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Original Research Paper

Contamination and Distribution of Tetracyclines, Sulfonamides, Quinolones and Macrolides in the Haihe River, China

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ABSTRACT

Ten kinds of antibiotics belonging to tetracyclines, sulfonamides, quinolones and macrolides were detected in water samples from Haihe River, China. Sulfamethoxazole and sulfachlororyridazine had largest detection frequencies (100% and 86%), with the maximum concentrations of 330 ng/L and 380 ng/L, respectively. Friedman and Moranȇs I test indicated that the target antibiotics at different sampling sites were significantly different ($p < 0.05$), suggesting that the antibiotics detected in the Haihe River were primarily depended on proximal sources of antibiotics. Municipal wastewater plants, fishponds, and livestockȇs industries along the river were confirmed to import antibiotics to the tributaries, and then distribute into the mainstream. By studying the variations of relative compositions of antibiotics in both surface water and sediment, we found that sulfonamides had a much higher migration capacity than other antibiotics, quinolones and macrolides deposited in sediment after travelling short distance from sewage outfall and tetracyclines in pollution sources was strongly absorbed in the sediment.

INTRODUCTION

In recent years, many categories of antibiotics such as tetracyclines, sulfonamides, quinolones and macrolides are abused in both human medicine and stockbreeding industries for disease therapy and growth promotion. It is estimated that approximately 25 million pounds of antibiotics are produced per year in USA, and roughly 70% of these antibiotics are applied in stockbreeding industries as veterinary drugs (Union of Concerned Scientists 2001). China ranks the top in the world in production capacity of antibiotics. It is estimated that approximately 210000 tons of antibiotic materials are produced every year, among which 180000 tons are used in agriculture and medicine. The consumption of antibiotics/person/year is estimated approximately 138 g, which is 10 times higher than that in USA (Gorvett 2013).

A significant fraction of antibiotics fed to animals (25-75%) are excreted in an unaltered form in faeces and persist in soil after land application (Galvachin 1994), which increases the antibiotic residues in the environment. Antibiotics from the domestic sources and pharmaceutical industries can be transmitted to the wastewater treatment plants (WWTPs). Unfortunately, the wastewater treatment techniques do not reveal sufficient removal efficiency. Increasing evidences have confirmed that a considerable amount of antibiotics are detected in active sludge, effluent water of WWTPs and faeces (Golet et al. 2003, Kathryn 2004, Campagnolo et al. 2002, Ye et al. 2007). As a result, antibiotics are introduced into the aquatic environment. Meanwhile, faeces from confined animal feeding operations (CAFOs), which are often used as fertilizer, can also be the potential sources of antibiotics migrating into the aquatic ecosystem.

Previous studies in many regions such as in USA (Kathryn et al. 2006, Kim et al. 2007, Campagnolo et al. 2002), in Germany (Wiegel et al. 2004), in Spain (Meritxell et al. 2006), and in China (Hu et al. 2008, Chang et al. 2010, Tong et al. 2009, Peng et al. 2006, Xu et al. 2007, Wang et al. 2009, Lu et al. 2007, Jiang et al. 2014, Jiang et al. 2013) have reported the determination of antibiotics in hospital sewage, WWTPs, swine wastewater, animal slaughters, aquaculture ponds and rivers. However, nowadays, no comprehensive regional field study has ever been conducted to concurrently characterize the occurrence of potential antibiotic sources, and the corresponding transport of these antibiotics from pollution sources to the aqueous systems.

In the economically fast developing area of Haihe River basin, animal husbandry and aquaculture industries approximately share 57% of total agricultural gross domestic products (GDP), which is twice as the average level in China (30%) according to the estimation (Tianjin Municipal Bureau of Statistics 2005). Livestock and aquaculture wastes are the potential inputs of antibiotics. Meanwhile, due to the rapid growth of population in this region, intensive inputs of human used antibiotics via WWTPs should be gained the concern in this region. The complicated combination of antibiotic utilization in this region may contribute to the unique pattern of antibiotic pollution in the Haihe River.

The Haihe River, the largest water system in Northern China, flows through an agricultural area before discharging into the Bohai Sea. It has a drainage area of 265,000 km^2, including 120,000 km^2 of farmland, numerous CAFOs, WWTPs and fish ponds. In order to protect the water quality, a strict regulation by the government was issued to forbid discharging pollutants to the mainstream of the Haihe River; however, this regulation does not cover the tributaries of the Haihe River system. A hypothesis is that selected antibiotics may migrate to the Haihe River via its tributaries.

