Environmental Science
Processes & Impacts

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Environmental Impact statement: Antibiotic residues in environment have become an important environmental issue because it poses a potential threat to human health. Wastewater treatment plants are regarded as one of the most important sources of antibiotics in the environment. It is very important to study fate of antibiotics and its metabolites in wastewater treatment plants, which is contribute to assess potential environmental risk. This paper was investigated to study occurrence, seasonal variation and removal efficiency of 21 antibiotics and 10 metabolites in five wastewater treatment plants in different seasons in Jiulongjiang River Region, South China.

Occurrence, seasonal variation and removal efficiency of antibiotics and its metabolites in wastewater treatment plants, Jiulongjiang River Basin, South China

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Abstract

Wastewater treatment plants (WWTPs) are regarded as one of the most important sources of antibiotics in the environment. The occurrence, seasonal variation and removal efficiency of 21 antibiotics and 10 metabolites, including five sulfonamides and three their metabolites, six quinolones, two macrolides, two β-Lactams and five tetracyclines and seven their metabolites, were investigated in five WWTPs in different seasons in Jiulongjiang River Region, South China. 16 antibiotics and 6 metabolites in summer and 14 antibiotics and 6 metabolites in winter were found, respectively. The most frequently detected antibiotics were sulfamethazine, sulfamethoxazole, n-acetyl sulfamethazine, n-acetyl sulfamethoxazole, ofloxacin, cephalexin monohydrate and cephradine; of these, the concentration of cephradine was the highest in most of the influent and effluent samples. The highest level of total antibiotics was found in Longyan City WWTPs, where there are more population and swine farms. Seasonal variation of the antibiotics in wastewater samples was also studied. The concentrations of antibiotics in winter were higher than those in summer. The antibiotics could not be removed completely by the WWTPs, and the mean removal efficiency ranged from -71.6 to 56.3%. Of all the antibiotics, the tetracyclines were removed comparatively more efficiently, probably due to their adsorption to sludge. The low removal efficiency of antibiotics in WWTPs could become one of the important reasons of antibiotics in environment in Jiulongjiang Region.

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Key words: Antibiotics, Metabolites, Wastewater treatment plants, Seasonal Variation, Removal frequency, Jiulongjiang River

1. Introduction

Antibiotics have important uses in both human and veterinary medicine for their antibacterial properties and as growth promoters. As their consumption increases, they are being detected in all the sectors of the environment. Antibiotics in environmental residual have attracted much public attention in the last decade[1]. Previous studies have clearly shown that the municipal wastewater treatment plants (WWTPs) are an important pollution source of antibiotics released into the environment[2]. Antibiotics are suspected to present an environmental risk, e.g., fostering bacterial resistance[3,4]. For this reason, antibiotics in WWTPs have been subject to increased investigation in the last decade[5-7]. The conventional wastewater treatment plants appear unable to completely eradicate these antibiotics. For example, the removal efficiency of tetracycline varied from 12%[8] to 80%[9] in wastewater treatment plants. The incomplete removal of some of these antibiotics during conventional wastewater treatment plant can be directly linked to their presence not only in surface waters and sediments but also in soils.

Most antibiotics are excreted unaltered or as metabolites in feces and urine[10,11]. As much as 30-90% can be excreted, this having been poorly absorbed or metabolized. However, the current studies indicate that metabolites of antibiotics can be persistent, and accumulate in foods[12] and drinking supplies[13], including ground waters[14] which were originally expected to be impervious to considerable contamination. For example, approximately 40% of a sulfamethoxazole (SMZ) is transformed into N_4-acetyl-sulfamethoxazole (ASMZ) in urine[15]. But there is evidence that ASMZ may be transformed back to the parent compound during wastewater treatment[16]. Oxytetracycline, tetracycline, and chlortetracycline may be metabolized into their 4-epimers of 4-epioxytetracycline (EOTC), 4-epitetracycline (ETC) and 4-epichlortetracycline (ECTC). Although the antimicrobial effects of the 4-epimers are lower than that of tetracyclines or even disappeared, their toxicities are heavier than that of tetracyclines (TCs)[17]. With increasing and poorly regulated use of antibiotics in the world, the accumulation of antibiotics and their metabolites in

animals and humans can thus represent an environmental and toxicological threat, with wastewaters as central transfer mediums.

The Jiulongjiang River is the second largest water system in Fujian Province, southern China. It is the most important source of drinking water and industrial and agricultural activities. With the rapid growth of the urban population in the Jiulongjiang River basin, there are numerous inputs of medical antibiotics from municipal wastewater treatment systems into the river. In our previous studies[18], we found levels of antibiotics in water of Jiulongjiang River were very high. Besides, it was found that antibiotics in upstream of Jiulongjiang mainly came from swine wastewater, but those in downstream of Jiulongjiang from WWTPs[19]. However, little information on antibiotics in wastewater treatment plant of Jiulongjiang River is available. In this study, we examined the occurrence and fate of selected antibiotics and its metabolites in influent and effluent from five WWTPs in Jiulongjiang River Basin. Specifically, we collected and analyzed the samples from both summer and winter seasons. The results were used to evaluate distribution characteristics and seasonal variation of the selected antibiotics and its metabolites from WWTPs.

