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Occurrence, Seasonal Variation and Risk Assessment of Antibiotics in Qingcaosha Reservoir

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Abstract: Qingcaosha Reservoir is an important drinking water source in Shanghai. The occurrence of five groups of antibiotics was investigated in the surface water of this reservoir over a one-year period. Seventeen antibiotics were selected in this study based on their significant usage in China. Of these antibiotics, 16 were detected, while oxytetracycline was not detected in any sampling site. The detected frequency of tylosin was only 47.92% while the other 15 antibiotics were above 81.25%. The dominant antibiotic was different in four seasons: norfloxacin was dominant in spring, and penicillinV was dominant in summer, autumn and winter, with medium concentrations of 124.10 ng/L, 89.91 ng/L, 180.28 ng/L, and 216.43 ng/L, respectively. The concentrations and detection frequencies of antibiotics were notably higher in winter than in other seasons, demonstrating that low temperature and low flow may result in the persistence of antibiotics in the aquatic environment. Risk assessment suggested that norfloxacin, ciprofloxacin, penicillinV, and doxycycline in the surface water presented high ecological risks.

Keywords: antibiotics; Qingcaosha reservoir; risk assessment

1. Introduction

Since their discovery, antibiotics have been an effective means of treatment or prevention of bacterial infections. Currently, antibiotics are not only widely used as human medicine, but also as veterinary medicine and animal growth promoters [1]. In fact, the annual consumption of antibiotics for both human use and for veterinary use is substantial. The total usage of 36 frequently detected antibiotics in China was estimated to be 92,700 tons in 2013; 48% human use and 52% veterinary use [2]. However, the excessive use of antibiotics inevitably leads to the resistance of bacteria [3], which means that the microbes once susceptible to antibiotics are increasingly difficult to treat [4].

With increasing attention being paid to antibiotics as contaminants, antibiotics are found to be ubiquitous in wastewater treatment plants [5,6], surface water and sediments [7,8]. It has been demonstrated that the estuary zone may act as a reservoir for antibiotics coming from multiple sources because antibiotics are transported from terrestrial sources into the estuary region through river runoff. There are various sources of antibiotics in the aquatic environment. Wastewater from residential facilitates, hospitals, animal husbandry and the pharmaceutical industry is considered as the main source of antibiotics [9–11]. The antibiotics taken by humans or animals cannot be fully metabolized, and consequently enter sewage or manure via excreted urine or feces [12]. Since antibiotics can only be partially removed in wastewater treatment plants [13], the antibiotic residues will be discharged into the aquatic environment. Other important sources of antibiotics in the environment include the manure and sludge used in agricultural sites [7], as well as discarded pharmaceuticals, sludge and solid waste of the pharmaceutical industry in landfills [14].

It is notable that the antibiotics were found in surface water and groundwater which serve as drinking sources [15,16]. This gives rise to the concern that antibiotics may occur in drinking water and threaten human health. After all, the conventional drinking water treatment processes were proved not to be effective in removing all antibiotics [17]. As a matter of fact, trace-level antibiotics have been detected in tap water and drinking water samples [18,19].

Qingcaosha Reservoir, located at the estuary of the Yangtze River, is one of the major drinking water sources in Shanghai. The daily water supply is about 7.19 million m^3, which contributes to more than 50% of the total water supply of the city. The good water quality of Qingcaosha is essential to human health. Florfenicol and thiamphenicol were detected in the tap water of Shanghai by screening 21 antibiotics in the water samples [19]. The contamination of antibiotics in the Huangpu River, another drinking water source in Shanghai, has already been investigated, which showed its moderate contamination level of antibiotics [20,21]. The occurrence of antibiotics in the Yangtze Estuary and the coastal areas nearby was also reported [22,23]. In Qingcaosha Reservoir, the sedimentation and sorption release characteristics of phosphorus fractions [24], the effects of sudden salinity changes on physiological parameters and related gene transcription in M. aeruginosa [25] have been reported. However, the presence of antibiotics has still not been discussed.

The objective of this study was to determine the occurrence of 17 selected antibiotics in the surface water of Qingcaosha Reservoir (sulfonamides, tetracyclines, fluoroquinolones) or seldom reported antibiotics in this region (β-lactams, macrolides) and evaluate the ecological risk of these antibiotics in the reservoir. The detection of the water samples in Qingcaosha Reservoir was over a one-year period to understand the seasonal variation of the antibiotics.

2. Materials and Methods

2.1. Chemicals and Standards

Antibiotics standards of sulfonamides (SAs) including sulfadiazine (SDZ), sulfamonomethoxine (SMM), and sulfaquinoxaline (SQX); fluoroquinolones (FQs) including norfloxacin (NFX), ciprofloxacin (CFX), and ofloxacin (OFX); β-lactams including cefalexin (LEX), penicillinG (PENG), and penicillinV (PENV); macrolides (MLs) including tylosin (TYL), and erythromycin-H_2O (ETM-H_2O); tetracyclines (TCs) including oxytetracycline (OTC), tetracycline (TC), and doxycycline (DC); others including polymix-B (POL), vancomycin (VAN), lincomycin (LIN), and labeled compounds including ciprofloxacin-D_8 (CFX-D_8), norfloxacin-D_5 (NFX-D_5), amoxicillin-D_4 (AMX-D_4), sulfadiazine-D_4 (SDZ-D_4), and Doxycycline-D_3 (DOX-D_3) were purchased from Dr. Ehrenstorfer (GmbH, Augsburg, Germany). The purities of all the chemicals are >98%. The detailed properties of the target compounds are shown in Table A1. Each compound was prepared by diluting the stock solution with methanol at 1000 mg/L and mixture of working standards containing each compound at 10 mg/L. Methanol and acetonitrile were of High-Performance Liquid Chromatography (HPLC) grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Ourchem, Guangzhou, China). Ultra-pure water (MQ) was obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA). All standard solutions were stored in the refrigerator at −20 °C. Formic acid, hydrochloric acid, and disodium ethylenediamine tetracetate (Na_2EDTA) were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Tianjin, China).