Due to typically chemical characterization, antibiotics may behave differently during entering into the aquatic system. For example, sulfonamides are characterized by high solubility and chemical stability in water, and macrolides are liable to be hydrolysed. Quinolones reveal the tendency to be photodegraded. Tetracyclines have high affinity to organic matters in soil through cation bridging and cation exchange. Whether these distinguished chemical properties affect the transport behaviour of these different groups of antibiotics from potential sources to the Haihe River remains unclear.

The objective of this study is to characterize the unique occurrence and transport of selected human and veterinary antibiotics including tetracyclines, sulfonamides, quinolones and macrolides in the Haihe River in China by (1) characterizing the unique occurrence of tetracycline, sulfonamide, quinolone and macrolide residues in the Haihe River and its tributaries, to infer the unique pattern of antibiotic utility in this Haihe area, (2) confirming our hypothesis that selected antibiotics are transported into the Haihe River via tributaries, and (3) investigating the transport behaviour of these selected antibiotics from potential sources to the Haihe River. According to our current knowledge, this is the first regional field study to systematically explore the occurrence and fate of target antibiotics in the aqueous ecosystem of the Haihe River.

MATERIALS AND METHODS

Materials: Totally 12 standard antibiotics and Simatone (internal standard substance) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Standard trimethyl-13C3 (surrogate) was purchased from Cambridge Isotope Laboratories. HPLC grade methanol (99.9%), HPLC grade acetonitrile (99.9%) and analytical grade formic acid (99%) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Analytical grade sodium (99.8), analytical grade elhylene diamine tetraacetic acid (Na_2EDTA, 99.8%), analytical grade citric acid (99.5%) and sodium citrate (99.8%) were purchased from Tianjin University of China. Stock solutions of 12 antibiotics at the concentrations of 100 mg/L were prepared in methanol and stored at 4°C.

Study area and sampling sites: Nine sampling sites in mainstream (M1-M9) of Haihe River were investigated, and numbered sequentially in the direction of Haihe River flow, with the following characteristics: sites M1-M3, urban-influenced areas located WWTPs receiving domestic, pharmaceutical and hospital sewage; sites M4-M7, agricultural influenced areas (Jinnan and Dongli District) located fish ponds, feedlots and dairies; sites M8-M9, the end of the Haihe River to Bohai Sea. These sampling sites are shown in Fig. 1. In August and December 2013, surface water samples were collected at the main stream of the Haihe River (M1-M9) with three replicated samples.

In order to investigate the occurrence and fate of the antibiotics in the Haihe River, the potential input activities of antibiotics including hoggery wastewater, surface water of fishponds and effluent of WWTPs that were proximity to six tributaries of the Haihe River were sampled. In each tributary, both surface water and sediment samples representing potential sources, tributaries and their confluences with mainstream were collected.

Both water and sediment samples were kept at light-free environment at 4°C during sampling events and were immediately transported to the laboratory. Water samples were stored at 4°C and sediment samples were stored at -18°C until sample pretreatment within 24 hours.

Sample pre-treatment and solid phase extraction optimization: Approximately 1 mL of 0.5 mg/L trimethyl-13C3 was added to 400 mL of water samples as the surrogate, filtered through 0.45 μm glass fibre filter, and adjusted to pH 5 with citrate buffer. Then 0.2 g of Na_2EDTA was added to complex divalent cations such as Ca^{2+} and Mg^{2+}.

A total of 1 mL of 0.5 mg/L trimethyl-13C3 was added to 2 g of lyophilized and grinded sediment samples as the surrogate, and spiked with 40 mL of extraction buffer including 25 mL of methanol, 5 mL of 0.1 M Na_2EDTA and 10 mL of citrate buffer (pH = 5). The mixture was vortexed at 3000 r/min for 2 min, ultrasonic extracted for 25 min, centrifuged at 3000 r/min for 10 min, and supernatant was collected. The extraction procedure was repeated three times. The supernatant of the extractions from three times was blended and diluted to 500 mL with pure water.