2. Materials and Methods

2.1. Chemicals and Materials

The antibiotics and its metabolites in this study including five sulfonamides and three of their metabolites, five tetracyclines and seven of their metabolites, six quinolones, two macrolides, and two β-Lactams were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). More details are shown in Table 1. Stock solutions (100.00 mg/L) were prepared by dissolving 0.500 mg standard antibiotics in 5 mL 10% methanol solution, individually. They were kept in brown glass vials in a refrigerator (4°C). Oasis HLB (500 mg, 6 mL) was purchased from Waters Corporation (Milford, MA, USA). HPLC grade methanol and acetone were purchased from TEDIA Company (Fairfield, OH, USA). Milli-Q water (Millipore, USA) was used throughout the study. Unless otherwise indicated, chemicals used in the analysis were analytical grade.

2.2. Sampling Sites and Sample Collection

As is shown in Figure 1, five wastewater treatment plants (S1–S5) in Jiulongjiang River Basin were chosen. S1 is situated in Longyan City. S2 is located in Zhangping City. S3 is sited at Hua'an County. S4 is situated in Changtai County and S5 is located in Zhangzhou City. The WWTPs employ similar treatment processes: primary (mechanical) process using screens, settling tanks and skimmers to remove particles, coupled with secondary biological treatment. Detailed information of five WWTPs is shown in Table 2.

Wastewater samples were collected as ''grab samples'' from five WWTPs. For the grab sampling program, the raw wastewater influents and the final effluents from the WWTPs were sampled in sequence according to the hydraulic retention of the sewage water treatment. Three replicate samples were collected for laboratory analysis. During the sample collection, the sampling bottles were rinsed with sample three times before a final sample was collected. Every sample was collected for 4L. All samples were kept in the dark at -18^{o}C until analysis. All samples were collected in July 2012 (summer) and January 2013 (winter).

2.3. Extraction and analysis

The water samples were filtered through 0.45 μm glass fiber filters within 24h after the sample collection. Antibiotics compounds were extracted according to the method of Zhang et al[18]. Briefly, 500 mL of water sample was acidified to pH=3.0 by adding HCl, followed by addition of 0.2 g Na_2EDTA. The samples were extracted using Oasis HLB (500mg, 6mL) extraction cartridges. The HLB columns were conditioned with 6.0 mL of acetone, 6.0 mL of methanol and 6.0 mL of 0.1% formic acid and 5mmol/L ammonium acetate (CH_3COONH_4), followed by loading of the sample at a flow rate of 5 mL/min. The cartridges were dried under nitrogen and eluted with 6 mL methanol. Finally, the target fraction was collected in a 10 mL test tube, volume reduced to almost dryness under a gentle nitrogen stream, and then re-dissolved in 10% methanol solution to a final volume of 1.0 mL. Final extracts were transferred to 2 mL amber vials for LC–MS/MS analysis.

Sulfonamides (SAs), quinolones (QNs), macrolides (MLs) and β-Lactams (β-Ls)

were analyzed by LC-MS/MS system (ABI 3200Q TRAP) as described previously with slight revision. Briefly, an Inertsil ODS-SP column (4.6 mm × 150 mm, 5 μm, GL Science Inc. Japan) was maintained at 40°C, with injection volumes of 20 μL. Purified water with 0.1% formic acid (v/v) (phase A) and methanol (phase B) were used as chromatographic mobile phases at a total flow rate of 1 mL/min. Tetracyclines and their metabolites were analyzed by UPLC-MS/MS system with the same mobile phases at a total flow rate of 0.2 mL/min. Detailed information on the method of antibiotics detection has been described previously [19].

2.5. Quality assurance and quality control

Recoveries of the 31 target compounds were determined for wastewater by the standard addition method at 100 ng/L in triplicate. Limits of detection (LOD) of the antibiotics were determined as the lowest concentrations resulting in a signal-to-noise (S/N) ratio of 3. Limits of quantification (LOQ) were calculated with a S/N ratio of 10. Main analytical parameters were shown in Table 3.

All data generated from the analysis were subject to strict quality control procedures. With each set of samples to be analyzed, a solvent blank, a standard and a procedure blank were run in sequence to check for background contamination, peak identification and quantification. In addition, surrogate standards were added to all the samples to monitor matrix effects.

2.6 Statistical analysis

Two-way analysis of variance (ANOVA) was used to examine the seasonal differences. The relationship analyses were performed using SPSS 15.0 for windows (SPSS Inc.).

3. Result and Discussion

3.1 Occurrence of antibiotics in summer

Table 4 shows the concentrations of selected antibiotics in influent and effluent samples from five WWTPs in summer. Among 31 analytes, 16 antibiotics and 6

metabolites including three SAs and three their metabolites, five QNs, two macrolides, two β-Lactams and four TCs and three their metabolites were detected.

In the five WWTPs, three out of five SAs were detected in influent and effluent samples, and SDM and SM1 were not found in all WWTPs. SD and SMZ were detected in all influent and effluent of WWTPs. The detection frequencies of SM2 were both 80% in the effluent and influent, with the maximum concentration of 251 ng/L in the influent sample. The concentration levels of SAs in the present study coincide with those in WWTP effluents in Spain[20] and Beijing[21]. Three metabolites of SAs were ubiquitous in all samples of influent and effluent of WWTPs. ASM, ASD and ASMZ concentrations in influent samples were in range of 8.50-51.5 ng/L, 2.70-19.3 ng/L and 72.7-232 ng/L, respectively.

