Occurrence, distribution and potential affecting factors of antibiotics in sewage sludge of wastewater treatment plants in China

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HIGHLIGHTS

- High level of quinolones in sludge indicated that antibiotics widely existed in China.
- Antibiotic levels in sewage sludge depend on wastewater characteristics.
- TOC could affect the sludge adsorption capability to the antibiotics to some extent.
- Antibiotic levels in sewage sludge increased with longer solid retention time.

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ABSTRACT

The occurrence and distribution of eight quinolones, nine sulfonamides, and five macrolides were investigated in sewage sludge from 45 wastewater treatment plants in 23 cities in China. Among all the antibiotics considered, quinolones were the dominant antibiotics detected in all samples [total concentrations up to 8905 µg/kg, dry weight (dw)], followed by macrolides (85.1 µg/kg, dw), and sulfonamides (22.7 µg/kg, dw). High concentrations of quinolones in sewage sludge indicated that antibiotics are widely used and extensive pollutants in China. Significant differences were observed for the total concentrations of antibiotics in sludge samples among the 45 WWTPs. To evaluate the potential factors affecting the antibiotic levels in sewage sludge, wastewater and sludge characteristics, as well as the operational conditions and treatment techniques in WWTPs were investigated. The results indicated that the antibiotic levels in sewage sludge depend to a great extent on wastewater characteristics. Significant correlation between total organic carbon (TOC) and total concentrations of antibiotics was also found in studied WWTPs, indicating that TOC could affect the sludge adsorption capability to the antibiotics to some extent. Moreover, the relation between treatment techniques and the total concentrations of antibiotics in sludge showed that antibiotic levels in sludge increased with longer solid retention time.

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1. Introduction

Antibiotics, an emerging group of environmental contaminant, are widely used as human medicine and veterinary drugs to prevent or treat infective diseases, and as growth promoter in livestock farming, aquaculture and agriculture (Gao et al., 2012; Zuccato et al., 2010). Many antibiotics administered to humans and animals cannot be completely absorbed or metabolized in the body, and most of them were directly discharged into the sewage system through excretion (Bendz et al., 2005; Carballa et al., 2005).

Municipal wastewater systems are a major pathway for the disposal of antibiotics, and the concentrations measured in wastewater treatment plant (WWTP) influents can reflect the levels of antibiotics in its serving region (Al-Rifai et al., 2011; Jelić et al., 2009). In WWTPs,

antibiotics were removed mainly through sorption and biodegradation by the activated sludge (Hyland et al., 2012; Jelic et al., 2011; Maeng et al., 2011; Yang et al., 2012). For some antibiotics, especially hydrophobic antibiotics, with their limited mobility and low biodegradation in the sludge, they are prone to adsorb onto sewage sludge and more stable than those in wastewater (Baquero et al., 2008; Cruz Moreno-Bondi et al., 2009; Zhou et al., 2009). Several studies have reported that many antibiotics in wastewater were considerably eliminated by sorption and transported to sewage sludge during sewage treatment (Batt et al., 2007; Beausse, 2004; Escher et al., 2011; Hörsing et al., 2011), indicating that sludge can serve as an important reservoir of antibiotics. Therefore, concentrations of antibiotics in sewage sludge can give an important indication on their pollution levels to some extent. In addition, it has been recognized that the disposal of sludge, such as agricultural application and land-fill, can potentially release the antibiotics into the environment. Peng et al. (2011) have reported that about 22,800 kg fluoroquinolones

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may enter the terrestrial environment via disposal of sewage sludge in Guangzhou in 2007. Once they enter the environment, antibiotics may pose potential risks to human and health ecosystems (Sapkota et al., 2008; Wu et al., 2010). Thus, the occurrence and fate of antibiotics in sewage sludge should be emphasized.

