3.7
SA	27	77%	4.3	<MDL–12.0	28	78%	2.0	<MDL–7.9
STZ	19	54%	3.9	<MDL–8.5	–	–	–	–
SIM	2	6%	–	0.4	–	–	–	–
SDM	1	3%	–	1.0	–	–	–	–
Total			192.1	–		–	63.4	–

[a] Full names and structures of chemicals are listed in Table 1.
[b] Number detected.
MDL = method detection limits.

included animal husbandries and mariculture sources, and four livestock discharges from swine, duck, chicken, cattle farms (termed AS1 to AS4) and one mariculture sample (MQ) were collected. All chemicals except for SCP, SIM, and SDM found in the Liao River basin were detected, with SME (0.7 ng/L) and SMP (21.5 ng/L) newly found in hospital wastewater, and SMR (1.0 ng/L) found in the STP influent. Especially, SMP in swine farm discharge reached 3,393 ng/L, and a relatively high concentration of SME (5.3 ng/L) was also detected in untreated sewage compared with hospital wastewater. (Fig. 2; Supplemental Data, Table S3).

As for the human sources, the highest total concentration was observed in hospital wastewater (24,346 ng/L), followed by discharge from untreated wastewater HS2 (5,382 ng/L), STP influent (2,374 ng/L), untreated wastewater HS1 (1,727 ng/L), and STP effluent (1,724 ng/L). Because chemical compositions were similar within these samples, their median composition was estimated as 46.6% for NAcSMX, 25.9% for SMX, 22.9% for TMP, and 1.4% for SPD, accounting for 96.8% (95.5–98.6%) of the total percentage in the human source profile (Fig. 3; Supplemental Data, Table S3). Both SMX and TMP are widely used in human medicines, and their high contributions were also reported in hospital, STPs effluent, and regional discharges in Taiwan [37].

With regard to animal husbandry sources, the total concentration in wastewater from swine farms (96,206 ng/L) was much higher than that in chicken (2,431 ng/L) and duck (92.4 ng/L) farms, while no compounds were found in cattle farm discharge. The total level of swine discharge was also higher than those reported in Taiwan and Vietnam (147–19,464 ng/L) [14,37], but lower than that from USA swine farm lagoons (up to 400 μg/L) [38]. Chemical profiles were different in the three distinct

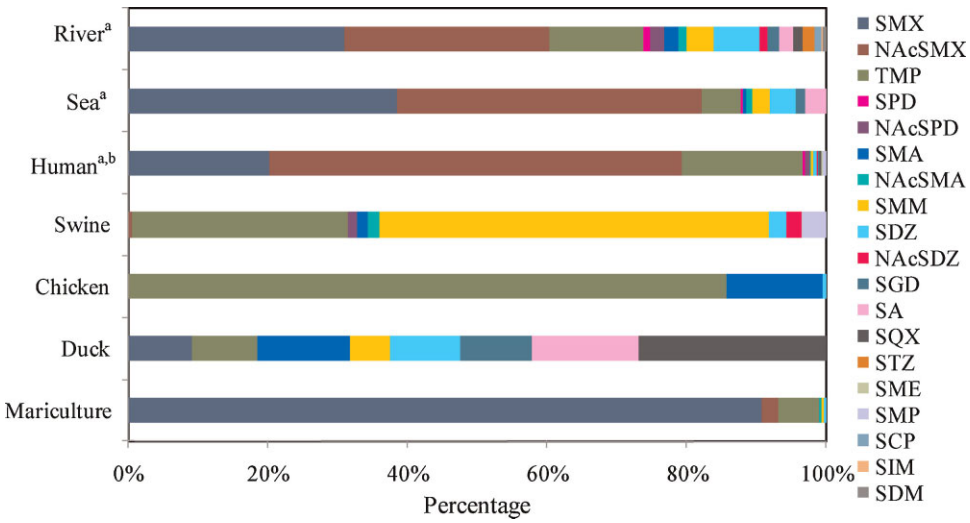


Fig. 3. Comparison of chemical profiles detected in Liao River basin and Liaodong Bay, China with those of human, animal husbandry, and mariculture sources. [a]Median values were used because most detected chemicals followed lognormal distribution within these samples. [b]The human profile excluded the sewage treatment plant (STP) influent (HS3) sample, since it was not directly discharged into the environment. Chemical full names and structures are listed in Table 1. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