There were four QNs observed in the wastewater of the investigated WWTPs. In influent samples, OF was the most frequently detected (100%) with the maximum concentration of 91.5 ng/L. The second in influent samples is NOF (80%), with a mean concentration of 34.8 ng/L and a maximum concentration of 130 ng/L. The detection frequencies of CIP in influent and effluent were 60% and 40%, respectively. To QNs, OF and NOF were the dominant compounds, which have been proven to be widely used in medical treatments[22]. Compared to our results, NOF in WWTPs effluents in Sweden, France, Greece, and Italy were lower (30-80 ng/L) but OF levels were higher (120-580 ng/L)[23]. A similar concentration range for NOF has been recorded as 0.10-0.46 μg/L in influent samples from five WWTPs in Hong Kong and Shenzhen[24] and 0.054-0.26 μg/L[25] in influent samples from four WWTPs in the Pearl River Delta. In addition, the reported concentration of CIP (0.028-0.32μg/L) is similar to our result (0.015-0.14 μg/L). In contrast, in Spain, much higher concentrations of CIP have been reported, with 0.160-13.6 μg/L for CIP[26].

ERY and ROX as the macrolides were detected in all influent and effluent samples in this study. The concentrations of ERY ranged from 1.10 to 4.40 ng/L in influents and from 1.20 to 1.80 ng/L in effluents. ROX was found with ranging from 12.7 to 54.5 ng/L in influents and from 6.54 to 42.0 ng/L in effluents. However, higher concentration ranges such as 54-360 ng/L for ROX and 51-300 ng/L for ERY in WWTPs effluent samples in Beijing have been reported[21]. In this study, β-Lactams including CEFM and CEFD were detected in all samples and the highest concentrations were found with 147 and 175 ng/L, respectively.

Among five targets of TCs, there were four TCs found including TC, OTC, CTC and DXC. In influent samples, the detection frequencies of four TCs were all 40%. In effluent samples, the highest detection frequency was OTC (60%), with the maximum concentration of 37.8 ng/L and the lowest of detection frequency was DXC (20%), with the maximum concentration of 87.3ng/L. In South China, levels of TCs in WWTPs were higher than those in this study[27].In this study, levels of TCs were much lower than those in the raw WWTPs influent in the USA at concentrations between 0.1 and 0.6 mg/L[28]. In Canada, the remaining concentration of tetracycline in WWTPs effluent was reported to be nearly 1.0 μg/L[29]. Once TCs were extensively used for both human and veterinary medicine in China. But now TCs are mostly used in treating animal disease in this region. To metabolites of TCs, three out of seven were detected in influent samples. Although detection frequency of TC was (40%), ICTC, as a metabolite of TC, was found in all WWTPs influent. This is probable because that CTC is converted to isochlortetracycline (ICTC) under alkaline conditions, while the epimerisation has been found to be catalyzed in acidic solutions in a pH range from 2 to 6[30].

3.2 Occurrence of antibiotics in winter

The concentrations of selected antibiotics are shown in Table 5 in influent and effluent samples from five WWTPs in winter. 20 of 31 analytes were detected in the analyzed samples of WWTPs including three sulfonamides and three their metabolites, four quinolones, one macrolides, two β-Lactams and four tetracyclines and three their metabolites.

Among the five SAs, three out of five were observed in all influent samples including SM2, SMZ and SD. The high concentration was SM2 up to 259 ng/L in influent sample. In effluent samples, SD and SMZ were detected in all samples. The detected frequency of SM2 was 80% in effluents. At the same time, metabolites of three SAs were also found in WWTPs. Among three metabolites of SAs, the concentration of ASMZ was the high up to 297 ng/L. To QNs, OF was the most frequently detected among QNs (100%), with the maximum of 53.4 ng/L. The second was ENX (80%), with a maximum concentration of 18.5ng/L in S5 effluent. However, SARA and DALA were not found in all samples. The detected frequency of CIP was

60%, while NOF was only 40%. Macrolides and β-Lactams were detected in all WWTPs samples except ERY. Level of CEFD was the highest up to 1185 ng/L. A similar level (670 – 2900ng/L) was observed in WWTPs influent from Hong Kong and Shenzhen, southern China[24].

There were four TCs found in WWTPs in winter. Among four TCs, DXC was the most frequently detected (100%) in effluent samples. TC, OTC and CTC were found with the detection frequency of 60%. In effluent samples, OTC was the most frequently detected (80%), with the maximum concentration of 178ng/L, and then the detected frequencies of TC, DXC and CTC were 60%. MTC was not detected in all samples. Similar to metabolites of TCs in summer, there were three MTCs found in winter. ICTC, as a metabolite of TCs, was found in all WWTPs except S5 with the maximum of 651ng/L. Similar to ICTC, EOTC in S1 was up to the highest concentration of 296 ng/L. The main degradation products of TCs detected in WWTPs were their epimers ETC, ECTC and EOTC, which is in agreement with the reported literature that TCs can be excreted in the form of their 4-epimers [31]. Especially for ETC, the concentration was nearly as much as that of TC. Dehydrated products and 4-epianhydrotetracyclines of TC and CTC tended to be lower than their parent TCs.