So far, extensive studies have been conducted on the occurrence of antibiotics in the influent and effluent of WWTPs (Gulkowska et al., 2008; Karthikeyan and Meyer, 2006; Zorita et al., 2009), while only limited information was available on the occurrence and distribution patterns of antibiotics in sludge mainly due to its high complex matrix (Jelic et al., 2011; Kümmerer, 2009). It was reported that the concentrations of antibiotics ranged from $\mu\text{g/kg}$ to a few mg/kg in sewage sludge from different countries, such as Estonian (Lillenberg et al., 2009), Italy (Zuccato et al., 2010), Spain (Nieto et al., 2010; Radjenović et al., 2009), Switzerland (Göbel et al., 2005), USA (Kinney et al., 2006), Japan (Okuda et al., 2009) and China (Xu et al., 2007). In China, there are over 30 million tons of sewage sludge (moisture content: 80%) generated in 2011, and 75–85% of them cannot be safely disposed (Zou and Wang, 2012). Moreover, high levels of antibiotics have been found in sewage sludge, in which the concentration was up to several mg/kg (Gao et al., 2012). Therefore, antibiotic contamination in sewage sludge has become a severe problem in China, and it is necessary to monitor their concentrations on a large scale. The aim of this study was to investigate the occurrence and distribution of 22 common antibiotics in the sewage sludge in China, and to assess potential factors affecting the antibiotic levels in sewage sludge in 45 WWTPs. We hope that this study would provide useful information to indicate the antibiotic pollution levels in China. As far as we know, this is the first study demonstrating the ubiquitous occurrence of antibiotics in China.

2. Materials and methods

2.1. Standards and reagents

HPLC-grade methanol and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA, USA); formic acid (98%) was purchased from Fluka; ammonium formate (99%) and ammonium hydroxide (v/v, 50%) were purchased from Alfa Aesar. All purity water ($>18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) was prepared with Milli-Q Advantage A10 system (Millipore, USA).

Oxfloxacin (OFL, 99.9%), norfloxacin (NOR, 99.9%), ciprofloxacin (CIP, 99.9%), sarafloxacin (SAR, 95.0%), fleroxacin (FLE, 99.5%), lomefloxacin (LOM, 98.0%), difloxacin (DIF, 98.0%), enrofloxacin (ENR, 99.9%), sulfadiazine (SDZ, 99.7%), sulfamerazine (SMR, 99.9%), sulfadimethoxine (SDM, 99.4%), sulfisoxazole (SIA, 99.0%), sulfamonomethoxine (SMM, 99.0%), erythromycin (ERY, 99.1%), roxithromycin (ROX, 90.0%), josamycin (JOS, 98.0%), tylosin (TYL, 82.4%), and spiramycin (SPI, 88.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfathiazole (STZ, 99.0%), sulfapyridine (SPD, 99.0%), sulfamethoxazole (SMX, 99.0%), and sulfamethazine (SMZ, 99.0%) were purchased from KaSei Industry Co., Ltd. (Tokyo, Japan).

The following isotopically labeled compounds were used as surrogates standards (100.0 $\mu\text{g/L}$ in methanol): ofloxacin- d_3 (OFL- d_3), norfloxacin- d_5 (NOR- d_5) and sarafloxacin- d_8 (SAR- d_8) that were purchased from Sigma-Aldrich (St. Louis, MO, USA); and sulfamethoxazole- d_4 (SMX- d_4), sulfamethazine- d_4 (SMZ- d_4), spiramycin I- d_3 (SPI I- d_3), and erythromycin- $^{13}\text{C}_3$ (ERY- $^{13}\text{C}_3$) that were purchased from Toronto Research Chemicals (Oakville, ON, Canada).

2.2. Sample collection

The sampling map and locations are shown in Fig. 1. A total of 45 sewage sludge samples were collected from different WWTPs in 23 cities in China during the period of October 2010 to March 2011.

Considering the impact of precipitation on the antibiotic concentrations, the sampling campaign was carried out during the dry season to avoid the dilution effects of precipitation. The selected cities cover 13 economically developed provinces in China. Details of each WWTP about treatment technique, sewage sources, treatment capacity, actual daily loads and serving population are supplied in Table S1.

All sludge samples were collected in polyethylene bags. They were centrifuged and immediately freeze-dried after being delivered to the laboratory. The sludge was ground and sieved to smaller than 0.44 mm, and then stored at -20°C until analysis.