Strata strong anion exchanger (SAX) cartridge (3 mL/200 mg, Thermo, USA), and Oasis hydrophilic-lipophilic balance (HLB) cartridge (6 mL/500 mg, Waters, Watford, UK) were set up in tandem. The cartridges were conditioned with methanol (5 mL) and pure water (5 mL). The water samples and supernatant of sediment samples were passed through the cartridges at a loading rate of approximately 5 mL per minute. After the entire sample was loaded, the SAX cartridge was removed and then HLB cartridge was then washed with 5 mL of pure water, as well as HLB cartridge were air dried for 10 min. Finally, elution was conducted by 5 mL of methanol. The 5 mL of eluate was collected in a glass tube and evaporated in a gentle nitrogen stream, spiked with 10 μL of 10 mg/L Simatone as the internal standard, and then filled up to 1 mL with initial mobile phase and stored at -18°C prior to the analysis by HPLC-MS/MS.

HPLC-MS/MS analysis: HPLC-MS/MS analysis system was consisted of an Alliance 2695 HPLC from (Waters; Manchester, UK) and a Waters Micromass Quattro MicroTM detector with electrospray ionization (ESI). Individual standard solutions of 12 antibiotics, internal standard substance, and surrogate were injected directly into MS to optimize MS parameters including cone voltage generated the most intense signal of a selected precursor ion, and collision gas energy generated the most intense signal of product ions resulting from fragmentation of the selected precursor ion for each antibiotics.

MS parameters for the analysis were as follows: Function, MRM; Ionization mode, ES+; Capillary voltage, 4.0 kV; Extractor voltage, 2.0 V; Radio frequency lens, 0.5 V; Source temperature, 90°C; Desolvation temperature, 350°C; Cone gas flow, 50 L/h; Desolvation gas flow, 500 L/h; Low mass (LM) 1 resolution, 15.0; High mass (HM) 1 resolution 1, 15.0; Entrance, -1; exit, 0; LM 2 resolution, 15.0; HM 2 resolution 1, 15.0; Multiplier, 650.00 V.

Simultaneous chromatographic separation of 12 antibiotics including trimethoprim (TMP), sulfadiazine (SD), sulfamethazine (SM2), sulfamethoxazole (SMZ), sulfachlororyridazine (SCP), ciprofloxacin (CIP), enrofloxacin (ENR), ofloxacin (VFX), tetracycline (TC), oxyetracycline (OTC), eythromycin (ERY) and roxithromycin (ROX), internal standard substance, and surrogate was performed on a 2.1 × 250 mm Intersil ODS-3 column (5 μm particle size, Sciences, JPN). The column was maintained at 40°C with a flow rate of 0.2 mL/min, and the injection volume was 10 μL. Acetonitrile (A) and purified water containing 0.3% formic acid (v/v) (B) were used as mobile phases. The gradient conditions were initiated with 10% A followed by a linear increase to 20% A within 1 min, and then to 30% within 4 min. Then A was increased to 35% within 5 min and maintained for 5 min. Another 4 min later, A was changed back to the initial proportion with concentration-holding time for 6 min until the next injection. Results showed that the chromatographic reproducibility, resolution and sensitivity of detection were applicable for analysis.

Method validation: The recovery rates of 12 target compounds were determined for surface water and sediment using the samples spiked at gradient concentration levels (20, 50, 200 and 500 ng/L for surface water, and 2, 10, 100 and 500 ng/g for sediment) to validate the method. The recovery rates of sulfonamides, quinolones, tetracyclines and macrolidesin in water samples were 74-91%, 63-80%, 75-87%, and 66-82%, respectively, and 74-90%, 53-78%, 69-79% and 77-81% for sediment samples. Recovery rates of trimethyl-13C3 for both water and sediment samples were 78-90%.

Limit of quantification (LOQ) was determined as the lowest concentration that generated a signal to noise ratio (S/N) larger than 10. The LOQs of 12 compounds calculated with the statistical method were 1.5-8 ng/L for water samples and 0.10-3.5 ng/g for sediment samples. Reproducibility of response (peak area RSD, %) was calculated from the average of three replicated injections from each of three replicated samples spiked at gradient concentration levels. RSDs of 12 compounds were 0.5-2.9% for water samples, and 0.5-5.0% for sediment samples.