3.3 Seasonal variation of antibiotics in influent and effluent

As shown in Fig.2, the total levels of antibiotics showed significant seasonal fluctuations between in winter and in summer in all wastewater samples ($p<0.05$, $F=9.97$). The total levels of antibiotics in winter were higher than those in summer in all WWTPs. For example, the total concentration of antibiotics in wither season in S4 influent was 6.5 times higher than that in summer.

Statistical analysis showed that there were not significant seasonal ($p>0.001$) variations for five kinds of SAs, except SDM and SM1. To three metabolites of SAs, no significant variations were found between the two sampling events, although the total levels of SAs metabolites in winter were slightly higher than those in summer. To six quinolones, two quinolones (SARA and DALA) were not detected in winter, but only SARA was not found in summer. The total concentrations of quinolones in summer were higher than those in winter, although the frequencies of detection were

higher in winter than those in summer. This is probable because that quinolones are frequently treated with intestines infection in summer. Two macrolides showed significant (p<0.05, F=4.18) seasonal variations between two sampling events. The levels of ROX in winter were significantly (p<0.05, F=6.87) higher than those in summer. ERY was not detected in winter, but detected frequency of ERY was 100% in summer. For two β-Lactams, there was similar to the macrolides. Four TCs showed significant seasonal variations (p<0.05, F=6.24). The total concentrations of tetracyclines at inffluent samples in winter (ranging from 17.3 to 482 ng/L) were 2 times those in summer season (from ND to 231 ng/L). Each detected frequencies of four tetracyclines were 40%, and they were only observed at S1 and S2 influent samples in summer. However, four TCs were all detected in all influent samples, except S4 and S5. To metabolites of TCs, there was similar to TCs. The total concentration of metabolites in summer was slightly lower than that in winter, although there was not significant variation between in winter and in summer.

The different occurrences of antibiotics between the two sampling events may be explained by the consumption level of antibiotics and climatic conditions. Antibiotics showed highest concentrations in winter and lowest concentrations in summer in the WWTPs influents. Previous reports have also revealed that the level of antibiotics in the WWTPs influents is greater in winter than in other seasons[32-34], indicating a higher consumption of antibiotics in winter. Because of the cold weather during the winter, human beings and livestock used much more antibiotics to cure the infection of the respiratory tract. In addition, water consumption of urban residents usually is much more in summer than that in winter. Besides, in summer is the typical flood and high water season of Jiulongjiang River and winter is the typical low water season. So, another factor contributing to lower concentrations in winter might be much water consumption and the high flow conditions，which might result in a great dilution on the concentrations of antibiotics in WWTPs influent.

3.4 Spatial distribution of antibiotics in five WWTPs

Spatial distributions of 31 antibiotics and its metabolites in the current study were also compared among five WWTPs along the Jiulongjiang River Region (Fig.2).

Of all WWTPs, the total level of antibiotics in S1 was relatively higher. To SAs and its metabolites, levels in effluent and influent of S5 were the highest among five WWTPs in both seasons. Contributed to the total level of SAs in S5 biggest was SM2 up to 258.7 ng/L in influent of S5 in summer. To QNs, the highest total concentration of QNs was found in S1, however, the second was found in S5. NOF was the most abundant QNs in S1. NOF, which has been proven to be widely used in medical treatments[22], was commonly detected in WWTPs. Two macrolides and two β-Lactams were detected in all WWTPs samples. The total level of macrolides and β-Lactams were the highest in S4 in winter. This is probable because in S4 region there was higher the population density, while macrolides and β-Lactams were mainly used to treat human disease. TCs were found only in S1-S3. The highest total concentration of TCs and metabolites of TCs was found in S1 in winter. Now TCs were mostly used in treating animal disease in China. S1-S3 are located in upstream of Jiulongjiang River where there are a lot of swine farms[19], discharging of untreated swine breeding wastewaters into environment.

The concentrations of antibiotics in different WWTPs influents and effluents vary significantly, depending on consumption patterns and the types of wastewater treatment processes employed. In S1, there is more population than that in S2-S5. Besides, there is much more livestock breeding in S1. Thus, more population and livestock may result in more usage of antibiotics and more input into water in S1.