2.3. Sample preparation and analysis

The total organic carbon (TOC) content was measured as CO_2 on acid treated samples with a Solid TOC Analyzer (O.I. Analytical Co., USA). TOC concentrations were calculated by the difference between total carbon and inorganic carbon. Chemical analysis was performed following the EPA method 1694 with some modifications (EPA, 2007). Target antibiotics from sludge samples were extracted with an ASE 350 pressurized liquid extraction (PLE) system (Dionex, Sunnyvale, CA, USA), and then purified using AutoTrace SPE 280 (Dionex, USA) with an Oasis HLB cartridge (6 cm^3 , 200 mg; Waters, USA).

A total of 0.1 g pretreated lyophilized sample was spiked with 20 ng of surrogate standard (ofloxacin- d_3 , norfloxacin- d_5 , sarafloxacin- d_8 , sulfamethoxazole- d_4 , sulfamethazine- d_4 , spiramycin I- d_3 and erythromycin- $^{13}\text{C}_3$) before being thoroughly mixed with 3 g of EDTA-washed diatomite from Dionex (Sunnyvale, CA, USA). The mixture was placed into a 10-mL stainless steel extraction cell with 100% methanol as the extracting solvent. The extraction conditions were as follows: extraction temperature, 70°C ; extraction pressure, 10.34 MPa; preheating period, 5 min; static extraction, 10 min; final extraction volume, 60 mL; flush volume, 60% of the cell volume; nitrogen purge, 100 s; and number of extraction cycles, 2.

After extraction, 25 mL extract was transferred to a 100-mL round-bottom flask, and then concentrated with an RE-2000 rotary evaporator (Yarong, Shanghai, China) at 37°C and 0.08 MPa to a final volume of about 1 mL. The extract was immediately transferred to a 100-mL conical tube and diluted to a final volume of 100 mL with DI water.

The SPE procedure was performed on an AutoTrace SPE 280 system (Dionex, USA). The HLB cartridges were conditioned with 5 mL methanol and 5 mL DI water. The 100-mL diluted extract was passed through the cartridge at a flow rate of 2 mL/min. After all extracts were loaded, cartridges were washed with 10 mL DI water, and then dried under a nitrogen stream for 20 min. Finally, the analytes were eluted with 6 mL of methanol containing 5% ammonium hydroxide. The eluate was concentrated to 1 mL under a gentle stream of nitrogen at 35°C before being dissolved with 1 mL of a mixture of methanol/aqueous solution of 0.05% formic acid (10:90 v/v). Then the extract was centrifuged for 5 min at 12,000 rpm. Finally, the supernatant was filtered through a 0.22- μm nylon membrane, and an aliquot (15 μL) of the filtered supernatant was injected into the HPLC-ESI MS/MS system for analysis.

2.4. Quantitation and quality control

The concentrations were determined by an internal standard method. The calibration curve was prepared within a wide range of concentrations (0.01–500 $\mu\text{g/L}$) to reveal strong linearity ($r^2 > 0.99$). The limits of detections (LODs) and recoveries for sludge are listed in Table S2. LODs were determined as the amounts for which signal-to-noise ratios (S/N) were higher than 3.

By measuring the concentrations before and after 1 month's storage in the dark at -20°C , most antibiotics have no or small degradation loss, except erythromycin (10.7%). Field blanks and procedural blanks were analyzed with extraction to control travel contamination

Fig. 1. Sampling locations of municipal sewage sludge samples in China.

and laboratory contamination, and all equipments and containers were rinsed with methanol and water before use to avoid analytical interference and/or cross contamination. A 10 µg/L standard was set as the quality control concentration, which was checked every 10 injections to ensure analysis stability and to verify calibration. The calibration curve was used for quantification only when the quality control standard was within 20% of its initial value. Detailed information was listed in Table S3 in the Supplementary materials.

2.5. Statistical analysis

All statistical analyses were performed with IBM PASW Statistics 20.0 (IBM Corp., 1989–2011). Comparisons of concentrations were performed using Two-Sample Kolmogorov–Smirnov nonparametric tests. It was considered as statistically significant difference when $p < 0.05$ for this test. PCA on the analytical data was executed in order to obtain a visual representation of the main characteristics, and the principal components (PCs) were extracted for eigenvalues that were greater than 1.