Statistical analysis: Friedman's test and Spatial Autocorrelation Analysis (Moran's I Test) were combined to explore the spatial distribution of 12 compounds at mainstream in both the seasons. Friedman Test was used to investigate the pollution sources of antibiotics (at different sampling sites) contributing to the occurrence of antibiotics in Haihe River. The significant difference in concentrations of antibiotics among different sampling sites were considered at $p < 0.05$. However, Friedman Test can not effectively describe the distribution characteristics of pollution sources presenting equivalent input to Haihe River. Therefore, Moran's I Test was introduced in the present study. Moran's I fall in the range of -0.5-0.5, indicating that the spatial autocorrelation for the concentrations of antibiotics at different sampling sites was weak, which suggested that the input of antibiotics from different sources plays a dominant role, rather than the migration of antibiotics in the river. Moran's I is calculated from the following formula:

$$\text{Moran's I} = \frac{n\sum_{i=1}^{n}\sum_{j=1}^{n} w_{ij}(c_i - \bar{c})(c_j - \bar{c})}{\sum_{i=1}^{n}\sum_{j=1}^{n} w_{ij}\sum_{i=1}^{n}(c_i - \bar{c})^2}$$

Where, c_i and c_j refer to antibiotic concentration in sampling sites i and j, respectively. $\bar{c}$ is the overall mean antibiotic concentration, and w_{ij} as the weight matrix is 1 if stations i and j are within the neighbour threshold, otherwise it is 0. If the number of detectable sites in mainstream were less than 4, no calculation for target compounds is required.

RESULTS AND DISCUSSION

The occurrence of antibiotics in the Haihe river: In the surface water samples from mainstream of Haihe River at summer and winter seasons, except SM2 and ENR, 10 antibiotics including TMP, SD, SMZ, SCP, CIP, VFX, TC, OTC, ERY and ROX were detected. The sulfonamides ranked the highest detection frequencies with 100%, 86%, 71% and 86% for SMZ, TMP, SD and SCP, respectively, and the highest concentrations of 10-380 ng/L for these antibiotics. For quinolones, the detection frequencies of CIP and VFX were 18% and 32%, and the detected concentrations were 180 ng/L and 273 ng/L, respectively. Compared with sulfonamides and quinolones, detection frequency and concentration of tetracyclines and macrolides were much lower. For tetracyclines, the detection frequencies of TC and OTC were 10% and 18%, and the highest concentrations were 35 ng/L and 50 ng/L, respectively. The detection frequencies of ERY and ROX were 14% and 7%, and the highest concentrations were 42 ng/L and 45 ng/L, respectively.

These results were different from the recent surveys conducted in the Pearl River and Shenzhen River (in China), Elbe River (in Germany), Youngsan River (in Korea), where 4, 4, 4, and 3 kinds of antibiotics (Table 2) were detected respectively.

The detected concentrations of these antibiotics in the Haihe River were much higher than those in other rivers, especially for TMP and VFX, whose concentrations were 1-2 magnitude orders higher than those in other rivers. SCP and CIP were detected with high concentrations in the Haihe River, while these antibiotics were not detectable in other rivers. Although the concentrations of TC and OTC were lower when compared with other compounds in Haihe River, both were not detectable in other rivers.

The obvious higher detection frequencies and concentrations of antibiotics for both human and veterinary drugs in the Haihe River are derived from the high-density application of antibiotics in this highly populated river basin located in the development centre of economics in northern China. Moreover, lots of animal husbandry and aquaculture industries share approximately 57% of total agricultural GDP, which is twice as the average level (30%) in China (Tianjin Municipal Bureau of Statistics 2005).

Temporal and spatial distribution of antibiotics in the Haihe river: A clear trend of temporal distribution of antibiotics in the Haihe River was observed (*t*-Test, $p < 0.01$). Except TC and OTC, the average concentrations of TMP, SD, SMZ, SCP, CIP, VFX, ERY and ROX in December were 127, 161, 175, 213, 129, 39 and 38 ng/L, which were significantly higher than that of 82, 95, 110, 135, 84, 30 and 31 ng/L in August. The lower concentrations of antibiotics in the summer may be due to faster photolysis (Huang et al. 2001, Thiele-Bruhn 2003), thermal degradation (26°C in summer versus 5 °C in winter) and biodegradation (Jerold et al. 2003, Ingerslev & Sorensen 2000) as well as higher dilution from runoff.