3.5 Aqueous removal percentages of antibiotics

The removal efficiency of antibiotics was calculated from the analyte concentration in influent (C_{in}) and effluent (C_{ef}): $[(C_{in} - C_{ef})/C_{in}]\times 100\%$. As shown in Table 6, the average removal efficiencies of SAs ranged from -6.20 to 26.9%. To three metabolites of SAs, the average removal efficiencies were from -19.4 to 47.8%. The average removal efficiency of SD, SM2 and SMZ in summer was 32.8, 15.6 and 17.8%, respectively. Similar removal efficiency of SD (20–82%) has also been detected in four WWTPs from Taiwan[35] and in two WWTPs from the Pearl River Delta[23]. The removal efficiency of SMZ (71% in summer and 0–84% in winter) in WWTPs in Italy has also been reported[33]. The negative removal efficiency of SMZ in WWTPs may be caused by the presence of metabolites in the influents, which can

subsequently be transformed to their parent compounds during biological treatment[36]. N_4-acetylsulfamethoxazole usually accounts for more than 50% of an administered dose in human excretion and can occur in WWTPs influents at concentrations of 2.5-3.5 times higher than concentrations of the parent compound[32]. Significant removal efficiencies (81-96% and 68-92%, respectively) of N_4-acetylsulfamethoxazole during secondary treatment were reported by Göbel et al[32]. and Joss et al[37]. N_4-acetylsulfamethoxazole can also deconjugate into sulfamethoxazole during wastewater treatment[36], leading to an underestimation of removal efficiency for sulfamethoxazole if this metabolite is not considered. This might be a reason for the highly varying observed elimination rates.

To QNs, the mean removal efficiency (-71.6-28.7%) was lower than those of SAs. The overall removal percentages were very variable. The mean removal efficiency of OF was 50%, which was similar to the value (56%) by the WWTPs in Sweden[38] and lower than that (83%) by the WWTPs in Finland[38]. For NOF, the mean and maximum removal efficiency was 6.4 and 100%, respectively. The mean removal efficiency of 28.7% for CIP in our study is lower than Rosal's observation (57.0%)[26] and 84% from WWTPs in Finland[39]. In Sweden[40], the mean elimination degrees of NOF, NOR and CIP were estimated to be 86, 87 and 87%, respectively. Several studies have also reported that the predominant removal mechanism of quinolones in the WWTPs is adsorption to sludge[41-42]. For example, approximately 80% of the total mass of both NOF and CIP are absorbed to particles in the raw sewage water[43].

Compared with the other detected antibiotics, the average removal efficiencies of two macrolides were very lower. The mean removal efficiency of ERY and ROX were 24.2%, which was similar to the reported removal efficiency (26%) of ERY–H_2O in four WWTPs in the Pearl River Delta[25] and higher than the elimination (4.3%) of ERY in WWTPs in Spain[26]. The removal efficiency of ROX ranged from -223 to 48.5% (-42.9% on average) in our study. The negative removal efficiency of macrolides by WWTPs had also been reported in previous works[24, 25]. One of the possible reasons for these findings is that particles larger than 0.45μm are not included in the analysis, which may lead to an underestimation of the concentrations of the relevant compounds in the influents. Moreover, the conjugated metabolites in raw influent samples can be de-conjugated during the treatment process, or analyte behavior such as adsorption to particles may be altered by changing physicochemical

parameters during the treatment process, thus influencing the removal efficiency[26,40]

For four TCs, the mean removal efficiency ranged from 25.62% to 50.74%. In the USA, CTC and DXC have been reported after secondary treatment and chlorination with removal efficiencies of 78% and 67%, respectively[44]. Li and Zhang[45] reported removals of 24-36% at two plants while higher removals (67.9-100%) were reported by Karthikeyan and Meyer[9] and four Taiwanese WWTPs (66-90%) by Lin et al[35]. TCs are reported to interact strongly with clay, nature organic matter and metal oxides by cation exchange, surface complexation/cation, bridging hydrophobic partitioning, and electron donor–acceptor interactions[45,46]. Besides, other processes such as hydrolysis, epimerization and photolysis may also contribute a certain degree to the degradation of this class of antibiotics in wastewater[47,48]. So, the higher removal efficiency of TCs was compared to others antibiotics. To metabolites of TCs, the negative removal efficiency was found such as ICTC. One of the possible reasons for these findings is that CTC was transformed to ICTC during the wastewater treatment process.

Removal efficiencies for antibiotics appear to vary with wastewater treatment plants, affected by their operations, geographic locations, and environmental factors. The main operational factors that can influence the biological removal of antibiotic residues in wastewater treatment are biochemical oxygen demand (BOD5), existence and size of anoxic and anaerobic compartments, suspended solids (SS) loading, hydraulic retention time (HRT), sludge retention time (SRT), food/microorganism ratio (F/M ratio), mixed liquorsuspended solids (MLSS), pH, temperature of the raw sewage and the plant's configuration[24, 32, 49,50]. In this study, there are differences in treatment process and operational factors of five WWTPs. So the removal of antibiotics was also diversity in five studied WWTPs.

4. Conclusion

Out of 31 antibiotics and their metabolites, in summer 16 antibiotics and 6 metabolites were found in the influents and effluents at the five studied WWTPs, while in winter 14 antibiotics and 6 metabolites were detected. The most frequently detected antibiotics were SD, SMZ, ASD, ASMZ, OF, ROX, CEFM and CEFD. The concentrations of antibiotics are higher in winter than that in summer. The highest

level of total antibiotics was found in Longyan City WWTPs, where there are more population and swine farms. The removal of antibiotics by the five studied WWTPs is incomplete. TCs were removed relatively more efficiently compared to other studied antibiotics. As for the occurrence and removal of antibiotics from wastewater at the five studied WWTPs, these were found to be similar to reports on other WWTPs. So, antibiotics from studied WWTPs maybe be one of the important reasons in environment of this region.