3. Results and discussion

3.1. Concentrations of antibiotics in sewage sludge

The dry-weight-based concentrations of 22 antibiotics in sewage sludge are summarized in Table 1. The concentrations of individual quinolones, macrolides, and sulfonamides are illustrated by box-and-whisker plots in Fig. 2a, b, and c, respectively. A total of 18 antibiotics, including seven quinolones (NOR, CIP, ENR, FLE, OFL, LOM, and SAR), six sulfonamides (STZ, SMX, SPD, SMZ, SDZ, and SMM), and five macrolides (SPI, JOS, TYL, ROX and ERY), were detectable in the 45 sludge samples. Concentrations of $\sum$ QNs, $\sum$ MCs and $\sum$ SAs ranged

from 124 to 29,647 µg/kg [mean 8905 µg/kg, dry weight (dw)], from 0.34 to 392.56 µg/kg (mean 85.1 µg/kg, dw) and from BDL to 75.6 µg/kg (mean 22.7 µg/kg, dw) respectively.

As illustrated in Fig. S1, the sewage sludge samples from 45 WWTPs showed very similar composition profiles of antibiotics, indicating their universal usages and extensive pollution in China. Composition analysis also indicated that the predominant antibiotics in sludge samples were quinolones, which accounted in average for

Table 1
Concentrations of targeted antibiotics in sludge samples (µg/kg, dw).

Groups	Analytes	Minimum	Maximum	Mean	Frequency (%)
Quinolones	NOR	75.5	21,335	5399	100
	CIP	0.00	926	285	97.8
	DIF	0.00	0.00	0.00	0.00
	ENR	0.00	41.9	10.5	95.6
	FLE	0.00	1840	407	97.8
	OFL	33.9	7788	2686	100
	LOM	0.00	502	118	97.8
	SAR	0.00	14.6	0.87	6.67
Sulfonamides	STZ	0.00	0.75	0.02	2.22
	SMX	0.00	17.0	3.90	71.1
	SIA	0.00	0.00	0.00	0.00
	SPD	0.00	47.7	8.57	91.1
	SDM	0.00	0.00	0.00	0.00
	SMZ	0.00	11.7	0.72	64.4
	SDZ	0.00	51.9	8.95	75.6
	SMR	0.00	0.00	0.00	0.00
Macrolides	SMM	0.00	11.1	0.58	17.8
	SPI	0.00	13.3	3.64	62.2
	JOS	0.00	6.30	0.60	11.1
	TYL	0.00	33.8	0.89	6.67
	ROX	0.00	342	69.6	95.6
	ERY	0.00	55.8	10.4	97.8

Fig. 2. Box-and-whisker plots of antibiotics in sludge samples collected from 45 WWTPs in China. The horizontal black line in the box represented the median value and the low and upper edges of the box mark the 25th and 75th percentiles. The whiskers extending from the box show the highest and lowest values. “★” represents extreme values, which were beyond the triplication of the difference between 25th and 75th percentiles, and singular values are represented by “O”, which were beyond the 150th percentile of the difference between 25th and 75th percentiles.

98.80% of the total antibiotics. In contrast, the other two groups of antibiotics, macrolides (0.94%) and sulfonamides (0.25%), contributed little to the total antibiotics. In general, the concentration of antibiotics in sludge mainly depends on the solid–water distribution coefficient (K_d) (Baquero et al., 2008). It has been reported that K_d s of quinolones were relatively high (Okuda et al., 2009), which were up to two or three orders of magnitude higher than those of macrolides and sulfonamides (Li et al., 2012). Compared with macrolides and sulfonamides, quinolones showed high persistence in sludge due to their strong adsorption onto sewage sludge by chelating with cations and binding with particulate matter, which delay their degradation

(Cruz Moreno-Bondi et al., 2009; Robinson et al., 2005; Tamtam et al., 2008).

3.1.1. Quinolones

NOR (mean 5399 $\mu\text{g/kg}$) and OFL (mean 2686 $\mu\text{g/kg}$), two quinolones widely used in hospital in China, were detected in all sludge samples. FLE (mean 407 $\mu\text{g/kg}$), CIP (mean 285 $\mu\text{g/kg}$), LOM (mean 118 $\mu\text{g/kg}$) and ENR (mean 10.5 $\mu\text{g/kg}$) were also frequently detected in most samples (95.6–97.8%). It should be noted that, FLE, and LOM, which had not been reported in sludge before, were also detected at relative high levels in the sludge samples in the present study. The mean concentration of SAR was below 1 $\mu\text{g/kg}$, while DIF was not detected in any sludge sample.