2.2. Sample Collection

QCS reservoir is located at Yangtze River Estuary and supplies Shanghai with more than 7.19 million $m^3 \cdot d^{-1}$ of drinking water. The geographical coordinates of the reservoir are 31°48′ N, 121°57′ E. In order to improve the channel flow conditions and avoid damaging the flood control of

Yangtze Estuary, the reservoir has been constructed in a narrow and long shape. The inlet and outlet sluices and the output pipe station of the reservoir are managed by operators. When the inlet sluice was open, the flux of inflow water was set from 700 to 900 $m^3 \cdot s^{-1}$. When the outlet sluice was open, the flux of outflow water was set from 100 to 300 $m^3 \cdot s^{-1}$. The output pipe was located at the water pump station; it transported water from the reservoir to a drinking water treatment plant and the flux was about 60–83 $m^3 \cdot s^{-1}$ [26].

Twelve sampling campaigns were conducted monthly from May 2016 to April 2017. The water quality parameters are shown in Figure A1. The location of sampling sites is illustrated in Figure 1. Sampling point S1 was located in the Yangtze Estuary, in front of the inflow sluice and outside the reservoir, where the velocity and turbidity of water are different from the site inside the reservoir. S2 was located downstream of the outflow sluice in the reservoir, which is used to control the water level together with the inflow sluice. S3 was located at the water pump station, which transported the water to the water plant. S4 was located in the middle of the reservoir, which is an important inspection location. Before sampling, the bottles were sequentially cleaned by methanol and Milli-Q water. All of the water samples were collected 0.5 m below the surface using a water grab sampler, then stored in bottles. The samples were immediately transported to the laboratory and filtered through 0.45 μm filters (Anpel, Shanghai, China) and stored in the refrigerator at 4 °C.

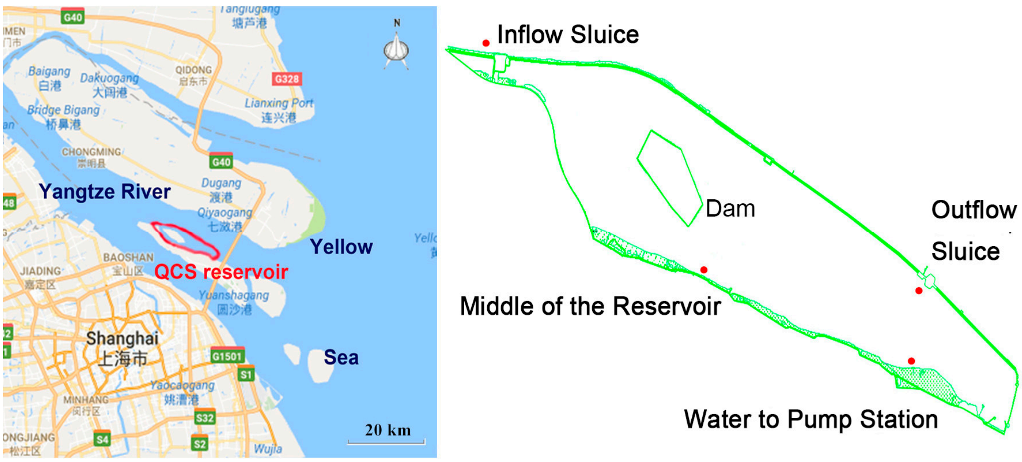

Figure 1. Sampling sites in Qingcaosha Reservoir.

2.3. Sample Preparation and Extraction

The filtered water samples (1 L) were extracted using Poly-Sery Hydrophile-Lipophile Balance Solid-Phase Extraction (HLB SPE) (Anpel, Shanghai, China) cartridges (200 mg, 6 mL). All cartridges were preconditioned with 10 mL methanol and 10 mL Milli-Q water at a flow rate of 1 mL/min. Before extraction, the samples were acidified to pH = 3 with hydrochloric acid, followed by the addition of 0.5 g Na_2EDTA as the chelating agent, then spiked with 50 ng labeled compound (1 mg/L). The extraction rate by SPE was 3–5 mL/min. After being extracted, antibiotics were eluted from SPE cartridges with 10 mL methanol, and the eluent was condensed to dryness under a gentle stream of N_2. Finally, 0.5 mL of the external standard (NFX-D_5, 80 μg/L) was added.

The target antibiotics were performed by a TSQ Quantum Access Max ultra-performance liquid chromatograph-tandem mass spectrometry system (UPLC-MS/MS) (Thermo Fisher Scientific, Waltham, MA, USA). The separation was performed with an Agilent C18 column (50 mm × 2.1 mm, 1.8 μm) (Agilent, Santa Clara, CA, USA). The temperature of the column was maintained at 30 °C, the flow rate was 0.3 mL/min, and the injection volume was 10 μL. Mobile phase: eluent A is ultrapure water containing 0.1% formic acid (*v*/*v*) and eluent B is acetonitrile. The gradient program was as follows: 95% A (0–1.3 min), 95–60% A (1.3–8 min), 60% A (8–10 min), 95% A (10–12 min). The mass spectrometric analysis was operated with a positive electrospray ionization (ESI^+) source in multiple reaction monitoring (MRM) modes. Tandem mass spectrometric parameters for the target antibiotics are shown in Table A2.