Acknowledgements: This work is financially supported by International Cooperation of Ministry Science and Technology of China (2011DFB91710) and Natural Science Foundation of Fujian Province, China (Y1F0511E0, 2013R01020064).

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474 Table1 The target compounds and abbreviation

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Class	Compound	Abbreviation
Sulfonamides (SAs)	Sulfadiazine	SD
	Sulfadimethoxine	SDM
	Sulfamerazine	SM1
	Sulfameter	SMT
	Sulfamethazine	SM2
	Sulfamethoxazole	SMZ
Metabolites of Sulfonamide (MSAs)	n-acetyl sulfamethazine	ASM
	n-acetyl sulfadiazine	ASD
	n-acetyl sulfamethoxazole	ASMZ
Quninolones (QNs)	Ciprofloxacin hydrochloride	CIP
	Norfloxacin	NOF
	Sarafloxacin hydrochloride	SARA
	Ofloxacin	OF
	Danofloxacin mesylate	DALA
	Enrofloxacin	ENX
Macrolide (MLs)	Roxithromycin	ROX
	Erythromycin	ERY
β-Lactams (β-Ls)	Cephalexin monohydrate	CEFM
	Cephradine	CEFD
Tetracyclines (TCs)	Tetracycline	TC
	Oxytetracycline	OTC
	Chlorotetracycline	CTC
	Methacycline	MTC
	Doxycycline	DXC
Metabolites of Tetracyclines (MTCs)	Anhydrotetracycline	ATC
	4-epitetracycline	ETC
	4-epi-anhydrotetracycline	EATC
	α-apo-oxytetracycline	α-OTC
	4-epioxytetracycline	EOTC
	Isochlortetracycline	ICTC
	Demeclocycline	DMCTC

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480 Table 2 Five WWTPs information

Sequence	City	WWTPs	Treatment techniques	Sewage source	Treatment capacity [a] (10^4 m^3/d)	Population (10^4)
1	Longyan	S1	A2/O	dom	13.1	30
2	Zhangping	S2	A2/O	dom	1.5	5
3	Hua'an	S3	A2/O	dom	0.8	3
4	Changtai	S4	OD	dom	1.3	5
5	Zhangzhou	S5	A/B	dom	6.7	18

481 A2/O represents Anaerobic-Anoxic-Oxic

482 A/B represents Adsorption Bio- degradation

483 OD represents Oxidation Ditch

484 dom represents domestic

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497 Table 3 Main parameters of analytical method

Compounds	Recovery (%)(n=3)[a]	LOD [b](ng/L)	LOQ [c](ng/L)
SD	88.8±2.8	0.50	0.8
SDM	40.6±3.6	0.27	0.91
SM1	59±3.0	0.25	0.83
SMT	53.5±3.5	0.27	0.5
SM2	70.1±4.5	0.27	0.91
SMZ	50.7±2.3	0.04	0.14
ASM	92.3±1.3	0.67	1.18
ASD	70.0±2.4	0.50	0.87
ASMZ	73.7±5.1	0.20	0.7
CIP	120.9±1.2	5.0	8.7
NOF	78.4±7.6	5.0	10
SARA	65.8±3.0	1.0	3.22
OF	52.9±8.6	0.67	2.56
DALA	125±2.1	4.3	13.3
ENX	72.2±3.4	1.5	3.91
ROX	59.0±4.1	0.33	1.33
ERY	102±2.5	5.0	15.4
CEFM	89.4±4.7	5.0	11.1
CEFD	94.1±4.2	5.0	8.7
TC	118±5.6	0.67	2
OTC	126±2.5	0.32	1
CTC	64.2±7.0	2 .0	8
ATC	66.6±1.8	12	40
MTC	112±3.7	5.0	10
ETC	109±5.1	1.82	6.4
EATC	64.2±4.3	5.0	13. 9
DXC	120±3.6	3.0	10
α-OTC	65.7±5.1	4.2	13.7
EOTC	125±4.2	0.67	2
ICTC	124±7.1	1.0	3.35
DMCTC	97.9±8.2	5.0	32.3

498 [a] Recovery –at 100 ng/L
499 [b] LOD--limit of detection
500 [c] LOQ--limit of quantitation
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502 Table 4 Range of Concentration of antibiotics in WWTPs in summer （in nanograms per liter）

