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Occurrence, Distribution, and Risk Assessment of Antibiotics in a Subtropical River-Reservoir System

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Abstract: Antibiotic pollutions in the aquatic environment have attracted widespread attention due to their ubiquitous distribution and antibacterial properties. The occurrence, distribution, and ecological risk assessment of 17 common antibiotics in this study were preformed in a vital drinking water source represented as a river-reservoir system in South China. In general, 15 antibiotics were detected at least once in the watershed, with the total concentrations of antibiotics in the water samples ranging from 193.6 to 863.3 ng/L and 115.1 to 278.2 μg/kg in the sediment samples. For the water samples, higher rain runoff may contribute to the levels of total concentration in the river system, while perennial anthropic activity associated with the usage pattern of antibiotics may be an important factor determining similar sources and release mechanisms of antibiotics in the riparian environment. Meanwhile, the reservoir system could act as a stable reactor to influence the level and composition of antibiotics exported from the river system. For the sediment samples, hydrological factor in the reservoir may influence the antibiotic distributions along with seasonal variation. Ecological risk assessment revealed that tetracycline and ciprofloxacin could pose high risks in the aquatic environment. Taken together, further investigations should be performed to elaborate the environmental behaviors of antibiotics in the river-reservoir system, especially in drinking water sources.

Keywords: antibiotics; river-reservoir system; water; sediment; risk assessment

1. Introduction

Antibiotics have been extensively and effectively used for several decades not only to relieve symptoms and treat human and animal diseases, but also to promote growths in the livestock, aquaculture and plant agriculture [1,2]. Estimated annual antibiotic consumption in the world ranged from 100,000 to 200,000 tons, and antibiotic consumption is on the rise [3,4]. Based on the market survey, China is considered as the largest producer and user of antibiotics in the world, with approximately 162,000 tons consumed in China (in 2013) [5]. In addition, it has been reported that antibiotics could only be partially metabolized by humans or animals [3]. Then, active substances associated with the antibiotics pass through artificial environments, and may end up in different environmental

compartments including water, sediment, and soil, attributed to the fact that most of these chemicals are water soluble and not susceptible to degradation and transformation [6–8]. Consequently, it is inevitable that environmental organisms are more or less exposed to these compounds, which are still active with ecotoxic effects even in a very low concentration. Moreover, antibiotics also can contribute to the dissemination of antibiotic resistance genes (ARGs) and subsequently pose potential risk to human health [9,10].

Aquatic environments play a vital role in maintaining the biogeochemical processes in the world. In recent years, especially natural surface water sources have been universally threatened by the continuous pressure of anthropogenic contamination and are in need for better protection for the drinking water quality. One of the most particular concerns in drinking water sources is the widely detected antibiotics due to their persistence and negative effects [11]. Unfortunately, even advanced drinking water treatment cannot thoroughly remove all antibiotics [12]. Although considerable research has developed regarding the presence of antibiotics in the environment [13–15], there has been comparatively few investigations on their characteristics in drinking water sources. In addition, a large number of reservoirs were built in the upstream rivers for various purposes, including drinking water supply, flood control, irrigation, and the generation of hydropower. The number of reservoirs have increased dramatically over the past several decades, reaching about 16.7 million dams and over 50,000 large dams in the current world [16]. Undoubtedly, these dams have the potential to disrupt the original geochemical processes and ecological connectivity of rivers [16,17]. Based on the geographical distribution from the river to reservoir, river-reservoir systems are commonly used to describe its hybrid environmental properties. Previous studies mainly focused on the dissolved organic matter, pesticides, phthalate and heavy metals [18–21]. However, very few published papers have reported the pollution characteristics of antibiotics in the river-reservoir system and the impact of reservoirs on the spatiotemporal distribution of riverine antibiotics. Therefore, characterizing the differences in the occurrence, distribution and ecological risks of antibiotics from river system to reservoir system will be of great significance for a better understanding of their biogeochemical behaviors along with the environmental gradients.

To achieve these mentioned points, the present work, was experimentally performed in a subtropical river-reservoir system, which was located on the Headwater Region of the Dongjiang River (HRDR). It is also a vital drinking water source for about 40 million people living in several metropolitan cities (e.g., Guangzhou, Shenzhen, and Hong Kong) [10]. Thus, the objectives of this study were to: (1) comprehensively investigate the occurrence, spatiotemporal distribution, and ecological risks of 17 commonly used antibiotics in bulk waters and surface sediments of the HRDR and; (2) perform a comparative study on the dynamic characteristics of antibiotics in the river-reservoir system, as well as to provide new insights into the impact of the reservoir on the biogeochemical behaviors of riverine antibiotics.

2. Materials and Methods

2.1. Field Sites and Sampling

The HRDR is located in southern Jiangxi Province and northern Guangdong Province in China (Figure 1), covering an area of about 5161 km^2. In general, this catchment consists of two primary rivers located in the anthropic zone and a reservoir (Fengshuba Reservoir) located in the natural environment. Moreover, the rivers of Beiling River (BLR) and Xunwu River (XWR) are characterized by typical nonpoint source pollution from 467,000 rural residents with a large number of livestock breeding [22]. Interestingly, the reservoir could be regarded as a large pool with the average depth of 70 m to impound the upstream waters from the two rivers. As a result, apart from the antibiotic inputs from the rivers, we hypothesized that the reservoir as a natural environment was less directly polluted with antibiotics due to the forestry projects and reservoir resettlement. Detailed description of the basic hydrological information of the catchment can be found in the Supplementary Materials Text S1.