2.4. Quality Assurance/Quality Control

Standard solutions (from 5 to 400 μg/L in seven points), spiking with the same internal standards, showed strong linearity; correlation coefficients (r^2) of the standard curves were higher than 0.99. The limits of detection (LODs) for antibiotics were defined as the concentrations corresponding to the signal-to-noise (S/N) ratio of 3 and ranged from 0.21–2.41 ng/L. The limits of quantification (LOQs) for antibiotics were defined as the concentrations corresponding to the signal-to-noise (S/N) ratio of 10 and ranged from 0.46–8.32 ng/L. The mean recoveries of 16 antibiotics, spiked to the filtered surface water (n = 3), were between 69–125% [27,28]. Moreover, triplicate Milli-Q water samples, used as field blank samples, were all detected below the LOD.

2.5. Risk Assessment

Risk quotient (RQ) has been widely used to evaluate the ecological risk of individual antibiotics in the aquatic environment. RQ was calculated from the ratio between the measured environmental concentration (MEC) and the predicted no effect concentration (PNEC). The value of the PNEC was obtained from acute toxicity data (EC50 or LC50) in Environmental Protection Agency (EPA) and divided by a safety factor of 1000. The equations are shown as follows:

$$\mathrm{RQ} = \frac{\mathrm{MEC}}{\mathrm{PNEC}} \tag{1}$$

$$\mathrm{PNEC} = \frac{\mathrm{EC}_{50}\ \text{or}\ \mathrm{LC}_{50}}{1000} \tag{2}$$

In Equation (1), the highest MEC of all sampling sites and lowest PNEC of each trophic level was used.

3. Results

3.1. Occurrence of Antibiotics in Qingcaosha Reservoir

Of the 17 antibiotics compounds from six groups, only one antibiotic (OTC) was not detected at any sampling site; TYL was detected with the lowest detection rate of 47.92% while four antibiotics (SQX, NFX, TC, DC) were the most frequently detected compounds with a detection rate of 100%, and the detection rates of the other twelve antibiotics were all above 80%. The concentration and detection frequencies of antibiotics in Qingcaosha Reservoir are shown in Figure 2.

In the group of sulfonamides (SAs), SQX showed 100% detection frequency, while SDZ and SMM showed detection frequency over 89%. With the properties of recalcitrance and hydrophilicity, SAs were prevalent in the surface water [6,29]. In the group of fluoroquinolones (FQs), three antibiotics (NFX, CFX, OFX) still frequently showed detection frequency over 81.25%. Among FQs, CFX showed the highest concentration (283.5 ng/L) in November. The mean concentrations of antibiotics in this group followed the rank order: NFX (129.63 ng/L) > CFX (25.12 ng/L) > OFX (7.64 ng/L). In the group of β-lactams, the detection frequencies of LEX, PENG, PENV were more than 89.58%. The highest concentration was observed for PENV, reaching 404.9 ng/L. In the group of macrolides (MLs), TYL and ETM-H_2O were detected at a low concentration level, in the range of not detedted (n.d)–6.2 ng/L and n.d–37.4 ng/L, respectively, which were notably lower than Australian urban water (0–60 ng/L) [29] but higher than the South Yellow Sea (n.d–1.7 ng/L) [30]. The detected frequency of TYL (47.92%) is lowest among 16 antibiotics. In the group of tetracyclines (TCs), TC and DC were 100% detected with concentrations up to 35.9 ng/L and 266.7 ng/L, respectively. In the group of others, the concentrations of the antibiotics were relatively low compared with the other five groups of antibiotics. LIN has been reported in ranges from 4–171 ng/L in Urban Water [31] and 1–50 ng/L in Surface water in Spain [29] compared to a range from n.d–11.5 ng/L in Qingcaosha Reservoir.

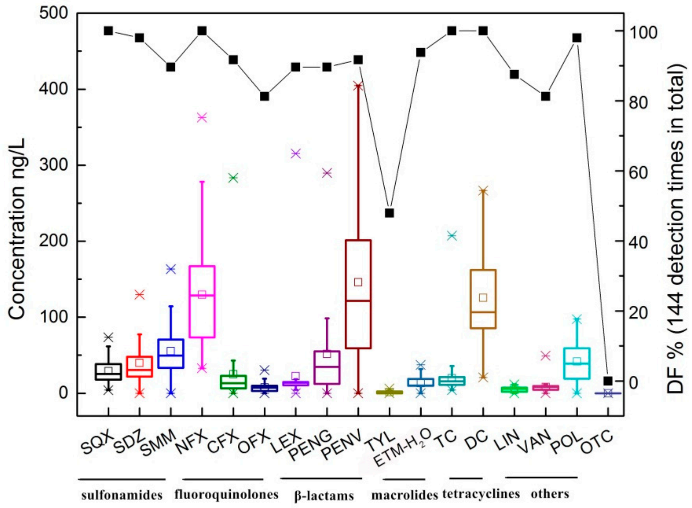

Figure 2. The concentration and detection frequencies of antibiotics in Qingcaosha Reservoir. Color "×" belongs to the box chart and the Y-axis of the box diagram is on the left, it represents the maximum and minimum concentration for each antibiotic. Color "□" belongs to the box chart and the Y-axis of the box diagram is on the left, it represents the mean value for each antibiotic. "■" represents the detection frequency (DF) of every antibiotic and the Y-axis of the DF is on the right. The line connecting with the box is up to 99% and down to 1% which lefts the maximum and minimum value of each antibiotic as Color "×":

3.2. Seasonal Variation of Antibiotics

The seasonal variations of six groups of antibiotics investigated at four sampling sites are shown in Figure 3. The concentrations of antibiotics in summer were notably lower than in winter, and slightly lower than in spring and autumn.