animal farm wastewater samples (Fig. 3). In swine discharge, the main compounds detected were SMM (55.8%), TMP (30.9%), SMP (3.5%), SDZ (2.5%), and SMA (1.4%), which differed substantially from previous reports from Vietnam, Japan, and the USA in which SMA was dominant and occupied 64.6 to 99.8% of the total concentrations [14,38], and from Taiwan in which STZ (82%) and SMR (14%) were dominant in swine waste effluents [37]. In the chicken farm lagoon, TMP (85.7%) was dominant followed by SMA (13.7%) and SDZ (0.6%), which also differed from a previous study in Vietnam, which reported SMA (60.1%) to be the main sulfonamide [14]. In the duck lagoon, SMX (9.1%), TMP (9.5%), SMA (13.2%), SMM (5.8%), SDZ (10.0%), SGD (10.3%), SA (15.2%), and SQX (27.0%) were detected with similar contributions. These results reveal that while the occurrence of veterinary sulfonamides depended largely on their different application in various animal feeding operations, the profiles in swine, duck, and chicken farm lagoons were all distinct from human sources. In particular, the proportion of SMM, SMA, SQX, SGD, SDZ, SA, and SMP were largely increased in animal husbandry samples compared to the human profile. As for mariculture sample from the shrimp pond, SMX (91%) was the main sulfonamide followed by TMP (5.9%) and NAcSMX (2.3%), of which the profile was quite different from terrestrial livestock sources, but similar to fish and clam aquaculture reported in Taiwan, where SMX and TMP accounted for 66.6% and 24.7% of the total sulfonamides, respectively [37].

The profiles of sulfonamides in the river basin and sea samples were more similar to human sources than agricultural sources (Fig. 3). However, the contributions by SDZ (6.6%), SMM (3.9%), SMA (1.9%), SGD (1.7%), SA (2.0%), and SQX (1.4%), major sulfonamides in animal husbandry, were much greater than the human profile in which their composition was generally below 0.4%.

PCA-MLR results. Because no statistics refer to any information on antibiotics use, species, and discharge volumes in this area, to further quantitatively describe the influences of human and agricultural activity on the marine environment based on target compounds, we performed source apportionment analysis using PCA-MLR method. All 36 sea samples, four river estuary samples (sites X2, D3, L6, and DL5), and the mariculture source (MQ) were included in the analysis. The 10 detected chemicals in the sea samples were included as variables in the PCA analysis, and concentrations below the MDLs were recorded as half of the MDL values in the datasheet. Three principal components were identified after varimax rotation, which accounted for 54.9, 20.5, and 15.3% of the total variance, respectively (Table 3). The first component was heavily weighted by SGD, SDZ, SA, and SMM. According to the above profile of human and agricultural sources, this component should represent the animal husbandry source. The second component was mainly associated with SPD, TMP, and NAcSMX, which were important chemicals in the human profile, suggesting that this component could be indicative of human sources. The third component correlated only with NAcSMA and SMX. Because SMX is a specific sulfonamide in both human and mariculture profiles (Fig. 3), a primary deduction is that this component could represent the combination of human and mariculture sources.

Multiple linear regression analysis with the factor score (FS_k) against the standard normalized deviate ($\hat{Z}_{sum}$) of the sum concentrations of the 10 chemicals was performed to determ ine the mass apportionment of the three components

Table 3. Rotated component matrix of sea, estuary, and mariculture samples[a]

	Component		
Variable[b]	1	2	3
SGD	*0.951*[c]	0.094	0.058
SDZ	*0.922*	0.287	0.123
SA	*0.910*	-0.047	0.039
SMM	*0.857*	0.304	0.115
SPD	0.182	*0.965*	0.019
TMP	0.001	*0.922*	0.323
SMA	*0.628*	*0.678*	0.171
NAcSMX	*0.648*	*0.656*	-0.068
SMX	0.018	0.161	*0.957*
NAcSMA	0.165	0.081	*0.950*
Percentage variance explained (%)	54.9%	20.5%	15.3%

[a] Extraction method: Principal component analysis. Rotation method: Varimax with Kaiser normalization.
[b] Full names and structures of chemicals are listed in Table 1.
[c] Numbers in italic refer to the variables with more significant loadings in each factor.

in all samples. The resulting equation was as follows:

$$\hat{Z}_{sum} = 0.228FS_1 + 0.428FS_2 + 0.845FS_3 \ (R^2 = 0.949) \quad (3)$$

By expanding $\hat{Z}_{sum}$, the MLR equation can be written as:

$$Z_{sum} = 0.228\sigma FS_1 + 0.428\sigma FS_2 + 0.845\sigma FS_3 + mean[Z_{sum}] \quad (4)$$

where σ was 78.51 ng/L; and $mean[Z_{sum}]$, was 82.21 ng/L. Thus the mean percentage contribution $(B_k / \sum B_k)$ was 15.2% for animal husbandry (Factor 1), 28.5% for human (Factor 2), and 56.3% for the combination of human and mariculture source (Factor 3).