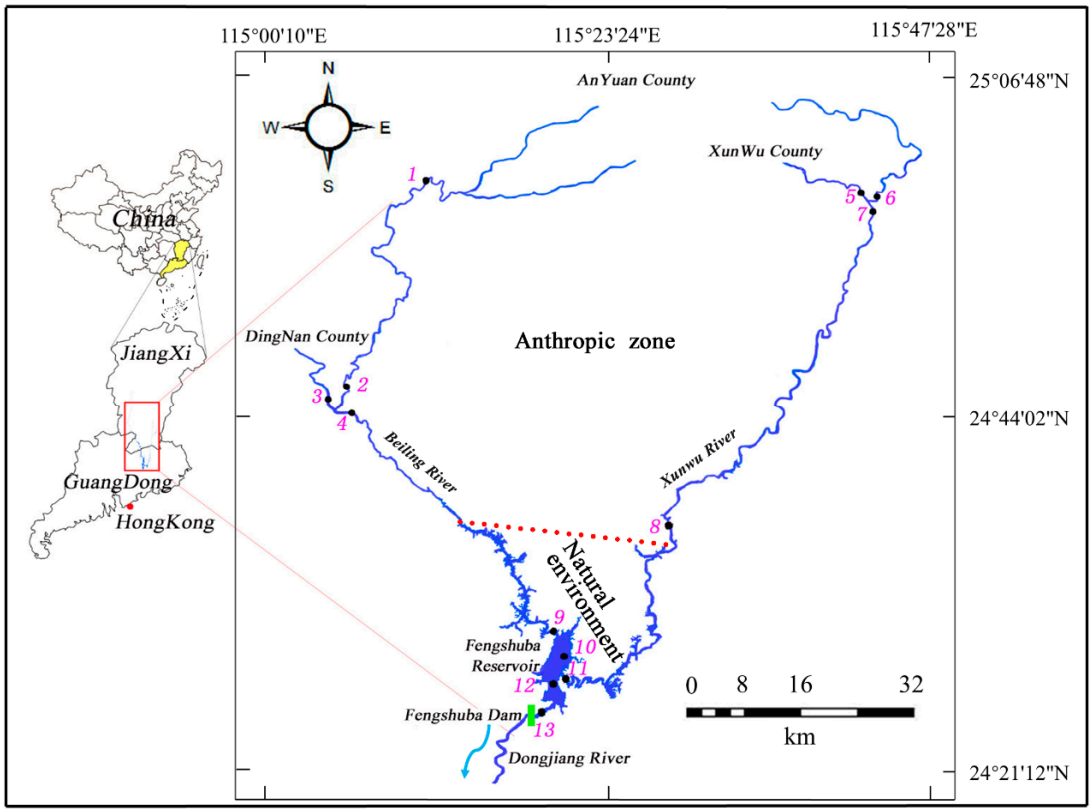

Figure 1. Map showing the sampling sites in the river system (Beiling River (S1–S4), Xunwu River (S5–S8)) and reservoir system (Fengshuba Reservoir (S9–S13)).

According to three typical hydrological periods (Figure S1), three campaigns took place on 18–20 July 2015 (wet-to-dry transition season), 25–27 November 2015 (dry season) and 14–16 March 2016 (wet season), respectively. In detail, eight surface water samples (0.5 m below surface) in the river system (S1–S8) were obtained, but five equal-mixed water samples (S9–S13) were obtained form the surface water (0.5 m below surface), middle water (half of the depth) and bottom water (about 2–4 m above bottom) of the Fengshuba Reservoir (FSBR). In particular, no representative sediment samples were obtained in the rivers due to the fact that rapid water flow was not conducive to the formation of sediment. Accordingly, surface sediment samples (about 15 cm) were only collected by a sediment sampler at the five stations (S9–S13) of FSBR. All the above samples were collected in 5 L acid cleaned glass bottles and were immediately transported to the laboratory in an ultra-low temperature storage tank. Before being analyzed, water samples and sediment samples were kept at 4 °C and −80 °C in the dark until extraction, respectively.

2.2. Chemicals and Standards

Antibiotic standards of three sulfonamides (including sulfadiazine (SDZ), sulfamonomethoxine (SMM) and sulfaquinoxaline (SQX)), three fluoroquinolones (including norfloxacin (NOR), ciprofloxacin (CIP) and ofloxacin (OFC)), four β-lactamases (including amoxicillin (AMX), cefalexin (CLX), penicillin G (PENG) and penicillin V (PENV)), three tetracyclines (including oxytetracycline (OTC), tetracycline (TC) and doxycycline (DC)) and four others (including tylosin (TYL), erythromycin-H_2O (ETM-H_2O), lincomycin (LIN) and vancomycin (VAN)) were purchased from the Dr. Ehrenstorfer (Augsburg, Germany). Furthermore, isotopic standard of ciprofloxacin-D8 is a surrogate standard for all the selected antibiotics, and norfloxacin-D5 was chosen as an internal standard for the quantification of all the samples. These two isotopic standards were purchased

from the TRC (Toronto, ON, Canada). The basic physicochemical characteristics and usages of these antibiotics are shown in Supplementary Materials Tables S2 and S3.

Methanol, acetonitrile, and formic acid (HCOOH) were all of high-performance liquid chromatography (HPLC) grade and purchased from the Thermo Fisher Scientific Inc. (Loughborough, UK). Phosphate-citric acid and disodium ethylenediamine tetraacetate (Na_2EDTA) were all of analytical grade and obtained from the Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Ultra pure water (10–18 MΩ·cm, 25 °C) was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

2.3. Sample Preparation

About 1 L triplicate water samples were filtered through 0.45 μm glass fiber filter (ANPEL Corp., Shanghai, China) and subsequently subjected to solid phase extraction (SPE) with Poly-Sery hydrophile-lipophile balance (HLB) cartridge (6 cc/200 mg, ANPEL Corp., Shanghai, China) following our previous method [23]. In particular, prior to extraction, all the filtered waters were acidified to pH = 3.0 with 6 mol/L HCI, and then spiked with 200 mg of Na_2EDTA and 50 ng of ciprofloxacin-D8. After extraction, the loaded cartridges were eluted with 12 mL methanol, subsequently the eluates were then concentrated to near dryness under a gentle stream of nitrogen. Then, 500 μL mobile phase (methanol and 50 ng of internal standard) were added to redissolve these target compounds, and stored in the refrigerator at −40 °C.

The sediments were freeze-dried and then sieved through a 100-mesh sieve (<150 μm) for further processing. Each sediment sample (2 g dry weight) was weighted into a 30 mL glass tube, followed by addition of 20 ng of ciprofloxacin-D8. Then, the samples were fully mixed and stored in a refrigerator at 4 °C overnight. Ten milliliters phosphate-citric acid (pH = 3.0 and 10 mL) and acetonitrile were added together into each glass tube, followed by mixing using a vortex oscillator for 20 min, then, in an ultrasonic extractor for 20 min. All the glass tubes were centrifuged at 12,000 rpm for 10 min to get the supernatants. In particular, the extraction processes were then repeated twice and the supernatants from the three extractions were combined together. All these supernatants were piped into a 200 mL round bottom flask and evaporated at 40 °C to remove organic solvents on a rotary evaporator. The concentrated residues in the flask were diluted to 200 mL with ultra-pure water to make sure that the organic solvent in the solution was less than 5% [24]. Then, the following method was equivalent to the above processes for the water SPE.