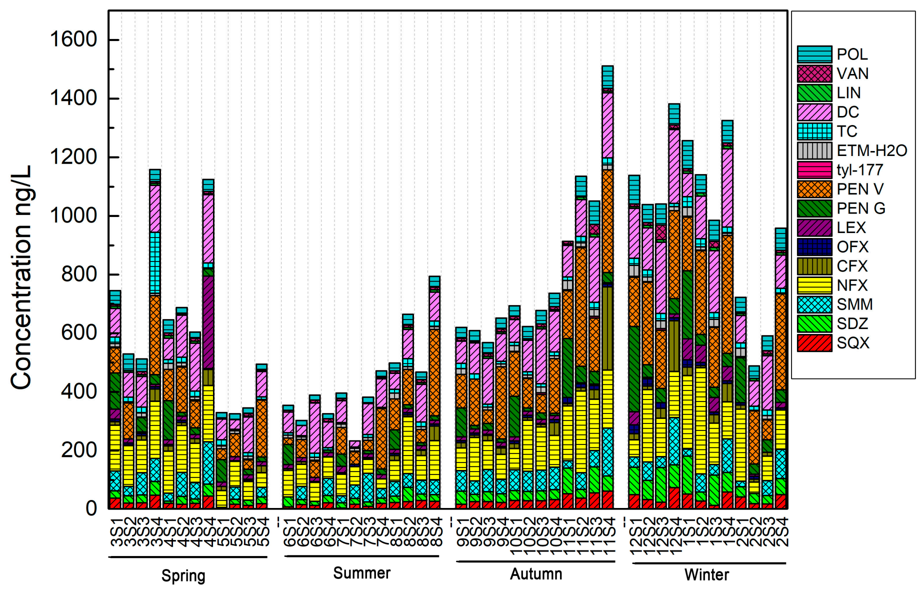

Figure 3. Total concentration of six groups of antibiotics in Qingcaosha Reservoir over four seasons. S1 to S4 represent the every sampling sites and the number before S1 to S4 represent the months.

Among all the antibiotics, the concentrations were highest in winter. The median concentrations of antibiotics in winter were approximately 1.10–5.32 times higher than in the other three seasons. Concentrations of SAs ranged from 0 to 143.8 ng/L in spring, from 0 to 97.0 ng/L in summer, from 17.2 to 163.2 ng/L in autumn, and from 12.92 to 161.3 ng/L in winter. Moreover, the result of this study showed that dominating antibiotics in different seasons were different. For example, NFX was found to be the main compound in spring while PENV was significantly high in the other three seasons.

3.3. Spatial Variation of Antibiotics

Among all sampling sites, the middle of the reservoir (S4) was most contaminated, with the total concentration of 1510.86 ng/L in October, followed by inflow sluice (S1) (1256.71 ng/L) in January, outflow sluice (S2) (1140.58 ng/L) in January and water pump station (S3) (1050.86 ng/L) in November. For each group of antibiotics, the highest concentration of macrolides was found at S1 (41.07 ng/L) in December, while the highest concentration of other groups was found at S4 (Figure A2).

4. Discussion

4.1. Occurrence, Seasonal Variation

Compared with previous studies (Table 1), the concentrations of SDZ and SQX are relatively higher than those in the Yangtze Estuary [22], Huangpu River [21], and Urban water [29]. The concentration of SMM (n.d–163.2 ng/L) in this study is lower than those in the Huangpu River (ranged from 2.05–623.27 ng/L) [32] and the Pearl River (ranged from n.d–1080 ng/L) [33].

CFX and NFX were significantly higher than those in Yangtze Estuary (n.d–14.2 ng/L and n.d–2.27 ng/L, respectively) [15] and Pearl River Estuary (n.d–34.2 ng/L and n.d–33.6 ng/L, respectively) [33]. OFX in Qingcaosha Reservoir was much lower than the South Yellow Sea (n.d–497.6 ng/L) [30] and Chaohu (n.d–182.7 ng/L) [34]. It is reported that FQs showed relatively low persistence in water and had strong sorption to the solid phase [35], so in many studies, the detection frequencies of FQs were relatively low, which is different to this study. Compared with other studies, the concentration of OFX was found in municipal sewage and animal wastewater in the area of the South Yellow Sea, so the concentration was relatively high, but the pollution level of OFX in Qingcaosha Reservoir was lower while NFX and CFX were higher than other water bodies.

PENG has been reported in ranges from n.d–250 ng/L [29] compared to a range from n.d–289.9 ng/L in this study. In many types of research, β-lactams were thought not to be a concern as environmental pollutants due to the characteristic of fast hydrolysis. However, the result showed that β-lactams account for a large proportion of the total. This phenomenon elucidates that although these antibiotics are, generally, considered to degrade easily, a pseudo-persistence may be occurring as a result of the continuous discharge.

ETM-H_2O is used not only in livestock as treatment and food additions, but also in the treatment of humans. Because of the strong sorption to sediments and high hydrophobicity of the MLs, the concentrations of the MLs in the aquatic environment were found to be very low. In previous investigations, TC and DC have also been detected in the Huangpu River [20] and Yangtze Estuary [21]. The detection frequencies of TCs in the Huangpu River were high; this might be due to the large usage and discharge in the river [20]. Normally, TCs were seldom reported in the natural water due to their strong degradation ability as well as absorption to particles or soil. However, these antibiotics were found to be ubiquitous in rivers in Shanghai; this may reflect the large usage and discharge of the TCs in this area.