Table 1: MRM parameters for 12 antibiotics, internal standard and substitute.

	Precursor-ion (m/z)	Product-ion 1 (m/z)	Product-ion 2 (m/z)	Cone voltage (v)	Collision energy (v)
SD	251.2	156.1	207.1	20	18
SMZ	254.2	156.1	188.1	25	15
SM2	279.3	156.2	186.1	30	18
SCP	285.0	156.0	108.1	27	18
TMP	291.1	230.3	258.3	33	25
CIP	332.1	314.2	231.0	35	30
ENR	360.0	342.0	245.1	35	22
VFX	362.1	261.0	318.4	18	28
TC	445.4	410.3	427.2	20	18
OTC	461.2	426.2	443.2	20	18
ERY	734.2	158.2	576.4	26	32
ROX	837.7	158.2	679.3	32	31
Simatone	198.2	170.1	128.0	35	25
Trimethyl-$^{13}C_3$	198.0	112.0	140.0	30	23

In surface water of Haihe River, *p*-value of Friedman Test was less than 0.05 for all detected antibiotics, as given in Table 3, which indicated that sampling sites have the significant characteristics. Furthermore, the Moran's I was -0.5-0.5, which further confirmed that the input of antibiotics from different sources (different sampling sites) played a dominant role in the spatial distribution of antibiotics in Haihe River, rather than migration.

The spatial distribution of selected antibiotics in August 2009 in Haihe River is illustrated in Fig. 2.

Due to the various pollution sources, 9 antibiotics were detected at sites M1-M3, which represented the urban area with a density of population of 3.79 million. CIP and VFX revealed the highest detection frequencies (67% and 100%), which reflected that the antibiotics were used more frequently in the urban area. An aggregate daily wastewater load, including domestic sewage, hospital wastewater, swine wastewater, and the sewage of pharmaceutical industries, is estimated to be around 2093 million litres in the urban area (Zhang 2006). Nearly 75% of the sewage entered into the domestic WWTPs through the drainage system. Unfortunately, antibiotic could not be effectively removed through WWTPs and released into the surface water through the effluent of WWTPs.

Except for TMP, the highest concentrations of

Table 2: The concentrations (ng/L) of antibiotics in surface water of Haihe River and other rivers.

	Haihe River China	Pearl River China	Shenzhen River China	Jiulongjiang River China	Ebro River Spain	Elbe River Germany	Rio Grande River USA	Youngsan River Korea
Reference		Ye et al. (2007)	Ye et al. (2007)	Sun et al. (2009)	Meritxell et al. (2006)	Wiegel et al. (2004)	Kathryn et al. (2006)	Kim&Carlson (2007)
TMP	210[a](104)[b]	nt	nt	nt	20(11)	30(10)	nt	5.3(4)
SD	270(129)	(218)	(259)	nt	Nt	nt	nt	nt
SMZ	330(145)	(143)	(776)	nt	Nd	70(50)	300	36(20)
SCP	380(176)	nt	nt	nt	Nt	nt	nt	nt
CIP	150(107)	nt	nt	nd	Nt	nt	nd	nt
VFX	225(133)	(74)	(38)	5.8	Nd	nt	nd	nt
TC	35(31)	nt	nt	nd	Nt	nt	nt	nt
OTC	50(41)	nt	nt	nd	Nt	nt	nd	nt
ERY	42(34)	nt	nt	nt	30(17)	40(35)	nt	4.8(3.4)
ROX	45(33)	(70)	(191)	nt	Nt	40(16)	nt	nt

Table 3: *p* values of Friedman Test and Moran's I for antibiotics in surface water.

	Water in August			Water in December		
	χ^2	*p* value	Moran's I	χ^2	*p* value	Moran's I
TMP	7.5	0.01	-0.13	6.4	0.02	0.08
SD	11.8	0.02	-0.21	7.0	0.03	0.13
SMZ	9.5	0.03	-0.09	8.0	0.01	0.17
SCP	8.0	0.01	0.22	7.8	0.02	-0.24
CIP	7.0	0.01	0.13	8.1	0.01	-0.17
VFX	10.0	0.01	-0.17	7.8	0.02	-0.13

Table 4: Concentrations of antibiotics (ng/L) in effluents of WWTPs, hoggeries, and fishponds.