The concentrations of quinolones detected in the sludge in this study were comparable to those previously reported in Japan (CIP: mean 243 $\mu\text{g/kg}$) (Okuda et al., 2009). However, the concentrations of some quinolones in the sludge were at least ten times higher than those in Hong Kong (OFL: 835 $\mu\text{g/kg}$; NOR: 372 $\mu\text{g/kg}$) (Xu et al., 2007), Japan (NOR: 87 $\mu\text{g/kg}$) (Okuda et al., 2009), Estonian (OFL: 4–10.9 $\mu\text{g/kg}$; NOR: 20.8–25.0 $\mu\text{g/kg}$; CIP: 32.8–35.5 $\mu\text{g/kg}$) (Lillenberg et al., 2009), Italy (OFL: 3.4 $\mu\text{g/kg}$) (Zuccato et al., 2010) and Spain (OFL: 8.4 $\mu\text{g/kg}$) (Radjenović et al., 2009), indicating that the levels of quinolone pollution in China were relatively high.

3.1.2. Macrolides

The concentrations of macrolides in sludge were much lower than those of quinolones. ROX, ERY and SPI were frequently detected in sludge samples with the mean concentration of 69.6, 10.4 $\mu\text{g/kg}$, and 3.64 $\mu\text{g/kg}$, respectively. This may be attributed to their high consumption in China (Chang et al., 2010). As for the other two macrolides, TYL and JOS, showed very rare detection and low concentration in sludge samples.

The concentrations of macrolides detected in the sludge in this study were lower than those previously reported in Hong Kong (ERY: 63 $\mu\text{g/kg}$) (Xu et al., 2007), USA (ERY: 41 $\mu\text{g/kg}$) (Kinney et al., 2006) and Spain (ERY: 62.5 $\mu\text{g/kg}$; TYL: 1074 $\mu\text{g/kg}$) (Radjenović et al., 2009), indicating that the levels of macrolides pollution in this study were relatively low.

3.1.3. Sulfonamides

As shown in Table 1, no sulfonamide possessed the mean concentration over 10 $\mu\text{g/kg}$. Similar low concentration of these antibiotics was also reported to be present in sewage sludge samples from various WWTPs in Estonian (Lillenberg et al., 2009), Spain (Nieto et al., 2010), and Japan (Okuda et al., 2009). This may be due to the fact that sulfonamides appeared to have little sorption affinities to sludge, as was evidenced by their low values of K_d s (Okuda et al., 2009). In addition, sulfonamides are primarily used for animals (Chang et al., 2010; Malintan and Mohd, 2006), and only a small percentage of them are used for human medicine. Therefore, the concentrations of sulfonamides in sludge were much lower than those of quinolones and macrolides.

3.2. Comparison of different WWTPs

The concentrations for total antibiotics in different WWTPs are summarized in Fig. S2. The highest level of total antibiotics (29,825 $\mu\text{g/kg}$) was found at Xianyang in Shanxi Province, while the lowest level (125 $\mu\text{g/kg}$) was found at Ningbo in Zhejiang province (Table 2). In general, significant differences were observed for the total concentrations of antibiotics in sludge samples among the 45 WWTPs ($p < 0.05$).

In order to assess the potential factors affecting the antibiotic levels in sewage sludge, related information, such as characteristics of sewage (domestic, industrial, or combined) and sludge (total organic carbon), and the design and operational conditions of WWTPs

(treatment technique, treatment capacity, processing volume, and serving population)(Table S3) were investigated.