	Influent				Effluent			
	Range	Mean	Median	Frequency(%)	Range	Mean	Median	Frequency(%)
SD	2.30-36.0	18.9	20.4	100	1.46-28.4	14.1	15.8	100
SM2	ND-251	60.7	13.1	80	ND-184	46.2	11.2	80
SMZ	5.41-14.5	44.5	32.9	100	2.96-137	42.7	28.5	100
ASM	8.50-51.5	22.6	9.36	100	2.50-52.9	24.7	12.2	100
ASD	2.70-19.3	10.9	10.9	100	4.30-17.6	8.10	5.70	100
ASMZ	72.7-2312	125	96.0	100	38.7-162	136	129	100
CIP	ND-55.8	22.3	12.5	60	ND-49.8	16.5	0	40
NOF	ND-130	59. 7	34.8	80	13.1-172	74.5	56.0	100
OF	129-91.5	20.1	21.7	100	12.0-24.6	37.6	18.9	100
ENX	ND-11.0	2.20	0	20	ND-10.9	2.20	0	20
ROX	6.50-42.0	18.8	16.1	100	12.7-54.5	23.5	15.6	100
ERY	1.12-1.67	1.40	1.50	100	1.20-4.40	2.00	1.50	100
CEFM	54.1-155	114	139	100	25.0-116	76.7	98.5	100
CEFD	92.5-214	161	165	100	21.4-136	105	132	100
TC	ND-175	47.9	0	40	ND-34.8	10.9	0	40
OTC	ND-75.8	19.8	0	40	ND-37.8	14.2	11.9	60
CTC	ND-103	12.3	0	40	ND-39.6	30.8	0	40
DXC	ND-51.0	13.1	0	40	ND-87.3	17.5	0	20
ETC	ND-48.8	9.8	0	20	0	0	0	0
EOTC	ND-40.3	12.4	0	40	ND-37.4	14.1	11.9	60
ICTC	3.9-131	56.4	48.3	100	ND-310	107	86.9	80

503 ND represents not detectable (below limit of detection)

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513 Table 5 Range of Concentration of antibiotics in WWTPs in winter (in nanograms per liter)

	Influent				Effluent			
	Range	Mean	Median	Frequency(%)	Range	Mean	Median	Frequency(%)
SD	8.60-32.1	20.0	25.5	100	3.60-26.3	16.8	22.7	100
SM2	5.30-259	67.0	31.0	100	ND-233	62.3	31.6	80
SMZ	13.5-152	59.3	46.8	100	11.4-145	52.1	34.5	100
ASM	4.00-43.5	19.3	12.2	100	5.0-39.8	17.2	6.70	100
ASD	ND-51.4	21.6	22.7	80	ND-51.4	21.6	22.7	80
ASMZ	82.7-299	149	136	100	88.7-208	131	123	100
CIP	ND-15. 5	7.50	9.50	60	ND-13.0	6.50	9.40	60
NOF	ND-46.2	16.5	12.2	60	ND-13.0	11.6	0	40
OF	6.0-39.5	23.0	17.7	100	5.70-53.4	35.1	38.2	100
ENX	ND-16.8	9.80	10.5	80	ND-18.5	10.5	10.9	80
ROX	29.7-63.7	41.5	35.9	100	59.3-104	78.2	71.0	100
CEFM	33.4-822	240	133	100	65.2-187	142	147	100
CEFD	131.0-11850	460	357	100	155.7-369	304	353	100
TC	ND-124	45.2	16.5	60	ND-101	41.0	19.9	60
OTC	ND-167	64.1	6.70	60	ND-178	65.1	7.10	80
CTC	ND-261	83. 7	35.3	60	ND-154	57.7	24.9	60
DXC	17.3-36.4	26.4	25.5	100	ND-36.2	14.0	16.1	60
ETC	ND-87.7	21.8	ND	40	ND-82.1	23.0	9.70	60
EOTC	ND-287	81.2	6.30	60	ND-296	81.5	5.60	60
ICTC	ND-233	98.6	31.0	80	ND-651	250	105	80

514 ND represents not detectable (below limit of detection)

Table 6 Removal efficiency (%) of antibiotics in five WWTPs

	S1		S2		S3		S4		S5		Max	Min	Mean
	summer	winter	summer	winter	summer	winter	summer	winter	summer	winter			
SD	21.0	11.0	22.4	11.1	36.2	33.1	59.4	32.2	24.9	17.9	59.4	11.0	26.9
SM2	-2.80	-1.80	14.3	5.70		100	39.6	-180	26.9	10.1	100	-180	-6.20
SMZ	-19.7	4.60	-40.4	26.3	84.6	43.0	44.8	15.6	19.9	8.70	84.6	-40.4	19.3
ASM	5.40	-23.4	30.9	-29.0	-233	71.4	0.100	2.10	-2.80	8.6	71.4	-233	-17.0
ASD	60.9		19.7	100	33.8	100	23.0	21.1	8.60	63.2	100	8.60	47.8
ASMZ	28.1	15.8	-141	19.1	33.6	30.3	-123	-7.30	1.90	-51.2	33.6	-141	-19.4
CIP	10.6	20.1			100	0.600			25.0	15.8	100	25.0	28.7
NOF	-32.3	22.2	-61.2	100	-1.60				2.2	15.1	100	-61.2	6.40
OF	-272	-14.8	-20.8	-137	-19.3	-84.8	7.10	4.90	-132	-48.6	7.10	-272	-71.6
DALA	10.2												10.2
ENX	0.700	6.30		5.60		3.70				9.4	9.4	0.700	5.10
ROX	-20.1	-63.0	48.5	-224	22.9	-97.8	16.2	-82.1	3.10	-32.7	48.5	-224	-42.9
ERY	11.4		2.80		61.7		37.4		7.50		61.7	2.80	24.1
CEFM	32.9	-79.4	33.9	15.3	22.5	-18.8	66.0	70.2	16.3	20.2	66.0	-79.4	17.9
CEFD	21.1	-79.4	36.4	15.3	38.4	-18.8	76.8	70.2	19.8	20.2	76.8	-79.4	20.0
TC	46.4	18.6	88.7	1.4		-20.4					88.7	-20.4	26.9
OTC	50.1	-6.3	8.40	8.5	100	-7.00					100	-7.00	25.6
CTC	61.4	40.9	57.0	10.5		29.4					61.4	10.5	39.8
DXC	41.5	0.7	100	47.7		100		14.5			100	14.5	50.7
ETC	100	6.30		-8.7							100	-8.70	32.5
EOTC	7.40	2.80	0.100	5.4	100			100		100	100	0.100	45.1
ICTC	57.6	64.2	56.1	52.8	100	84.3	38.3	30.8	22.0		100	22.0	56.3