2.4. Chemical Analysis

For all water samples, the measurement of water properties including basic indictors (e.g., temperature, pH, dissolve oxygen (DO), and oxidation reduction potential (ORP)) and wet-to-dry transition parameters (e.g., dissolved organic carbon (DOC), dissolved total nitrogen (DTN), and dissolved total phosphorus (DTP)) could be found in Supplementary Materials Text Section 2. In addition, the contents of total carbon (TOC), total nitrogen (TN) and total phosphorus (TP) in the sediment samples were in accordance with standard methods [25]. Heavy metals (e.g., V, Ag, As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sn and Zn) for the sediment samples (<150 μm) were quantified by inductive coupled plasma-mass spectroscopy (ICP-MS) according to previous methods [18].

The antibiotics extracted from these samples were analyzed using ultra-high-performance liquid chromatography (UHPLC, Agilent 1290 Infinity, Santa Clara, CA, USA) coupled with tandem mass spectrometry (MS/MS, Agilent 6490 Triple Quadrupole, Santa Clara, CA, USA) [23]. An Agilent C18 column (50 mm × 2.1 mm, 1.8 μm) was used to separate and quantify the target analytes. All the target analytes were separated using a gradient method and is described in detail as follows: 0.1% formic acid in Milli-Q water (mobile phase A) and acetonitrile (mobile phase B) were prepared and the gradient elution started with 5% mobile phase B and kept isocratic for 1.3 min, and then linearly rose to 40% mobile phase B at 8 min and held until 10 min, subsequently dropped to 5% mobile phase B at 12 min. Flow rate of the mobile phase was 0.3 mL/min, the injection volume was 10 mL

and the column temperature was maintained at room temperature (25 ± 2 °C). Mass spectrometric analysis was operated in the positive electrospray ionization multiple reaction monitoring (MRM) mode. Tandem mass spectrometric parameters for each antibiotic are summarized in Supplementary Materials Table S4. In addition, detailed information on the quality assurance and control are shown in Supplementary Materials Text Section 3, Table S5.

2.5. Ecological Risk Assessment

The ecological risk quotient (RQ) was calculated for each antibiotic using the ratio between the measured environmental concentration (MEC) and predicted no effect concentration (PNEC). The PNEC of each antibiotic was calculated by acute and chronic aquatic toxicity, dividing the lowest short-term L(E)C_{50} or long-term non-observable effect concentration (NOEC) respectively through the division of an appropriate factor (AF). The appropriate assessment factors were used based on the methods and principles as described in detail in previous studies [26]. However, when NOEC values were unavailable, LC50 or EC50 values were used instead. In addition, typical taxons (algae, aquatic invertebrates and fish) from three trophic levels were chosen to represent the food chain in aquatic ecosystems. For most of the antibiotics, L(E)C_{50} and NOEC values were obtained from the ECOSAR V1.10. In general, RQs were classified into three levels of risk: when RQ $\geq$ 1.0, high risk; 0.1 $\leq$ RQ < 1.0, medium risk; RQ < 0.1, low risk [27].

2.6. Data Analysis

Statistical analyses and graphic visualizations including means ± standard deviation (SD), one-way ANOVA, and correlation analysis were performed using IBM SPSS 21.0 (IBM, Chicago, IL, USA) and OriginPro 2016 software (OriginLab, Northampton, MA, USA). All the test results with p-level $\leq$ 0.05 were considered to be statistically significant for pair comparisons. Spatial mapping of sampling sites, distributions and calculations of the mass balance of the antibiotics in the reservoir were performed using ArcGIS 10.2 (ESRI, Redlands, LA, USA) based on the Kriging analysis. Ecological risk assessment of the antibiotics were performed using HemI 1.0 [28].

3. Results and Discussion

3.1. Antibiotics in the Water Phase of the HRDR

Among the 17 target antibiotics, 15 antibiotics from five categories were detected in the water samples of the HRDR (Table 1). Five antibiotics (SDZ, SMM, SQX, DC, and Lin) were the most frequently detected compounds in 100% of the samples. NOR, CIP, OFC, CLX, PENG, PENV, OTC, and TC showed the second highest detection frequencies greater than 80%. These results suggest that the drinking water source has been severely disrupted by anthropic activities. The total concentrations of antibiotics in the water samples ranged from 193.6 ng/L located in the reservoir system to 863.3 ng/L located in the river system (Supplementary Materials Figure S2). The concentrations of Σ sulfonamides, Σ fluoroquinolones, Σ beta-lactamases, Σ tetracyclines and Σ others (defined as the sum of the corresponding antibiotics) ranged from 11.6 to 108.6 ng/L, from 26.8 to 597.2 ng/L, from 7.6 to 193.2 ng/L, from 9.1 to 300.7 ng/L, and from 0.3 to 15.4 ng/L, respectively. Additionally, fluoroquinolones were the dominant compounds in the water samples of the river system, which is consistent with previous studies in other water environments (Supplementary Materials Figure S3) [29,30]. In comparison, the information found in the water samples of the reservoir system was inconsistent with those in the river system. Taken together, these differences in the detection frequencies and levels of various antibiotics in the river-reservoir system may be attributed to the usage patterns of antibiotics in the surrounding catchments, as well as to the biogeochemical processes of antibiotics along with different hydrologic gradients, such as photo-degradation, adsorption, and biodegradation [3,27,31].

Table 1. Concentrations of antibiotics in the water samples of the HRDR (ng/L).

Classification	Compound	Range	Mean	Median	Detection Rate (%)
Sulfonamides	sulfadiazine	1.7–83.8	15.9	10.3	100 (39/39)
	sulfamonomethoxine	2.2–66.0	18.6	14.7	100 (39/39)
	sulfaquinoxaline	0.4–6.0	2.68	2.7	100 (39/39)
Fluoroquinolones	norfloxacin	<LOQ–156.3	62.3	58.9	97.4 (38/39)
	ciprofloxacin	<LOQ–442.1	169.2	156.7	94.9 (37/39)
	ofloxacin	<LOQ–17.2	7.1	6.7	97.4 (38/39)
Beta-lactamases	cefalexin	<LOQ–25.7	7.4	5.6	97.4 (38/39)
	penicillin G	<LOQ–97.0	19.2	10.8	92.3 (36/39)
	penicillin V	<LOQ–115.8	42.7	35.4	92.3 (36/49)
Tetracyclines	oxytetracycline	<LOQ–135.5	49.7	41.0	94.9 (37/39)
	tetracycline	<LOQ–111.5	44.9	43.3	92.3 (36/39)
	doxycycline	0.8~256.4	20.6	9.1	100 (39/39)
Others	tylosin	<LOQ–1.6	0.6	0.6	74.4 (29/39)
	erythromycin-H_2O	<LOQ–6.9	0.7	0.3	79.5 (31/38)
	lincomycin	0.13–10.4	1.4	0.7	100 (39/39)

Note: <LOD = values were below the level of detection (LOD).