3.2.1. Composition of the sewage

In general, domestic sewage and industrial wastewater were the main sources of WWTPs (Díaz-Cruz et al., 2009; Nieto et al., 2010). Among the 45 WWTPs investigated, 32 WWTPs were fed with mix of domestic sewage and industrial wastewater, 12 WWTPs were fed with domestic sewage, and only one was fed with industrial wastewater taken from an electroplating factory. As shown in Fig. S2, the lowest antibiotic levels in the sludge samples were detected in WWTP NB-3, which was only fed with industrial wastewater. We also found that the antibiotic levels detected in WWTPs fed with combined water (mean 10,162 µg/kg) were lower than those in WWTPs fed with domestic water (mean 13,788 µg/kg). Previous studies have reported that domestic sewage contains a vast majority of antibiotics from excretion of human drugs and release of hospital wastes (Ellis, 2006; Xu et al., 2007), which may transport to sewage sludge by sorption during sewage treatment. However, without the direct entry of antibiotics, it is assumed that industrial wastewater (excluding pharmaceutical wastewater) contains little or only a small quantity of antibiotics. As mentioned above, it can be concluded that the concentrations of antibiotics present in sludge samples

probably associated with the different sources of the wastewater in the studied WWTPs.

3.2.2. TOC in the sewage sludge

Total organic carbon is usually considered as one of the most important properties of solid environmental matrices (soil, sediment and sludge), because it may influence other physical, chemical, and biological characteristics. It has been reported that the adsorptions of some antibiotics, such as tetracycline and quinolones, are related to organic carbon content of the soil and sediment (Jia et al., 2012; Pomati et al., 2004; Zhang et al., 2011; Zhou et al., 2011). Therefore, the contents of total organic carbon (TOC, %) in sludge samples were also investigated to assess the potential influence on the antibiotic levels. As shown in Table S1, the TOC contents of 45 sludge samples varied from 7.05% to 45.0%. The Spearman's coefficients of the concentrations of antibiotics and TOC of the sludge are present in Table 3. Significant correlation between TOC and total concentrations of antibiotics was found in these samples ($R=0.481$, $P<0.01$), suggesting that antibiotics in sludge were significantly dependent on the sludge organic content. This may be related to their high potential to bind with organic matter in sewage sludge. Since the antibiotic concentrations in sludge increased with the increasing TOC contents, it can be assumed that TOC could affect the sludge adsorption capability to the antibiotics to some extent.

3.2.3. Treatment capacity, processing volume, and serving population

As the main design and operating parameters of WWTPs, treatment capacity, processing volume, and serving population were investigated to assess the pollutants of sewage sludge. This study showed that total concentrations of antibiotics have no significant relationships with the individual parameters ($p>0.05$). Similar results were also found in previous studies that the levels of targeted analytes, such as short chain chlorinated paraffins, and benzotriazole UV stabilizers, were not related to these WWTP characteristics (Ruan et al., 2012; Zeng et al., 2012). However, significant correlation between the total concentrations of antibiotics and the ratio of processing volume to serving population was found in this study ($R=0.333$, $P<0.05$) (Table 3). This provides a general indication that the levels of antibiotics in sludge tend to be higher in those WWTPs that have higher daily loading per capita (m^3 per person and day). It should be noted that no such relationships were found in other studies. This may be explained by that the ratio of processing volume to serving population could factually reflect the actual working conditions of the studied WWTPs. Regarding information on such factors is rather scarce, the relation between the levels of antibiotics and these factors needs further investigations.

3.2.4. Treatment technique

Due to their reasonable operating and maintenance costs, wastewater treatment systems that use activated sludge processes have been employed extensively throughout the world. A previous study has shown that concentrations of antibiotics in influents and effluents of WWTP vary significantly depending on the types of wastewater treatment technique (Yu and Chu, 2009). Table S1 summarizes the four main treatment techniques of the investigated WWTPs, which were mainly grouped into oxidation ditch (OD), sequencing batch reactor (SBR), anoxic/oxic (AO), and anaerobic/anoxic/oxic (AAO) activated sludge processes. Principal component analysis (PCA) was performed to assess the characteristics of antibiotics in sewage sludge of WWTPs with different treatment techniques. Six quinolones (NOR, CIP, ENR, FLE, OFL and LOM), three sulfonamides (SMX, SPD and SDZ), and three macrolides (SPI, ROX and ERY), whose detectable rates were over 60% and mean concentrations were above 1 µg/kg, were selected in this analysis. The varimax rotation reduced all variables to three principal components (PCs) with eigenvalues greater than 1, which represent 70.06% of the cumulative variances. Figs. 3 and 4

Table 2
Concentrations of targeted antibiotics in 45 WWTPs (µg/kg, dw).