	WWTP 1	WWTP 2	Hoggery 1	Hoggery 2	Fishpond 1	Fishpond 2
TMP	538	449	nd	nd	nd	nd
SMZ	917	1290	615	686	225	247
SM2	nd	nd	nd	nd	nd	nd
SCP	374	254	nd	nd	nd	nd
SD	741	382	nd	nd	nd	nd
CIP	281	450	nd	211	nd	nd
ENR	nd	Nd	nd	nd	nd	nd
VFX	542	861	nd	nd	nd	nd
TC	99	Nd	289	160	nd	nd
OTC	280	315	545	463	nd	nd
ERY	55	90	nd	nd	732	612
ROX	610	227	nd	nd	214	nd

nd = Not detectable

sulfonamides such as SMZ, SCP and SD were detected from sites M4-M7, locating in Dongli and Jinnan District, where animal husbandry and aquaculture industries were densely distributed. In Dongli and Jinnan District, the number of Confined Animal Feeding Operations (CAFOs) are as much as 72 and 105, respectively. In addition, lots of individual husbandries within this area made up 35% of the total stock-breeding scale. The aquaculture farms in Dongli and Jinnan District were 4000 ha and 2670 ha, most of which located along the Haihe River bank. Comparing with sulfonamides and quinolones, tetracyclines and macrolides recorded the less detected frequency and detected level in the surface water of mainstream of the Haihe River, which could be explained by relatively lower migration capability of tetracyclines and macrolides, as discussed in 3.5. Detected frequencies of tetracycline and macrolide in surface water of mainstream of Haihe River were 7-18%. Nearly 86% of these antibiotics were detected in the confluence of the tributaries entering into the main stream, implying that the input from the tributaries could be the important pathway of antibiotic occurrence in the mainstream of the Haihe River. Thereby, the migration of antibiotics from possible pollution sources to tributaries and final to the mainstream of the Haihe River was also included in this study.

Potential sources of antibiotics: In order to protect water quality, Haihe River is under supervision by prohibiting the discharging of pollutants to its mainstream, however, this supervision is not effective in discharging pollutants to the tributaries of Haihe River. We hypothesized that these antibiotics originated from potential sources flow through tributaries that were the potential pathways and then distributed into mainstream of the Haihe River. In order to discover the potential sources of selected antibiotics in the Haihe River, the concentrations of antibiotics from potential sources such as the effluent of WWTPs, lagoon wastewater from hoggeries and fish ponds that were adjacent to the tributaries of the Haihe River were determined. Results of HPLC/MS/MS analyses at effluent from potential sources are summarized in Table 4.

Ten and nine antibiotics (including four categories) were detectable in the effluent of WWTP1 and WWTP2, respectively, with the concentration range of 90-1290 ng/L. Comparing ten antibiotics in WWTP1 and nine antibiotics in WWTP2 were detectable, only four antibiotics were detect-

Table 5: Average pseudo-partitioning coefficients (P-PC) of selected compounds.

Compounds	P-PC (L/kg)
TMP	105
SMZ	114
SM2	NE*
SD	139
SCP	91
CIP	308
ENR	NE
VFX	299
TC	1410
OTC	1398
ERY	437
ROX	282

able in hoggeries and fishponds. SMZ, TC and OTC were detected in both lagoon wastewater from hoggeries with the concentration range of 160-732 ng/L. Because sulfonamides account for the extremely high concentration in the Haihe River (mainstream) when compared with other antibiotics, which may be due to the lagoon wastewater. Since it is the pollution source of these antibiotics, while CIP was detectable in one lagoon wastewater from hoggeries with the concentration of 211 ng/L. SCP and ERY were in the concentration range of 225-686 ng/L in two fishponds, while ROX in only one fishpond was detected with the concentration of 251 ng/L. The concentrations of TC, OTC and ERY in hoggeries and fishponds were higher than that in WWTPs, indicating that TC, OTC and ERY are mainly used as veterinary drugs. On the contrary, the concentrations of TMP, CIP, VFX and ROX in WWTPs were higher than that in hoggeries and fishponds, indicating that these antibiotics were mainly used as human pharmaceuticals. Sulfonamides is the most frequently prescribed antibiotics for humans, and some of them such as sulfamethazine and sulfathiazole are also used as the veterinary drugs.