It can be summarized that tetracyclines and fluoroquinolones are widely used as both human and veterinary medicines to treat diseases or to promote growth in livestock [22]. Because both human and livestock excreta, with metabolized or un-metabolized drugs, pass into sewage systems, the detection frequencies and concentrations of these antibiotics are high. SQX and TYL are only used in veterinary applications so they are less prevalent than other antibiotics in the aquatic environment.

Table 1. Comparison of antibiotics concentrations in surface water from different sites (ng/L).

Compounds	Sampling Locations							
	Qingcaosha	Yangtze Estuary	Huangpu River	Chaohu	Pearl River Estuary	South Yellow Sea	Urban Water	Surface Water
	China	China	China	China	China	China	Australia	Spanish
SDZ	n.d–129.8	0.28–71.8	1.39–112.5	n.d–45.6	n.d–726	-	n.d–30	-
SMM	n.d—163.2	0.53–89.1	2.05–623.27	n.d–8.8	n.d–1080	n.d–9.3	-	8–43
SQX	4.1–73.68	n.d–23.5	n.d–64.2	-	-	-	-	-
NFX	32.8–278.2	n.d–14.2	n.d–0.2	n.d–70.2	n.d–174	n.d–21.1	30–1150	-
CFX	n.d–283.5	n.d–2.27	n.d–34.2	n.d–23.3	n.d–33.6	-	n.d–1300	-
OFX	n.d–30.6	n.d–12.4	n.d–28.5	n.d–182.7	2.5–108	n.d–497.6	-	-
LEX	n.d–71.7	-	-	-	-	-	n.d–100	-
PENG	n.d–289.9	-	-	-	-	n.d–11.8	n.d–250	-
PENV	n.d–404.9	-	-	-	-	-	n.d–100	-
TYL	n.d–6.2	-	-	-	-	-	0–60	0.5–16
ETM-H_2O	n.d–37.4	-	-	-	-	n.d–1.7	n.d	-
OTC	n.d	n.d–22.5	n.d–219.8	-	-	-	n.d–100	-
TC	4.1–35.9	n.d–2.37	n.d–113.89	-	-	-	n.d–80	-
DC	32.4–266.7	n.d–5.63	n.d–112.3	n.d–42.3	-	-	n.d–40	-
POL	n.d–97.5	-	-	-	-	-	-	-
VAN	n.d–49.2	-	-	-	-	-	-	-
LIN	n.d–11.5	-	-	-	-	-	1–50	4–171
References	This study	[22]	[20,21]	[35]	[33]	[30]	[29]	[31]

n.d: not detected; - : not analyzed.

The varying presence of antibiotics between four seasons may be due to the usage and prosperities of antibiotics, flow conditions and water temperature. It is worth noting that the concentrations of detected antibiotics in high flow and warm conditions were lower than those in low flow and cold conditions [20,36]. From May to September, in order to prevent the eutrophication of water in the reservoir, the flow condition of Qingcaosha Reservoir was high, and from November to April, to restrain the invasion of the salt tide, the flow condition was low. The great dilution by the large flux of the Yangtze River in summer (normal above 50,000 m^3/s) led to the low concentration of antibiotics. Moreover, due to the higher microbial activity and stronger sunlight in summer, the bio-degradation and photo-degradation of antibiotics might be higher in summer than other seasons [37]. Therefore, lower concentrations were observed in summer than in the other three seasons for most antibiotics.

It is worth noting that S4 was located in the middle of the reservoir and was close to the suburb area and S3 was located at the water pump station, which transported the water to the water plant. The above data shows that the contamination level in S3 was less serious than in the other three sampling sites. The total concentration of antibiotics at S4 was very high; this result might be explained by the settled particles releasing antibiotics into the water. Doretto indicated that settled particles with low organic carbon contents had high antibiotics desorption capacity [38]. Sedimentation of the particles was remarkable in S4 while it was not significant downstream of the reservoir (S2/S3) (Figure A1), suggesting that there was a high potential for surface water contamination. Furthermore, the key factor affecting the sedimentation was flow rate; the rank order of the flow rate was as follows: S1 (0.2 m/s) > S4 (0.01~0.19 m/s) > S3, S2 (0.01~0.03 m/s) [39]. Particles were fully settled in S4 because of the relatively high velocity upstream of the reservoir. The suspended particles downstream of the reservoir were phytoplankton which would not release contaminants into the water. Qingcaosha Reservoir is located at the estuary of the Yangtze River and most domestic sewage effluents are now continuously discharged downstream of the Yangtze River since the completion of the wastewater control project. Yan claimed that wastewater treatment plants located in the upper reaches of the Yangtze River would be the primary reason for the higher concentration of antibiotics downstream of the Yangtze River [40]. Furthermore, several drain outlets were found in the Yuxi River which was located downstream of the Yangtze River; effluents from the pond and village area were discharged into the estuary without any purification treatment. Therefore, the point source of the antibiotics also exists [34].

Thus, although the tetracyclines had strong absorption to sediment, they were still detected with high frequency. The concentration of DC was found to be much higher than that of TC; the input contamination from upstream of the reservoir might be the main reason. The dominant contamination at S1 was PENV while at S2 and S4 it was PENG. This result might be due to the wide range of applications of PENG and PENV in clinical applications, considering their high effectiveness and low toxicity [29,30].

4.2. Environmental Risk Assessment of Antibiotics

In this study, the potential ecological risks of antibiotics were assessed by using the risk quotients (RQs) approach, according to the European technical guidance document (TGD) on risk assessment. The value of RQ was defined as the ratio of the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC). The value of PNEC was assessed based on the toxicity data which were obtained from the Ecological Structure Activity Relationships (ECOSAR) and shown in Table A3. In order to better distinguish the ecological risk levels, according to the individual RQ value, three risk levels were classified (0.01–0.1: low risk; 0.1–1: medium risk; >1: high risk) [22,41].