NO.	Provinces	Cities	WWTPs	QNs	SAs	MCs	Total
1	Beijing	Beijing	BJ-1	989	22.0	22.0	1033
2			BJ-2	4207	30.1	46.7	4284
3			BJ-3	8355	44.6	208.7	8608
4			BJ-4	5112	48.2	42.4	5202
5			BJ-5	10,096	58.6	40.9	10,195
6			BJ-6	8740	75.6	32.7	8848
7			BJ-7	5022	34.0	39.5	5096
8			BJ-8	1780	7.70	20.7	1808
9	Shangdong	Dezhou	DZ-1	350	1.22	13.9	365
10			DZ-2	1890	0.14	2.54	1893
11		Laiwu	LW-1	875	1.10	10.9	887
12			LW-2	2241	1.27	9.67	2252
13		Jining	JN-1	2284	7.67	140.4	2432
14			JN-2	1321	26.9	54.1	1402
15		Jining	JN-3	1923	22.9	392.6	2339
16		Linyi	LY-1	7478	16.7	46.7	7542
17			LY-2	7860	9.18	27.0	7896
18		Qingdao	QD-1	10,248	14.7	37.7	10,300
19			QD-2	15,144	10.7	100.0	15,255
20		Yantai	YT-1	8431	8.04	44.1	8483
21			YT-2	8332	29.6	313.5	8675
22		Rizhao	RZ	9800	22.9	190.7	10,013
23		Dongying	DY	5633	6.95	28.5	5669
24		Shanghai	SH	19,703	57.1	51.0	19,811
25	Zhejiang	Hangzhou	HZ-1	12,300	15.2	25.8	12,341
26			HZ-2	10,701	5.54	68.1	10,775
27		Ningbo	NB-1	639	0.00	5.89	645
28			NB-2	3762	11.8	29.2	3803
29	Anhui	Hefei	NB-3	124	0.00	0.34	125
30			HF-1	17,386	31.7	57.2	17,475
31			HF-2	9060	39.3	61.7	9161
32			HF-3	15,509	18.8	76.1	15,604
33	Hebei	Baoding	BD	1182	2.48	95.2	1280
34	Henan	Xinxiang	XX	13,683	20.0	85.6	13,788
35		Puyang	PY	11,444	26.6	102.6	11,573
36	Hubei	Wuhan	WH-1	5894	6.31	52.8	5953
37			WH-2	8397	14.5	41.3	8453
38	Hunan	Changsha	CS-1	4209	0.63	79.4	4289
39			CS-2	4717	0.39	48.3	4765
40	Guangdong	Zhongshan	ZS	18,096	12.4	129.9	18,238
41	Shanxi	Xianyang	XY	29,647	55.2	123.4	29,825
42		Xi'an	XA-1	27,043	42.0	288.0	27,373
43	Gansu	Lanzhou	XA-2	20,430	27.4	105.0	20,562
44			LZ	17,122	61.9	98.3	17,282
45	Heilongjiang	Jiamusi	JMS	21,587	73.6	339.6	22,000

Table 3

Pearson's correlation matrix for the concentrations of target antibiotics with parameters of 45 WWTPs and TOC in the sludge.

Parameters	QNs		SAs		MCs		Total	
	Pearson correlation	Sig. (2-tailed)	Pearson correlation	Sig. (2-tailed)	Pearson correlation	Sig. (2-tailed)	Pearson correlation	Sig. (2-tailed)
Treatment capacity (10^4 m ³ /d)	−0.045	0.772	0.150	0.331	−0.192	0.212	−0.047	0.764
Processing volume (10^4 m ³ /d)	−0.027	0.864	0.127	0.410	−0.169	0.272	−0.028	0.856
Population (10^4)	−0.068	0.659	0.039	0.802	−0.208	0.176	−0.070	0.649
Ratio ₁	0.175	0.251	0.348*	0.019	0.052	0.735	0.175	0.249
Ratio ₂	0.331*	0.026	0.393**	0.008	0.206	0.174	0.333*	0.026
TOC (%)	0.480**	0.001	0.661**	0.000	0.170	0.269	0.481**	0.001