For fluoroquinolones, the maximum concentration of CIP was found at site S3 (dry season) with the value of 442.1 ng/L, followed by site S5 (wet season) with the value of 417.6 ng/L (Supplementary Materials Figure S2). Unfortunately, the two sites of S3 and S5 are located in the rivers receiving directly integrated wastewater from the cities of Dingnan (urban population of 39,700) and Xunwu (urban population of 99,498) without effective domestic wastewater treatment facilities (Figure 1). Fluoroquinolones are used extensively in both human and veterinary medicines, and nearly 5340 tons of CIP were consumed in China in 2013 [5]. However, untreated wastewater related to high population density and the livestock units along the urban rivers may contribute to the CIP levels in the receiving rivers [32]. In general, it could be concluded that non-routine episodes associated with untreated effluents, landfills and medical wastewaters from urban inhabitants would exacerbate the pollution of antibiotics [33,34]. Furthermore, compared with previous studies, the mean CIP concentration for the water samples (169.2 ± 107.9 ng/L) was of similar levels to some other aquatic environments, such as the Wangyang River in China (205.5 ng/L) [35], Lebanese rivers (108 ng/L) [36], and the rivers in Northern Pakistan (110 ng/L) [37]. However, it was far greater than that in the water samples of Seine River (France) [38], Yangtze Estuary (China) [39], and Pearl River (China) [40], as well as other catchments in China (Poyang Lake, Chao Lake, and Liao River) [27,41,42]. Taken together, our results further support the concept that the CIP makes a major contribution to the burden of antibiotic pollutions and CIP contamination was at a moderate level in the HRDR, hence meriting preferential control.

For the water samples in the river system, the total concentration of antibiotics in the dry season (368.8 ± 66.9 ng/L) was significantly lower than that in the wet-to-dry transition season (567.0 ± 110.3 ng/L) and wet season (636.0 ± 138.8 ng/L) ($p < 0.05$) (Figure 2), suggesting that frequent rain runoff may contribute to the levels of total concentration especially in the rivers characterized as non-point source pollution watershed (Supplementary Materials Figure S2) [22,43–45]. However, this seasonal variation trend is inconsistent with previous investigations [31,39]. Furthermore, it was notable that the antibiotic compositions in the rivers did not exhibit a significant difference with the seasonal variation (analysis of similarities (ANOSIM), $p > 0.05$) (Supplementary Materials Figure S3), indicating that perennial anthropic activity, but not the seasons or hydrologic conditions, may be an important factor influencing the usage pattern of these antibiotics. That is to say, this result also suggests that these antibiotics probably have similar sources and release mechanisms from anthropogenic activities in the riparian environment [46]. In addition, for the river-reservoir system, the total

concentrations of antibiotics in the river system was markedly higher than those in the reservoir system along with the seasons ($p < 0.05$) (Figure 2), implying that river input was a potential important source of antibiotics in the reservoir. Also, this decreasing trend form the river system to reservoir system further suggests that the reservoir could be considered as a stable reactor to reduce the levels of total antibiotics from the river system. In addition, the antibiotic compositions changed significantly from the river system to the reservoir system in the dry and wet seasons (ANOSIM, $p < 0.05$). One plausible explanation for this is that long hydraulic retention time (HRT), increased transparency, and large surface area in the reservoir system, compared to those in the river system, would contribute to the attenuation in the total antibiotic concentrations of river system, probably via various geochemical processes (e.g., biotransformation, photolysis, sorption, and dispersion) [11,47]. In contrast, both the levels and compositions of antibiotics in the reservoir did not exhibit significant differences along with the seasons ($p > 0.05$). On the whole, these results suggest that hydrologic conditions or river input could not be the predominant factor influencing the environmental behaviors of antibiotics in the reservoir system. Meanwhile, it also implies that the reservoir can act as an ideal ecological barrier, which probably has a great capacity of water environment to balance and regulate the level and composition of antibiotics exported from the catchment.

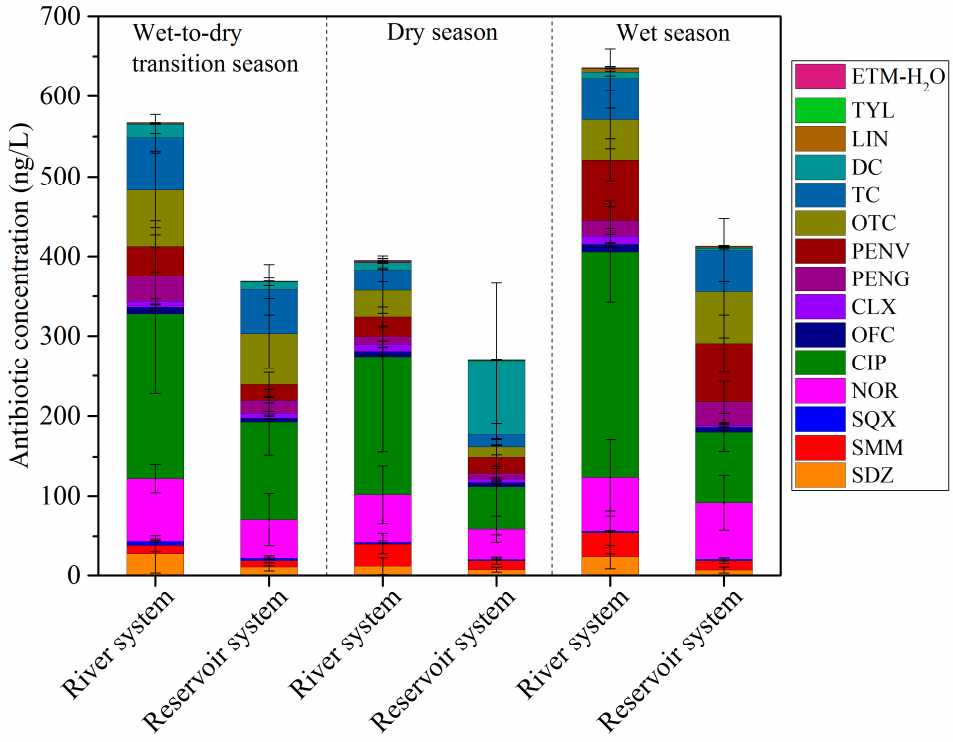

Figure 2. Accumulated concentrations of antibiotics in the river system (S1–S8) and reservoir system (S9–S13) over the three seasons. Error bars represent standard error of the mean.