The risk quotients (RQs) of antibiotics in the reservoir are shown in Figure 4. According to the RQs, seven antibiotics (SDZ, SMM, SQX, PENG, TYL, OTC and POL) posed a low risk to the relevant sensitive aquatic organisms (*S. capricornutum*, *S. vacuolatus*, *P. subcapitata*, *M. aeruginosa*) in four seasons; four antibiotics (NFX, CFX, PENV, DC) caused high risk. The RQ values of NFX, CFX, PENV, and DC suggested that these antibiotics might present a significant risk to the algae in Qingcaosha Reservoir.

Normally, RQs in winter, spring and autumn are remarkably higher than in summer. For example, OFX, ETM-H_2O and LIN caused high risk in winter, while they caused medium risk in summer, and the proportions of samples classified as high risk during the entire sampling period were 16.7%, 58.3%, 33.3%, respectively. However, LEX, may cause medium risk in the aquatic environment in April and low risk in other months.

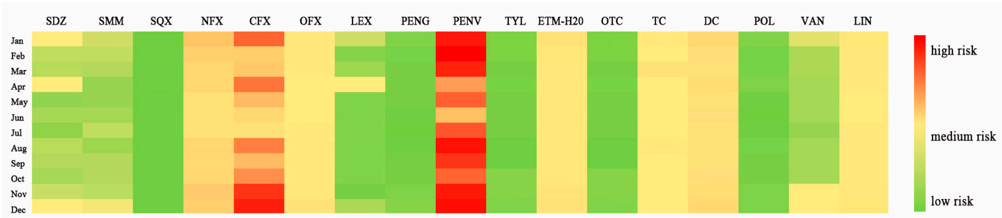

Figure 4. Risk assessment of antibiotics in Qingcaosha Reservoir from May 2016 to April 2017.

Studies have demonstrated that the residual trace antibiotics in the aquatic environment may impose selective stress on the microbe communities and accelerate the spread of antibiotic resistance genes (ARGs) [42]. ARGs such as *sul I*, *sul II*, *tet (C)*, *tet (G)* were the most prevalent resistance genes in raw water in Yangtze River Delta, and the absolute abundances of the *sul* and *tet* class genes ranged from 10^{10} to 10^{12} copies/L [43]. In another drinking water source in Shanghai, 11 ARGs were detected with high concentrations, and *sul II* was present at the highest concentration (4.19×10^8 copies/L). This phenomenon might reflect the widespread use of sulfonamides in this region [19]. Furthermore, ARGs could be transferred between bacteria through transposons, plasmids and integrons [44]. Xu observed that the abundances of ARGs were significantly correlated to the levels of mobile genetic elements, indicating that *intI-1* and transposons may contribute to the abundances of ARGs in drinking water [45]. Hence, the prevalence of the antibiotics in Qingcaosha Reservoir exhibited not only ecological risk in the water phase but also the risk of spread of the antibiotic resistance genes, which should be researched further in the future studies.

5. Conclusions

The occurrence and seasonal variations of six groups of antibiotics in Qingcaosha Reservoir were detected by using SPE and UPLC-MS/MS; antibiotics in all of the sampling sites were at the ng/L level. All antibiotics were frequently detected during the year-long period except for OTC and TYL. β-lactams showed the highest concentrations of antibiotics compared to other groups of antibiotics, suggesting its extensive use in this region, while macrolides exhibited relatively low concentrations. The seasonal variation of the total antibiotics indicated that residues of antibiotics in the surface water in winter were higher than in the other three seasons. The concentrations of the antibiotics at S4 were highest, suggesting that the antibiotics released by settled particles may be the major source. Since the completion of the wastewater control project, most domestic sewage effluents are discharged downstream of the Yangtze River; this might be the major antibiotics source of the reservoir. The risk assessment results based on the RQs clearly revealed that six antibiotics, which included NFX, CFX, PENV, DC, OFX, ETM-H_2O and LIN, posed a significant ecological risk to the relevant algae in the surface water of Qingcaosha Reservoir. Moreover, more attention should be paid to the fate of these antibiotics, considering the spread of antibiotic resistance genes in this region.

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Author Contributions: Yue Jiang, Cong Xu and Xiaoyu Wu conducted the experiments and wrote the paper; Yihan Chen designed the experiments; Wei Han analyzed the data; Karina Yew-Hoong Gin and Yilang He were in charge of the paper.

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

Appendix A

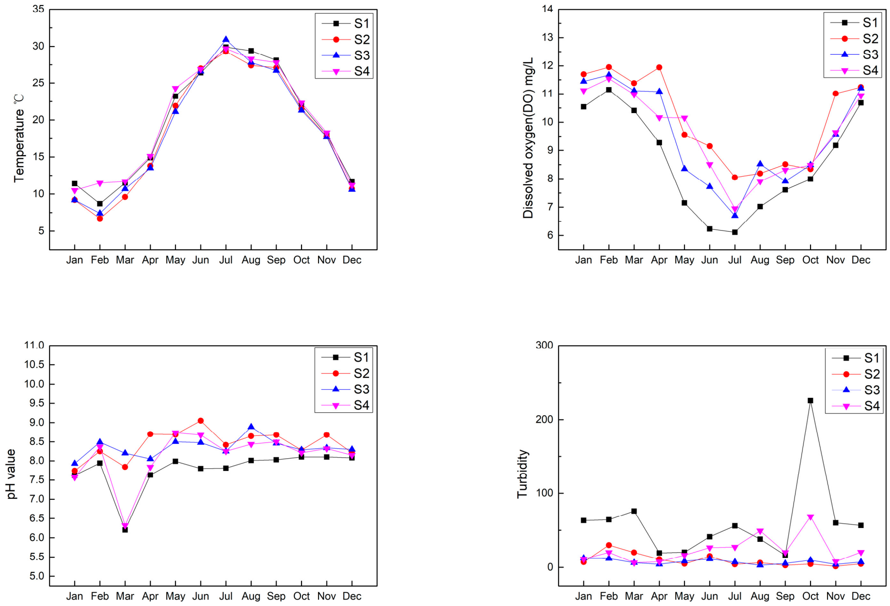

Figure A1. The water quality parameters in sampling sites in this study.