Figure 4 shows the estimated contributions for each source in all sea, estuary, and mariculture samples using Equation 2. The PCA-MLR analysis showed that animal husbandry mainly influenced the eastern sea area of Liaodong Bay, adjacent to the estuary of Shuangtaizi River and Daliao River. The result is consistent with local industry structure: livestock farms are primarily distributed on the Shuangtaizi River basin, while the Xiaoling River and Daling River mainly flow through the residential areas of Jinzhou and Linghai City, respectively (Fig. 1).

Because Factor 3 might be influenced by both human activity and mariculture, we were unable to distinguish their separate contributions by PCA-MLR analysis. According to the available statistical data, the mariculture area around Liaodong Bay is approximately 49,558 hm^2 [31], consisting of shellfish (64.4%), shrimp (29.1%), algae (2.5%), fish (0.4%), and other species (3.6%) ([39]; www.cnki.net). Of these mariculture species, shrimp and fish are mainly bred in bait casting ponds. Considering that the mariculture area for fish is much lower than shrimp and the shrimp breeding period is from May to August (120 d), the occurrence of sulfonamides in Liaodong Bay would be influenced by shrimp farming during our sampling dates. The average shrimp pond depth and daily water exchange rate are 1.0 m and 8%, respectively [40], thus the daily mass flux of sulfonamides from shrimp pond to seawater was estimated to be 5.45 kg/d. The sampling period for the present study was nearing the flood season, and the discharge from the four rivers that flow into the Liaodong Bay is approximately 0.47, 0.40, 0.20, and 0.04 10^8 m^3/d for Daliao River, Shuangtaizi River,

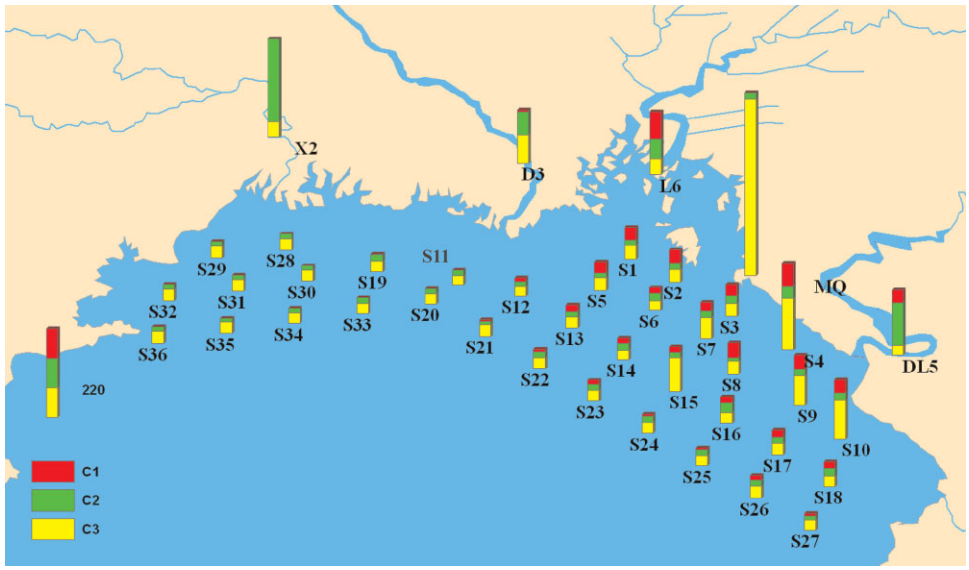


Fig. 4. Source contributions (ng/L) based on principal component analysis with multiple linear regression (PCA-MLR) for sea, estuary, and mariculture samples. C1: animal husbandry; C2: human; C3: human and mariculture.

Daling River, and Xiaoling River, respectively [39]. Taking into account the chemical concentrations in the four estuary sites, an estimated 17.1 kg/d of total sulfonamides flux was discharged into the Liaodong Bay though the rivers. Thus, the contribution by mariculture to total sulfonamides in Liaodong Bay was approximately 24.1%, and humans account for the most discharge (60.7%) of sulfonamide antibiotics in Liaodong Bay.

Overall, the present study reported the occurrence of sulfonamides, their available *N*-acetylated metabolites, and trimethoprim in human and agricultural sources, river, and marine samples from Liao River basin and its adjacent Liaodong Bay, north China. In addition, an estimated source apportionment was determined to understand the influence of human and agricultural activities on seawater for these chemicals.