Ratio₁ = Treatment capacity/Population; Ratio₂ = Processing volume/Population.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

illustrate the loading and score plots of the three PCs. As shown in the loading plot, PC1 accounted for 28.98% of the total variance and had strong positive loadings on NOR, SMX, SDZ, SPI, ROX, and ERY; PC2 accounted for as much as 23.61% of the total variance and had moderately positive loadings for CIP, OFL, LOM, and SPD; PC3 accounted for 17.47% of the total variance and had high correlation with ENR and FLE. The score plots of PCA in Fig. 4 were grouped by treatment techniques to visualize potential relationships. PCA results show that OD has higher loading values of PC1 and PC3 in the component plot, indicating higher concentrations of NOR, SMX, SDZ, SPI, ROX, ERY, ENR and FLE in these OD WWTPs, while SBR has higher loading values of PC2, suggesting higher level of CIP, OFL, LOM, and SPD in SBR WWTPs. In contrast, the other two treatment techniques, AAO and AO, show very low loading values of three PCs. This result indicated that low levels of these antibiotics were found in the two kinds of WWTPs.

The relation between treatment techniques and the total concentrations of antibiotics in sludge was also investigated. Fig. S3 showed that the average total concentrations of antibiotics treated by four activated sludge processes in the sludge samples were in the order of “OD (15,089 $\mu\text{g/kg}$) > SBR (11,217 $\mu\text{g/kg}$) > AAO (7453 $\mu\text{g/kg}$) > AO (4510 $\mu\text{g/kg}$)”. With the different operating parameters, the solid retention time (SRT) in the four types of treatment techniques was variable. In general, OD usually uses the extended aeration, which has a long SRT (20–40 days); Due to its big and batch reactor, SBR often operates with the SRT of 15–25 days; AAO usually operates with the SRT of 10–20 days; With the relatively simple process, AO possesses the relative short SRT (3–10 days). As an important parameter for activated sludge process design and operation (Batt et al., 2007; Clara et al., 2005), SRT may influence the levels of antibiotics in sludge.

It has been proved that longer SRT influences and improves the elimination of some pharmaceuticals by degradation processes during sewage treatment, but there is no clear conclusion as to whether SRT has an effect on the abiotic removal by sorption (Hyland et al., 2012). In the present study, the composition analysis indicated that the predominant antibiotics in sludge samples were quinolones, which accounted in average for 98.80% of the total antibiotics. Hence, the total concentrations of antibiotics in the sludge samples were mainly associated with the physiochemical properties and behaviors of quinolones. Many studies have shown that the dominant removal pathway for quinolones during wastewater treatment is sorption to sewage sludge rather than degradation. For example, approximately 80% of the total mass of both NOR and CIP sorbed to particles in the raw sewage water (Lindberg et al., 2006). In addition, it is known that quinolones have a high tendency for sorption to sludge, whose sorption coefficients are very high (Speltini et al., 2011). Thus, it was likely that quinolones would continually absorb onto the sludge during long contact time when biodegradative removal was not significant. Thus, longer SRT might cause the sludge to accumulate more quinolone antibiotics in wastewater. This study

showed that treatment techniques for WWTP with different SRTs have a significant impact on antibiotics levels in sludge, consistent with the report by Schaar et al. (2010).

Fig. 3. The loading plots of PCA by the database of antibiotics in sludge in China.

Fig. 4. The score plots of PCA by the database of antibiotics with different treatment techniques from the 45 WWTPs. a: PC1 vs PC2; b: PC1 vs PC3.

4. Conclusions

In the present work, a total of 18 antibiotics, including seven quinolones, six sulfonamides, and five macrolides, were detected in all sewage sludge samples collected from 45 WWTPs in 23 cities in China. The sludge samples from different WWTPs showed very similar composition profiles of antibiotics, indicating their universal usages and extensive pollution in China. Compared to previous studies, the levels of quinolones in this study were relatively high, while the concentrations of macrolides and sulfonamides were lower than or similar to those of other cities. This study found that the level of antibiotics in sewage sludge was associated with composition of the sewage, sludge organic content, the ratio of processing volume to serving population, and treatment techniques of the studied WWTPs. The risk of antibiotics in sludge needs further investigations, and the threat of these antibiotics in sludge should be of concern.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2012.12.050>.

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