Table A1. The physicochemical properties of the target antibiotic compounds.

Antibiotic	Usage	Molecular Formula	CAS Number	Log Kow	Log Koa	Vapor Pressure (Pascals)
Sulfadiazine	human	$C_{10}H_{10}N_4O_2S$	68-35-9	−0.09	8.1	2.29×10^{-4}
Sulfamonomethoxine	veterinary	$C_{11}H_{12}N_4O_3S$	1220-83-3	0.2	12.706	5.96×10^{-5}
Sulfaquinoxaline	veterinary	$C_{14}H_{12}N_4O_2S$	59-40-5	1.68	14.43	3.91×10^{-6}
Norfloxacin	human/veterinary	$C_{16}H_{18}FN_3O_3$	70458-96-7	−1.03	15.419	1.63×10^{-7}
Ciprofloxacin	human/veterinary	$C_{17}H_{18}FN_3O_3$	85721-33-1	0.28	16.962	7.27×10^{-8}
Ofloxacin	human/veterinary	$C_{18}H_{20}FN_3O_4$	82419-36-1	−0.39	17.301	4.11×10^{-8}
Cefalexin	human/veterinary	$C_{16}H_{19}N_3O_5S$	23325-78-2	−0.08	18.849	7.27×10^{-12}
Penicillin G	human/veterinary	$C_{16}H_{17}N_2O_4SK$	113-98-4	−3.01	6.05	4.96×10^{-13}
Penicillin V	human/veterinary	$C_{16}H_{17}N_2O_5S$	132-98-9	2.09	14.833	3.81×10^{-8}
Tylosin	veterinary	$C_{46}H_{77}NO_{17}$	1401-69-0	1.63	37.257	1.36×10^{-28}
Erythromycin-H_2O	human/veterinary	$C_{37}H_{65}NO_{12}$	23893-13-2	-	-	-
Oxytetracycline	human/veterinary	$C_{22}H_{25}ClN_2O_9$	2058-46-0	−3.6	24.561	2.09×10^{-22}
Tetracycline	human/veterinary	$C_{22}H_{25}CLN_2O_8$	64-75-5	−3.7	25.588	5.33×10^{-23}
Doxycycline	human/veterinary	$C_{22}H_{25}ClN_2O_8$	24390-14-5	-	-	-
Polymix-B	human	$C_{56}H_{100}N_{16}O_{17}S$	1405-20-5	-	-	-
Vancomycin	human/veterinary	$C_{66}H_{75}CL_2N_9O_{24}$	1404-93-9	−0.84	−0.995	-
Lincomycin	human/veterinary	$C_{18}H_{34}N_2O_6S$	154-21-2	0.2	21.111	7.09×10^{-13}

Table A2. Tandem mass spectrometric parameters for the target antibiotics in this study.

Group	Antibiotic	CAS Number	Acronym	Relative Molecule Mass	Precursor Ion M/Z	Product Ion M/Z	Collision Energy E/V
Sulfonamides (SAs)	Sulfadiazine	68-35-9	SDZ	250.05	251.1	156.0, 92.1	14, 26
	Sulfamonomethoxine	1220-83-3	SMM	280.06	281	108, 156, 92	23, 16, 26
	Sulfaquinoxaline	59-40-5	SQX	300.07	301	156, 92.1, 108	14, 24, 21
Fluoroquinolones (FQs)	Norfloxacin	70458-96-7	NFX	319.13	320.1	302.2, 276.2	22, 16
	Ciprofloxacin	85721-33-1	CFX	331.13	332.2	288.2, 245.1	20, 16, 22
	Ofloxacin	82419-36-1	OFX	361.14	362.2	318.0, 261.1	17, 26
β-lactmas	Cefalexin	23325-78-2	LEX	365.10	348.1	106.1, 158, 174	23, 8, 12
	Penicillin G	113-98-4	PEN G	372.05	335	176.1, 160, 114.1	10, 7, 28
	Penicillin V	132-98-9	PEN V	349.09	351.1	229.1, 106.1	14, 14
Macrolides (MLs)	Tylosin	1401-69-0	TYL	915.52	916.6	174, 772.6	34, 28
	Erythromycin-H_2O	23893-13-2	ETM-H_2O	715.45	716.3	158, 558	28, 17
Tetracyclines (TCs)	Oxytetracycline	2058-46-0	OTC	496.12	461.2	443.0, 426.0	11, 18
	Tetracycline	64-75-5	TC	480.13	444.82	410.2, 427.3	19, 10
	Doxycycline	24390-14-5	DC	480.13	445.2	428.2, 154	18, 28
Others	Polymix-B	1405-20-5	POL	1300.72	402	101.1, 120.1	20, 29
	Vancomycin	1404-93-9	VAN	1447.43	724.9	100.1, 144.1	27, 13
	Lincomycin	154-21-2	LIN	406.21	407.2	126.1, 359.3	35, 15
External standard	Norfloxacin-d5	1015856-57-1	NFX-d5	324.34	325.1	307.2, 281.2	22, 16
Internal standards	Ciprofloxacin-d8	1130050-35-9	CFX-d8	339.18	340.2	322.2, 296.2	21, 17
	Amoxicillin-d4	26787-78-0	AMX-d4	369.4	370.2	114, 212.1	19, 10
	Sulfadiazine-d4	1020719-78-1	SDZ-d4	254.28	255.1	160, 96.1	14, 25
	Doxycycline-d3	564-25-0	dox-d3	447.44	448.2	431.3, 323.2	21, 30