Contribution of potential antibiotic sources to mainstream of the Haihe river: In order to investigate the fate of antibiotics from potential sources to tributaries and finally to the mainstream of the Haihe River, the concentrations of four categories of antibiotics (in water) in potential pollution sources, tributaries and confluence of mainstream of the Haihe River were compared, as shown in Fig. 3.

The allocation of different species of antibiotics in potential sources, pathways and mainstream of the Haihe River was investigated. The results showed that the concentration of sulfonamides and quinolones in mainstream accounted for 17-39% and 21-26% of their potential sources, respectively, while these data were 1 magnitude order lower than the concentrations of tetracyclines and macrolides. These results indicated that migration capability of tetracyclines and macrolides was lower than that of sulfonamides and quinolones.

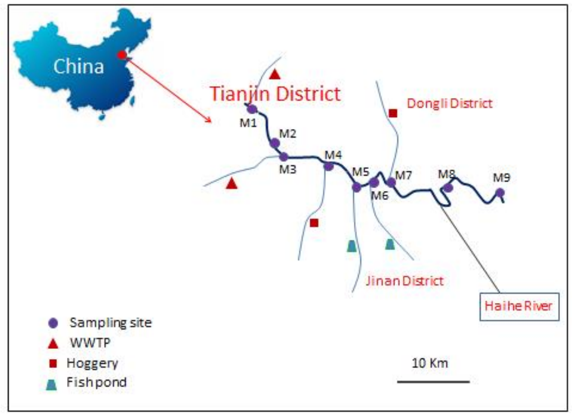

Fig. 1: Sampling sites.

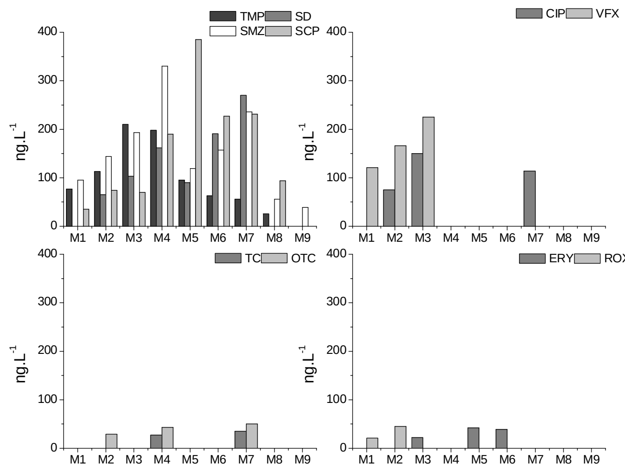

Fig. 2: Spatial distribution of detectable antibiotics in Haihe River in August 2013.

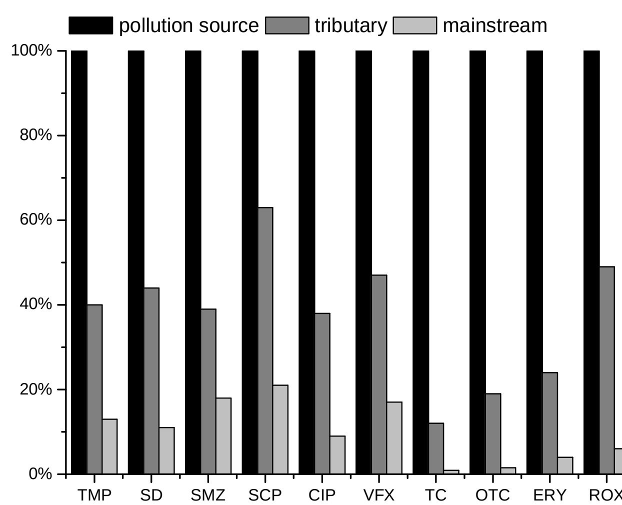

Fig. 3: Comparison of average concentrations of antibiotics in surface water following migration direction.