SUPPLEMENTAL DATA

Detailed sample preparation, LC-MS/MS analysis, method validation, and concentrations of target chemicals in river, sea, and source samples.

Fig. S1. LC-MS/MS chromatograms of one river sample (site L3) for 15 detected chemicals and the surrogate standards.

Table S1. LC-MS/MS multi-selected reaction monitoring (MRM) conditions of target compounds.

Table S2. Instrumental detection limits (IDL, pg), absolute recoveries ($n = 3$, %) and method detection limits (MDLs, ng/L) of target chemicals in various water samples.

Table S3. Concentrations (ng/L) of sulfonamides and trimethoprim in river, source and sea samples. (a) river and source samples (b) sea samples. (1.112 MB DOC)

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REFERENCES

1. Diaz-Cruz MS, Barcelo D. 2006. Determination of antimicrobial residues and metabolites in the aquaticenvironment by liquid chromatography tandem mass spectrometry. *Anal Bioanal Chem* 386: 973–985.
2. Gao JA, Pedersen JA. 2005. Adsorption of sulfonamide antimicrobial agents to clay minerals. *Environ Sci Technol* 39:9509–9516.
3. Bogialli S, Curini R, Di Corcia A, Nazzari M, Samperi R. 2003. A liquid chromatography-mass spectrometry assay for analyzing sulfonamide antibacterials in cattle and fish muscle tissues. *Anal Chem* 75:1798–1804.
4. Poe M. 1976. Antibacterial synergism — proposal for chemotherapeutic potentiation between trimethoprim and sulfamethoxazole. *Science* 194:533–535.
5. Neu HC. 1992. The crisis in antibiotic-resistance. *Science* 257:1064–1073.
6. Lee SH, Park YJ, Park ES, Kim YS, Choi YS, Kim BG, Park SJ, Chong SM. 2009. Effects of extremely low frequency electromagnetic fields on thyroid carcinogenesis induced by N-bis(2-hydroxypropyl)nitrosamine and sulfadimethoxine. *J Korean Surg Soc* 77:161–169.
7. Imai T, Hasumura M, Cho YM, Ota Y, Takami S, Hirose M, Nishikawa A. 2009. Inhibitory effects of aminoguanidineon thyroid follicular carcinoma development in inflamed capsular regions of rats treated with sulfadimethoxine after N-bis(2-hydroxypropyl)nitrosamine-initiation. *Cancer Sci* 100:1794–1800.
8. Hu JY, Shi JC, Chang H, Li D, Yang M, Kamagata YC. 2008. Phenotyping and genotyping of antihiotic-resistant Escherichia coli isolated from a natural river basin. *Environ Sci Technol* 42:3415–3420.
9. Knapp CW, Dolfing J, Ehlert P, Graham DW. 2010. Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. *nviron Sci Technol* 44:580–587.
10. Davison J. 1999. Genetic exchange between bacteria in the environment. *Plasmid* 42:73–91.
11. McGeer AJ. 1998. Agricultural antibiotics and resistance in human pathogens: Villain or scapegoat? *Can Med Assoc J* 159:1119–1120.
12. Yates CM, Pearce MC, Woolhouse M, Amyes S. 2004. High frequency transfer and horizontal spread of apramycin resistance in calf faecal Escherichia coli. *J Antimicrob Chemoth* 54:534–537.
13. Blahna MT, Zalewski CA, Reuer J, Kahlmeter G, Foxman B, Marrs CF. 2006. The role of horizontal gene transfer in the spread of trimethoprim-sulfamethoxazole resistance among uropathogenic *Escherichia coli* in Europe and Canada. *J Antimicrob Chemoth* 57:666–672.
14. Managaki S, Murata A, Takada H, Tuyen BC, Chiem NH. 2007. Distribution of macrolides, sulfonamides, and trimethoprim in tropical waters: Ubiquitous occurrence of veterinary antibiotics in the Mekong Delta. *Environ Sci Technol* 41:8004–8010.
15. Carr A, Tindall B, Brew BJ, Marriott DJ, Harkness JL, Penny R, Cooper DA. 1992. Low-dose trimethoprim-sulfamethoxazole prophylaxis for toxoplasmic encephalitis in patients with AIDS. *Ann Intern Med* 117:106–111.
16. Mermin J, Lule J, Ekwaru JP, Malamba S, Downing R, Ransom R, Kaharuza F, Culver D, Kizito F, Bunnell R, Kigozi A, Nakanjako D,