3.2. Antibiotics in the Sediment Phase of the FSBR

Among the target antibiotics, 16 antibiotics were detected in the sediment samples of the FSBR (Table 2), with total concentrations ranging from 115.1 to 278.2 μg/kg (Figure 3, Supplementary Materials Figure S4). The 11 antibiotic compounds (e.g., SDZ, NOR, CLX, OTC, and LIN) were detected with a high detection frequency of 100% (Table 2). The average concentrations of five categories decreased in the order: tetracyclines (88.8 ± 34.2 μg/kg) > fluoroquinolones (53.7 ± 33.6 μg/kg) > β-lactamases (33.7 ± 23.4 μg/kg) > sulfonamides (18.1 ± 12.8 μg/kg) > others (11.5 ± 9.8 μg/kg). The tetracyclines in the sediments showed the highest concentration levels, which is consistent with previous results in other catchments in China, such as the Huangpu River [48], Liao River [49],

Jiulong River and Nanliu River [50]. In addition, OTC, NOR, PENV, SMM and LIN were the dominant compounds in the corresponding categories, respectively (Table 2). It also followed the order: OTC > NOR > PENV > LIN > SMM. However, this finding in the sediments was markedly different from those in the waters. Probably, the reason for this is that total organic matter and cation exchange capacity in the sediment exert a greater affinity with the OTC, but the SMM may have relatively higher water solubility and weaker affinity to the sediments [51,52]. Furthermore, the mean concentration of OTC (44.6 ± 27.4 μg/kg) in the sediments was obviously higher than that in the Liao River (29.1 μg/kg) [49], Huangpu River (6.9 μg/kg) [48], and Yellow River Delta (4.9 μg/kg) [53], but comparable to that in the Taihu Lake [54]. However, it was dramatically lower than that in the Wangyang River (36,148.3 μg/kg) [35] and southern Baltic Sea [55]. In general, these results support the view that the OTC pollution is at a moderate level in the sediment phase of FSBR.

Table 2. Concentrations of antibiotics in the sediment samples of the FSBR (μg/kg).

Classification	Compound	Range	Mean	Median	Detection Rate (%)
Sulfonamides	sulfadiazine	0.7–19.5	7.6	3.8	100 (15/15)
	sulfamonomethoxine	1.5–20.1	7.8	5.5	100 (15/15)
	sulfaquinoxaline	0.8–6.6	2.8	1.9	100 (15/15)
Fluoroquinolones	norfloxacin	9.7–132.3	30.3	22.0	100 (15/15)
	ciprofloxacin	6.1–27.4	16.4	18.4	100 (15/15)
	ofloxacin	<LOQ–13.5	6.9	5.0	93.3 (14/15)
Beta-lactamases	cefalexin	3.7–21.9	12.6	10.8	100 (15/15)
	penicillin G	<LOQ–28.9	4.8	<LOQ	33.3 (5/15)
	penicillin V	<LOQ–73.2	16.3	11.3	80 (12/15)
Tetracyclines	oxytetracycline	12.2–102.4	44.6	44.2	100 (15/15)
	tetracycline	6.0–95.7	40.0	30.4	100 (15/15)
	doxycycline	0.8–20.9	5.2	3.1	100 (15/15)
Others	tylosin	<LOQ–4.8	0.6	<LOQ	46.7 (7/15)
	erythromycin-H_2O	0.4–9.1	3.0	2.0	100 (15/15)
	lincomycin	0.2–24.7	6.4	6.0	100 (15/15)
	vancomycin	<LOQ–7.0	1.4	1.1	60 (9/15)

Note: <LOD = values were below the level of detection (LOD).

In addition, it can be easily found that spatial distribution of the total concentration of the antibiotics exhibit a similar trend between the wet-to-dry transition season and wet season, which is contrary to the change trend in the dry season (Figure 3, Supplementary Materials Figure S4). Coincidentally, paired-sample t-tests indicated that antibiotic compositions in the dry season was significantly different from those in the wet-to-dry transition season (ANOSIM, $p < 0.05$) or wet season (ANOSIM, $p < 0.01$). However, no significant differences could be found between the wet-to-dry transition season and wet season ($p > 0.05$) (Supplementary Materials Figure S4). Based on these, it was notable that the change trend in the antibiotic levels and compositions of the sediments showed a consistent result along with the seasonal variations. Meanwhile, the hydraulic retention time in the dry season (November, 2015; HRT = 246 days) is much longer than that in the wet-to-dry transition season (July, 2015; HRT = 122 days) or wet season (March, 2016; HRT = 49 days) (Supplementary Materials Table S1). It can be concluded that hydrological factors in the reservoir may influence the antibiotic distributions in the sediments [52]. Taken together, generally, these results further suggest that longer HRT may contribute to the accumulation of antibiotics in the internal region of FSBR (Figure 3b), however shorter HRT contributes to the accumulation of antibiotics in the inlet or outlet region of FSBR (Figure 3a,c). Unfortunately, it was difficult to determine the complex mechanisms associated with the hydrological and chemical factors in the fate and transport of antibiotics in the reservoir studied, hence meriting further investigations.