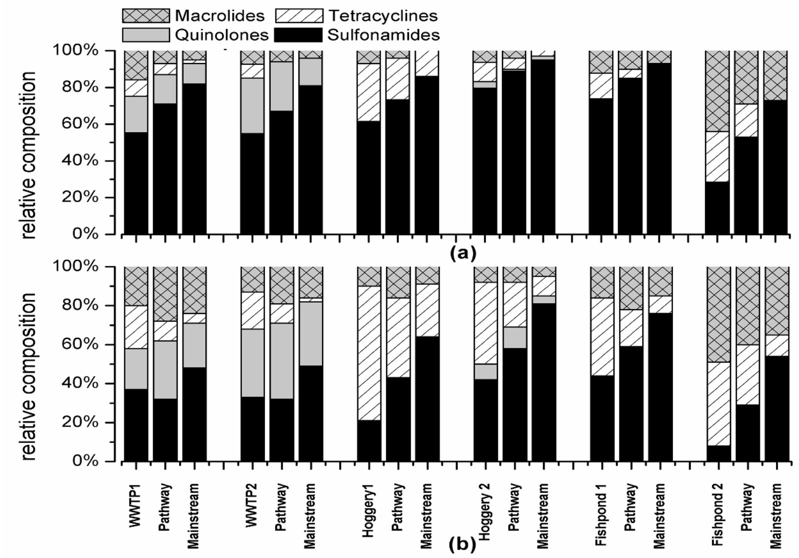

Fig. 4: (a) The relative compositions and contribution concentrations of antibiotics in surface water at different sampling sites following migration direction from pollution sources to confluence of the mainstream of Haihe River; (b) The relative compositions and contribution concentrations of antibiotics in sediment at different sampling sites following migration direction from pollution sources to confluence of the mainstream of Haihe River.

Antibiotics can be deposited and adsorbed in sediment during the river flow (Tolls 2001, Rabølle & Spliid 2000, Figueroa et al. 2004, Sarmah et al. 2006). The deposition and adsorption in sediment was one of the most important factors that influenced the migration of antibiotics in the river. An average pseudo-partitioning coefficient (P-PC) was valuable to assess the absorption capability of individual antibiotics to the sediment. Results from this study showed that the concentrations of antibiotics in sediment were significantly higher than that in water (Table 5). This indicates that antibiotics were adsorbed in sediment. In addition, these results also showed that tetracyclines present the strongest adsorption in sediment, while sulfonamides present the weakest adsorption in sediment.

The spatial distribution in water and sediment of four groups of antibiotics from potential sources to mainstream were combined to explore the effect of sediment adsorption on the migration of different categories of antibiotics.

The relative compositions were introduced to elucidate the proportion of one class of antibiotics accounting for total four selected classes of antibiotics in potential sources, pathways and mainstream of the Haihe River. Except in fishpond 1, relative compositions of sulfonamides in surface water were higher than those in sediment at sampling sites of other five pollution sources, while relative compositions of other three categories of antibiotics in surface water were lower than those in sediment. Among antibiotics discharged from the two WWTPs, only relative compositions of sulfonamides in surface water revealed a gradual increase from sewage outfall to mainstream, which was mainly due to the strong hydrophilicity and poor sediment adsorption capability of sulfonamides. In sediment, quinolones and macrolides in the pathways had higher relative compositions than those in sewage outfall and mainstream, indicating that these two categories of antibiotics deposited in sediment after travelling some distance from sewage outfall. Tetracyclines in the sewage outfall sediments had the largest relative compositions, indicating a short distance from their settlement, and strong adsorption in sediment. The migration of antibiotics from two hoggeries to mainstream was similar to that in WWTPs. The migration properties of tetracyclines in surface water were weaker than those of SMZ and CIP. Due to the strong adsorption, tetracyclines had higher relative composition in sewage outfall (80%) when compared with surface water (55%).

Although macrolides had stronger adsorption capability than sulfonamides, due to short distances from two fishponds to the mainstream (0.5 and 0.3 km, respectively), both ROX and ERY had a little proportion in the settlement. So the relative compositions of three antibiotics in water and sediment had no significant change from pollution sources to mainstream. These results showed that the migration capability of antibiotics to mainstream was also correlated with the migration distance.

CONCLUSION

Compared with other rivers referred in previous literature, more species, more frequencies and higher concentration of antibiotic residues in surface water of Haihe River were detected. Occurrence of detectable antibiotics in surface water was obviously influenced by the migration capability and spatial characteristics of pollution sources. Antibiotics in pollution sources had the higher concentration level than in mainstream. During the migration process, antibiotics can be adsorbed and accumulated in sediment at different degrees. Sulfonamides had the strongest migration capability in water, while tetracyclines had the weakest migration capability.

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