Wafula W, Quick R. 2004. Effect of co-trimoxazole prophylaxis on morbidity, mortality, CD4-cell count, and viral load in HIV infection in rural Uganda. *Lancet* 364:1428–1434.
17. Sarmah AK, Meyer MT, Boxall 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.
18. Gobel A, McArdell CS, Suter M, Giger W. 2004. Trace determination of macrolide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater using liquid chromatography coupled to electrospray tandem mass spectrometry. *Anal Chem* 76:4756–4764.
19. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000 : A national reconnaissance. *Environ Sci Technol* 36:1202–1211.
20. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Res* 42:3498–3518.
21. Lindsey ME, Meyer M, Thurman EM. 2001. Analysis of trace levels of sulfonamide and tetracycline antimicrobials, in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. *Anal Chem* 73:4640–4646.
22. Hartig C, Storm T, Jekel M. 1999. Detection and identification of sulphonamide drugs in municipal waste water by liquid chromatography coupled with electrospray ionisation tandem mass spectrometry. *J Chromatogr A* 854:163–173.
23. Xu WH, Zhang G, Zou SC, Li XD, Liu YC. 2007. 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* 145:672–679.
24. Gulkowska A, Leung HW, So MK, Taniyasu S, Yamashita N, Yeunq L, Richardson BJ, Lei AP, Giesy JP, Lam P. 2008. Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China. *Water Res* 42:395–403.
25. Shelver WL, Hakk H, Larsen GL, DeSutter TM, Casey F. 2010. Development of an ultra-high-pressure liquid chromatography-tandem mass spectrometry multi-residue sulfonamide method and its application to water, manure slurry, and soils from swine rearing facilities. *J Chromatogr A* 1217:1273–1282.
26. Mengelers M, Kleter GA, Hoogenboom L, Kuiper HA, VanMiert A. 1997. The biotransformation of sulfadimethoxine, sulfadimidine, sulfamethoxazole, trimethoprim and aditoprim by primary cultures of pig hepatocytes. *J Vet Pharmacol Ther* 20:24–32.
27. Vanderven A, Vree TB, Kolmer E, Koopmans PP, Vandermeer J. 1995. Urinary recovery and kinetics of sulfamethoxazole and its metabolites in HIV-seropositive patients and healthy-volunteers after a single oral dose of sulfamethoxazole. *Br J Clin Pharmacol* 39:621–625.
28. Gobel A, Thomsen A, Mcardell CS, Joss A, Giger W. 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. *Environ Sci Technol* 39:3981–3989.
29. Radke M, Lauwigi C, Heinkele G, Murdter TE, Letzel M. 2009. Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a water sediment test. *Environ Sci Technol* 43:3135–3141.
30. Berger K, Petersen B, Buningpfaue H. 1986. Persistence of drugs occurring in liquid manure in the food-chain. *Arch Lebensmittelhyg* 37:99–102.
31. *Panjin statistical yearbook*. 2008. Panjin statistical bureau, China.
32. Larsen RK, Baker JE. 2003. Source apportionment of polycyclic aromatic hydrocarbons in the urban atmosphere: A comparison of three methods. *Environ Sci Technol* 37:1873–1881.
33. Sofowote UM, McCarry BE, Marvin CH. 2008. Source apportionment of PAH in Hamilton Harbour suspended sediments: Comparison of two factor analysis methods. *Environ Sci Technol* 42:6007–6014.
34. Chang H, Wan Y, Hu JY. 2009. Determination and source apportionment of five classes of steroid hormones in urban rivers. *Environ Sci Technol* 43:7691–7698.
35. Yang SW, Cha J, Carlson K. 2004. Quantitative determination of trace concentrations of tetracycline and sulfonamide antibiotics in surface water using solid-phase extraction and liquid chromatography/ion trap tandem mass spectrometry. *Rapid Commun Mass Spectrom* 18:2131–2145.
36. Kim SC, Carlson K. 2007. Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. *Environ Sci Technol* 41:50–57.
37. Lin A, Yu TH, Lin CF. 2008. Pharmaceutical contamination in residential, industrial, and agricultural waste streams: Risk to aqueous environments in Taiwan. *Chemosphere* 74:131–141.
38. Campagnolo ER, Johnson KR, Karpati A, Rubin CS, Kolpin DW, Meyer MT, Esteban JE, Currier RW, Smith K, Thu KM, McGeehin M. 2002. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. *Sci Total Environ* 299:89–95.
39. Cui ZG. 2008. *Study on scheme of total emission control of main chemical pollutants in 13 cities around Bohai Sea* PhD thesis, Ocean University of China, Qingdao, China.
40. Cui Y, Chen BJ, Chen JF. 2005. Evaluation on self-pollution of marine culture in the Yellow Sea and Bohai Sea. *Chin J Appl Ecol* 16:180–185.