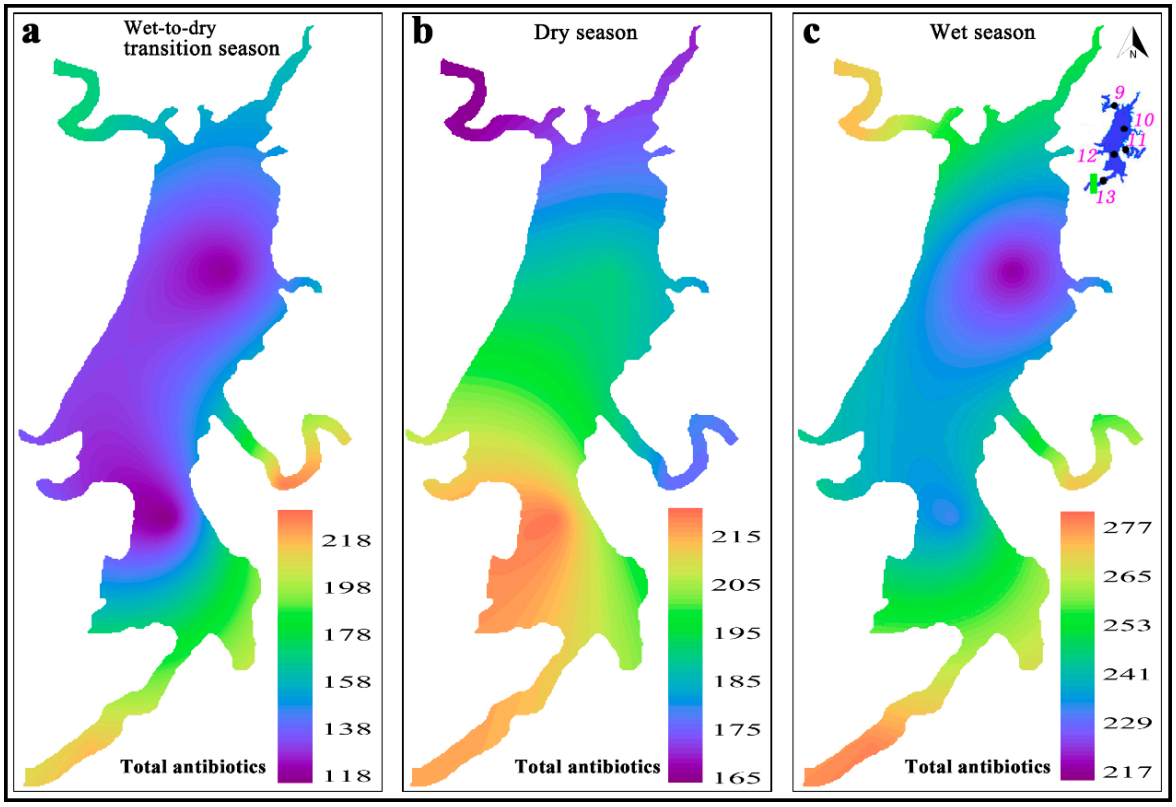

Figure 3. Contour maps showing the spatiotemporal distributions of total antibiotics in the sediment phase of FSBR (μg/kg). (**a**) Wet-to-dry transition season. (**b**) Dry season. (**c**) Wet season.

3.3. Relationships between Basic Parameters and Antibiotic Concentrations

Owing to the above-mentioned differences between the river system and reservoir system, the water and sediment samples were separately performed to explore the correlations between the basic parameters and antibiotic concentrations (Figure 4). For the water samples, the levels of NOR showed significantly positive relationships with the DTN (r^2 = 0.55, $p < 0.01$) and DTP (r^2 = 0.45, $p < 0.05$), indicating that these compounds may have an identical pollutant source from anthropogenic emissions in the river watersheds. In China, it has recently been reported that the total usage of NOR was 5440 tons, with only 18.62% consumed by humans and the rest consumed by animals. Furthermore, nitrogen and phosphorus pollutions are closely related to nonpoint source pollution (e.g., disperse domestic sewage, animal husbandry, and manure-fertilizer) in the rural catchment [22,52]. Based on these, it can be further inferred that nonpoint source pollution, especially untreated livestock wastewater and agricultural pollution, may contribute to most of the level of NOR in the river system. In addition, a positive relationship was found between the levels of SMM and TYL (r^2 = 0.47, $p < 0.05$) in the river system. Considering the fact that the TYL is only a veterinary antibiotic, it can be concluded that the SMM may be closely linked to the livestock and poultry farming in the catchment, which is consistent with the survey in China [5]. In contrast, all these relationships were not found in the reservoir system. Meanwhile, it was obvious that the relationship profiles in the river system was markedly different from that in the reservoir system. These results further suggest that the reservoir may have a potency to regulate the migration and transformation of antibiotics. On the other hand, compared to the pristine reservoir, it also implies that the concentration and distribution of antibiotics in the aquatic environment may be more related to human activities [56].