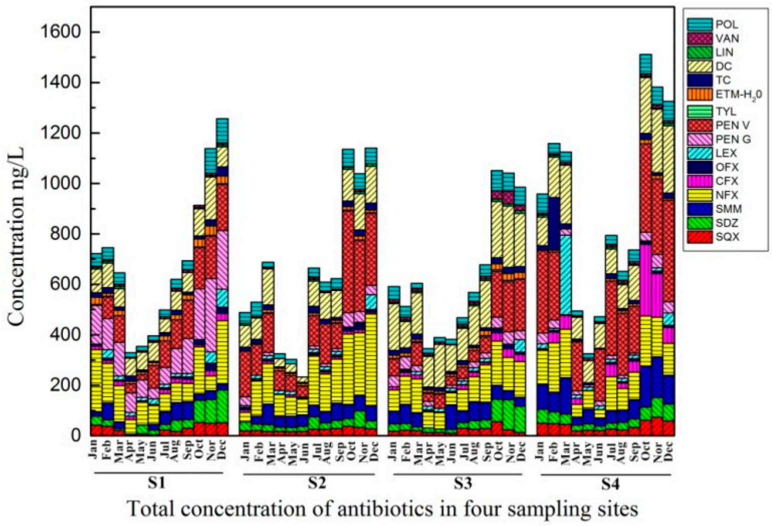

Figure A2. Total concentration of antibiotics in four sampling sites.

Table A3. Toxicity data on algae, invertebrates and fish.

Compounds		Species	Toxicity Data (mg/L)	PNEC (ng/L)	References
Sulfadiazine	Algae	*S. capricornutum*	2.2	2200	[46]
	Invertebrate	N.F	N.F	N.F	N.F
	Fish	N.F	N.F	N.F	N.F
Sulfamonomethoxine	Algae	*S. vacuolatus*	3.82	3820	[47]
	Invertebrate	N.F	2.259	2259	ECOSAR
	Fish	N.F	166.297	166,297	ECOSAR
Sulfaquinoxaline	Algae	N.F	131	131,000	[48]
	Invertebrate	N.F	N.F	N.F	N.F
	Fish	N.F	N.F	N.F	N.F
Norfloxacin	Algae	*M. wesenbergii*	0.038	38	[49]
	Invertebrate	*D. magna*	0.88	880	[50]
	Fish	N.F	20,081.355	20,081,355	ECOSAR
Ciprofloxacin	Algae	*P. subcapitata*	0.002	2	[51]
	Invertebrate	N.F	N.F	N.F	N.F
	Fish	N.F	N.F	N.F	N.F
Ofloxacin	Algae	*M. aeruginosa*	0.021	21	[51]
	Invertebrate	*C. dubia*	3.13	3130	[51]
	Fish	*D. rerio*	>1000	1,000,000	[51]
Cefalexin	Algae	N.F	2.5	2500	[52]
	Invertebrate	N.F	N.F	N.F	N.F
	Fish	N.F	N.F	N.F	N.F
Penicillin G	Algae	N.F	39.032	39,032	ECOSAR
	Invertebrate	N.F	193.241	193,241	ECOSAR
	Fish	N.F	375.923	375,923	ECOSAR
Penicillin V	Algae	N.F	0.006	6	[53]
	Invertebrate	N.F	N.F	N.F	N.F
	Fish	N.F	N.F	N.F	N.F
Tylosin	Algae	*P. subcapitata*	0.95	950	[54]
	Invertebrate	N.F	N.F	N.F	N.F
	Fish	N.F	N.F	N.F	N.F
Erythromycin-H_2O	Algae	*P. subcapitata*	0.02	20	[51]
	Invertebrate	*C. dubia*	0.22	220	[51]
	Fish	*D. rerio*	>1000	1,000,000	[51]
Oxytetracycline	Algae	*M. aeruginosa*	0.23	230	ECOSAR
	Invertebrate	N.F	3.08	30,800	ECOSAR
	Fish	*Oryzias latipes*	50	500,000	ECOSAR
Tetracycline	Algae	*M. aeruginosa*	0.09	90	ECOSAR
	Invertebrate	*B. calyciflorus*	5.6	5600	ECOSAR
	Fish	*Paracheirodon axelrodi*	2.5	25,000	ECOSAR

Table A3. *Cont.*

Compounds		Species	Toxicity Data (mg/L)	PNEC (ng/L)	References
Doxycycline	Algae	*M. aeruginosa*	0.062	62	ECOSAR
	Invertebrate	*C. dubia*	0.5	500	ECOSAR
	Fish	*D. rerio*	2.658	2658	ECOSAR
Polymix-B	Algae	*A. aeruginosa*	2	2000	ECOSAR
	Invertebrate	N.F	N.F	N.F	N.F
	Fish	N.F	N.F	N.F	N.F
Vancomycin	Algae	N.F	0.6	600	[50]
	Invertebrate	N.F	N.F	N.F	N.F
	Fish	N.F	N.F	N.F	N.F
Lincomycin	Algae	*P. subcapitata*	0.07	70	ECOSAR
	Invertebrate	*Thamnocephalus platyurus*	33	33,000	ECOSAR
	Fish	*Danio rerio*	1000	10,000,000	ECOSAR

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