Figures for

Seasonal variation of antibiotics and its metabolites in wastewater treatment plants, Jiulongjiang River Basin, South China

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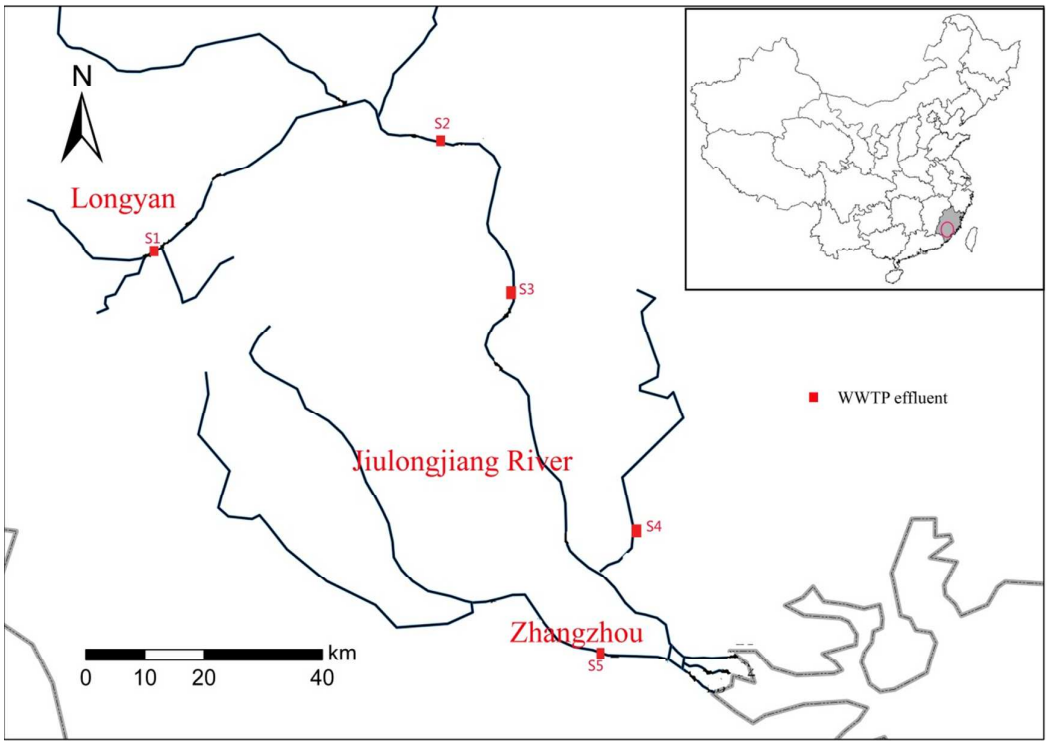
Captions

Fig.1 Sampling locations on the Jiulongjiang River Region of Wastewater Treatment Plant

Fig.2 Seasonal variation and spatial distribution of antibiotics in five WWTPs of Jiulongjiang River Region.



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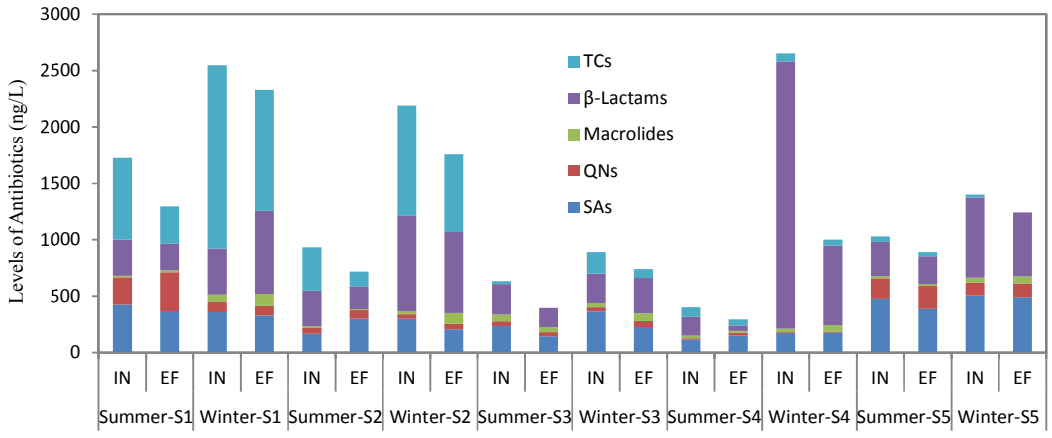

Fig.2 Seasonal variation and spatial distribution of antibiotics in five WWTPs of Jiulongjiang River Region.