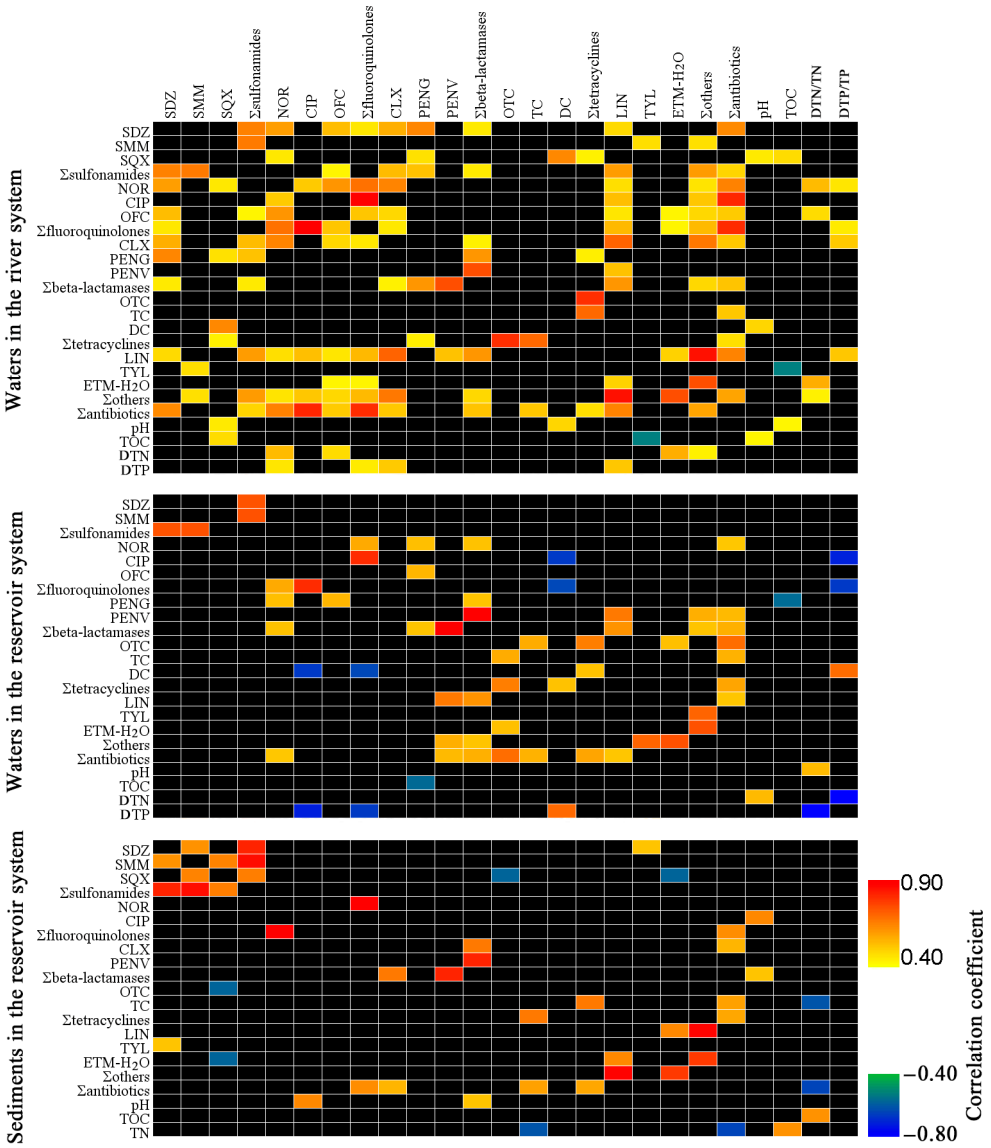

Figure 4. Pearson's correlation coefficients for antibiotic concentrations and basic parameters in the water and sediment samples in the river-reservoir system ($p < 0.05$). Bottom right shows the color band indicator of the correlation coefficient. Black boxes and no box shown in each line in the figure delineate no significant correlation for corresponding parameters ($p > 0.05$).

For the sediment samples, the levels of SMM showed significant relationships with the SDX ($r^2 = 0.63$, $p < 0.05$) and SQX ($r^2 = 0.67$, $p < 0.01$) (Figure 4). However, no significant relationships could be found between the sulfonamides and sediment property (e.g., pH, TOC, TN, and TP). These results suggest that similar chemical structures, but not the sediment property, may play an important role in the fate and residue of the selected sulfonamides in the sediments [57]. In addition, a positive relationship could be found between the level of CIP and pH ($r^2 = 0.66$, $p < 0.01$), probably suggesting that higher pH could accelerate the accumulation of CIP in the sediment particulates [58]. The levels of NOR showed significantly positive relationships with the heavy metals (e.g., As, Cd, Co, Cu, Mo, and Pb) ($p < 0.05$), respectively. This phenomenon associated with the concurrent presences of NOR and heavy metals may be attributed to the common mechanism of electrostatic adsorption in the sediments [59,60]. In comparison, no significant relationships were found between the heavy metals and other antibiotics. However, the heavy metal pollution could also enhance the level of bacterial

antibiotic resistance in the environment [9]. Hence, the co-selection potential of heavy metals and antibiotics in the dissemination of ARGs should not be neglected in any case.

In general, no significant relationships were found between the levels of total antibiotics and DOC/TOC in the three environmental compartments (Figure 4), suggesting that organic matter may not be the pivotal factor controlling the levels and distributions of antibiotics in the HRDR. A similar phenomenon was also found in the Baiyangdian Lake [61]. However, this finding is inconsistent with previous studies in the Yangtze Estuary [39], South Yellow Sea [29], Honghu Lake and East Dongting Lake [62].

3.4. Pseudo-Partitioning Coefficients of Antibiotics in the FSBR

The pseudo-partitioning coefficients (P-PC) is of great significance to get a better understanding of the dynamics of antibiotics between the sediment and water phases. In the study, P-PCs were calculated from the antibiotic concentrations in the sediments divided by their corresponding concentrations in the bulk waters, and the organic carbon normalized pseudo-partitioning coefficients (K_{oc}) were also calculated from the P-PCs divided by their corresponding fraction organic carbon content in sediments [48]. In general, the P-PCs and K_{oc} values were highly variable in the reservoir. For instance, the P-PC ranged from 145.46 to 5993.60 L/kg for SDZ, from 195.32 to 1470.89 L/kg for NOR, and from 15.61 to 8742.34 L/kg for DC. This suggests that NOR accumulates more easily in the sediments compared to SDZ and NOR, which is consistent with their corresponding chemical property associated with the Log K_{ow} (Supplementary Materials Table S2). Furthermore, the K_{oc} values ranged from 15,376.54 to 218,229.40 L/kg-oc for SDZ, from 18,172.64 to 140,804.50 L/kg-oc for NOR, and from 1973.08 to 817,805.80 L/kg-oc for DC. Taken together, such a variability suggests that although organic carbon content in sediments is an important parameter in sediment-water interactions of antibiotics, these different degrees of sediment-water interactions in the reservoir are likely driven by multiple factors (e.g., chemical characteristic, hydrological factor and mineral surface), rather than any single factor [48,52,53]. In other words, when the environmental conditions change, antibiotics adsorbed by the sediment phase would be released into the aqueous phase again.

3.5. Ecological Risk Assessment

Considering the fact that toxicity data of these antibiotics in sediment phase is very scarce, ecological risk assessment of the antibiotics were only evaluated in the water phase. The PNEC values of antibiotics were calculated by different assessment factors (Supplementary Materials Table S6), and risk quotients (RQs) corresponding to the spatiotemporal distribution of each antibiotic concentration detected in this study (shown in Figure 5a). In general, the RQs of ten antibiotics including SMM, SQX, OFC, CLX, PENG, PENV, DC, LIN, TYL, and ETM-H_2O were found to be below 0.1, indicating that these antibiotics may pose low ecological risks in the HRDR. The RQs of NOR and CIP ranged from 0.1 to 1.0, potentially suggesting that they pose medium ecological risks to the aquatic ecosystem. However, the RQs of TC were markedly higher than 1.0 due to the fact that some fishes are more sensitive to the TC (Supplementary Materials Table S6). This result suggests that the TC may pose a high ecological risk to the aquatic ecosystem and should be given priority controls.

Most importantly, selection pressure from antibiotics in the aquatic environment may accelerate the evolution and dissemination of ARGs [9]. Nevertheless, few minimal selective concentrations were currently determined from experimental research or natural ecosystems. Here, the PNEC (resistance selection) values of available antibiotics were obtained from an estimated research (Supplementary Materials Table S7) [63]. Likewise, risk quotients (RQs) of the antibiotics on potential selection for resistant bacteria corresponding to the spatiotemporal distribution of available antibiotics were shown in Figure 5b. The RQs of seven antibiotics including OFC, CLX, TC, DC, LIN, TYL, and ETM-H_2O were found to be below 0.1, implying that these antibiotics may not be selective for resistant bacteria. In addition, it is notable that the RQs of CIP are generally higher than 1. This suggests that the water concentrations of CIP may have a high probably to exert selection pressure

for resistant bacterial [63]. Hence, CIP should also be given priority controls considering this severe situation. Strikingly, antibiotics in aquatic ecosystems could have great impacts not only in aquatic organisms, but also in the dynamics of bacterial population and ARGs [64,65]. Unfortunately, the ecological risk assessments associated with the ARGs and bacterial population dynamics are still scarce. Taken together, more attention should be given in order to combat these challenges, especially in the drinking water sources that are closely linked to human health.

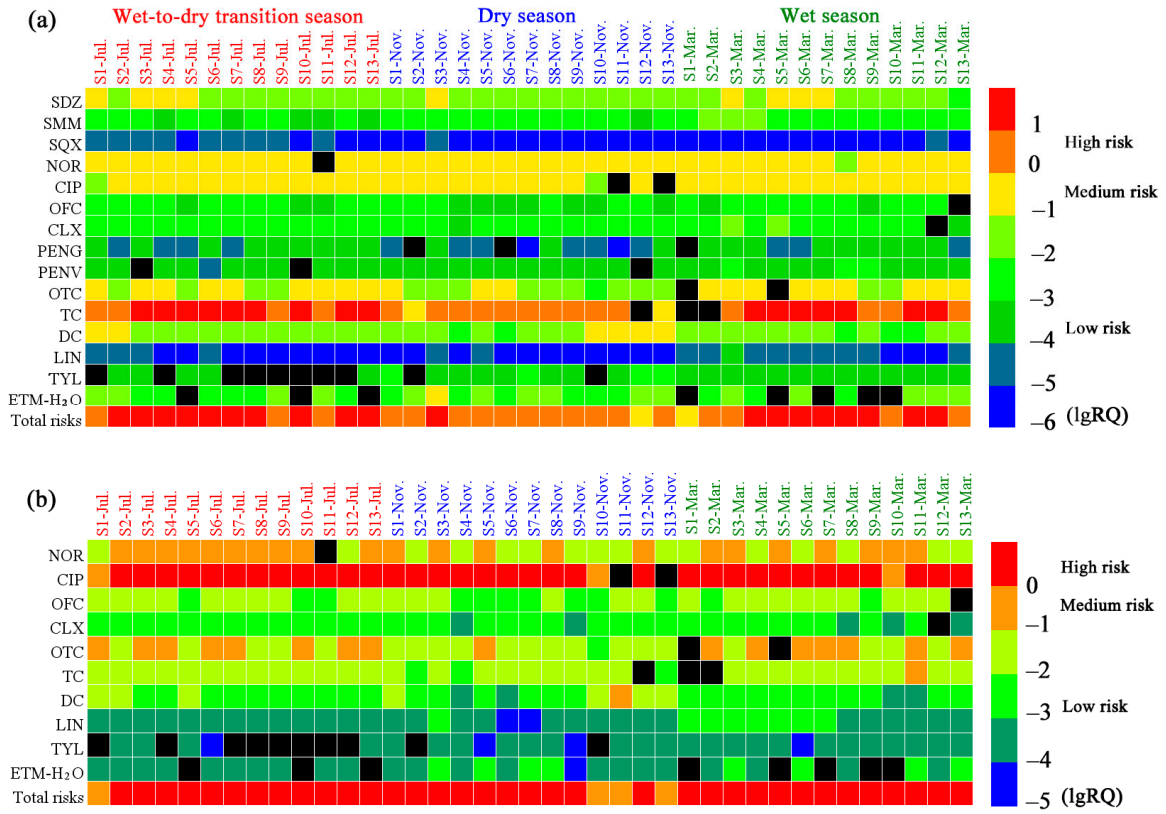

Figure 5. Calculated risk quotients (RQs) for the detected antibiotics in the water samples with the three seasons. (**a**) Ecological risk of the antibiotics on the typical taxons. (**b**) Ecological risk of the antibiotics on the selection for resistant bacterial. Bottom right shows the color band indicator of the level of ecological risk based on a log scale. Black boxes delineate no ecological risks due to the corresponding antibiotics undetected.

4. Conclusions

In this study, the occurrence and distribution of 17 antibiotics were investigated in a river-reservoir system, a key drinking water source in South China. Generally, 15 antibiotics were detected at least once in the watershed, with the total concentrations of antibiotics in the water samples ranging from 193.59 to 863.27 ng/L and 115.14 to 278.16 μg/kg in the sediment samples. For the waters, higher rain runoff may contribute to the levels of total antibiotics, while perennial anthropic activity may be an important factor influencing the usage pattern of antibiotics in the river system. However, the reservoir system can act as a stable reactor to balance and regulate the level and composition of antibiotics exported from the catchment. For the sediments, hydrological factors in the reservoir may influence the antibiotic distributions along with seasonal variation. Meanwhile, pH and heavy metals may control the accumulation of CIP and NOR in the sediment phase, respectively. Overall, tetracycline and ciprofloxacin could pose high risks in the aquatic environment, and should be preferentially controlled.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/10/2/104/s1, Figure S1: Monthly variations of rainfall and inflowing runoff for the HRDR from April 2015 to March 2016;

Figure S2: Antibiotic concentrations in the 39 water samples in the HRDR during the three seasons (July-2015, November-2015, and March-2016); Figure S3: Spatiotemporal variations of antibiotic compositions in the 39 water samples of the HRDR during the three seasons (July-2015, November-2015, and March-2016); Figure S4: Antibiotic concentrations and compositions in the 15 sediment samples of the FSBR with the three seasons. Table S1: Description of the basic hydrological information of Fengshuba Reservoir. Hydraulic retention times were calculated as volume of reservoir divided reservoir outflow; Table S2: The basic physicochemical characteristics of the target antibiotics; Table S3: Usage of selected antibiotics in China in 2013; Table S4: HPLC-MS/MS parameters for analysis of the analytes by MRM; Table S5: Method quantification limit (MQL), and recoveries of the antibiotics; Table S6: Ecotoxicity endpoints for fish, aquatic invertebrates, and algae and related PNEC values (μg/L) for these antibiotics; Table S7: Minimal selective concentrations (MSCs) for the available antibiotic (in μg/L).

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Conflicts of Interest: The authors declare no conflicts of interest.

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