

The occurrence of chloramphenicol and tetracyclines in municipal sewage and the Nanming River, Guiyang City, China

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The occurrence of antibiotics including chloramphenicol (CAP), oxytetracycline (OTC) and tetracycline (TC) was studied in municipal sewage, river water and sediment. Temporal and spatial variations of antibiotic concentrations in municipal sewage, river water and sediment were evaluated. In municipal sewage, CAP, OTC and TC concentrations were in the range of 5.8–47.4, 0.16–5.7 and 0.7–65.2 $\mu\text{g L}^{-1}$, respectively, and showed a temporal variation with high antibiotic concentrations appearing in the cold season. Untreated municipal sewage can seriously influence both river water and sediment. Generally, high antibiotic concentrations in river water appeared in winter owing to the low flow condition as well as the high antibiotic concentration in the sewage. However, high CAP and OTC concentrations in sediment were observed in summer most likely because runoff in high flow season can carry wastes from some origins (*e.g.* livestock farms in the countryside) into the river. The partitioning of antibiotics in river water and sediment suggests a lower sorption of TCs to the sediment compared to previous studies, which is believed to be caused by the high Ca^{2+} and Mg^{2+} concentrations, ionic strength and pH of the river water in the carbonate area.

1. Introduction

Antibiotics are widely used in large quantities in both human and animal medicine practices. For humans, antibiotics are used to prevent or treat disease; for animals, antibiotics are used in treating disease or as growth promoters. Therefore, human intake of antibiotics will include human medicines and food consumption.¹ A high percentage of these compounds are ultimately excreted unchanged or changed *via* urine and feces into domestic sewage, making municipal sewage a significant source of antibiotics.^{2–4} Whether domestic sewage is treated or untreated, the water environment will be impacted by antibiotics in the sewage, as antibiotics cannot be removed completely even after wastewater treatment.⁵

Regarding an urban river, the source of antibiotics may include municipal sewage, agricultural land (use of liquid manure), livestock farms and some surface waste. Understanding the occurrence of antibiotics in the different environmental compartments (*e.g.* sewage, river water and sediment) can be used to evaluate the transport and fate of antibiotics.⁶ Antibiotics may have potential adverse impacts on environment. For instance, they can accelerate the evolution of antimicrobial-resistant bacteria.^{7–9} Moreover, the presence of low levels of antibiotics and their transformation products in the environment could provide conditions for the transfer and spread of antibiotic resistant determinants among microorganisms, an emerging issue in public health.¹⁰ Therefore, knowledge about the occurrence and fate of antibiotics in the environment is of great significance.

Chloramphenicol (CAP) and tetracyclines (TCs) are broad-spectrum antibiotics, and TCs are the second most widely used antibiotics in the world, with applications in human therapy and the livestock industry.¹¹ In recent years, some publications reported the occurrence of CAP^{4,7} and TCs^{3,12} in municipal sewage or river water. However, the temporal and spatial variations of antibiotic concentrations in sewage and surface waters are not well understood. In particular, the sediment matrix has not been studied to a large extent.⁶ In this study, the occurrence of CAP, oxytetracycline (OTC) and tetracycline (TC) in municipal sewage, river water and sediment is described, so as to make an assessment about the occurrence and fate of antibiotics in municipal sewage and the surface water environment.

2. Materials and methods

2.1 Reagents and material

Methanol and acetonitrile (Dikma, USA) were of HPLC grade, and EDTA disodium salt, disodium hydrogen orthophosphate anhydrous (Na_2HPO_4), citric acid and oxalic acid were of analytical reagent grade. CAP, OTC and TC standards were obtained from Fluka (UK). McIlvaine buffer (pH = 4) was prepared by mixing 0.2 M citric acid and 0.4 M Na_2HPO_4 (60 : 40). The extraction buffer was a mixture of 0.1M EDTA and McIlvaine buffer (50 : 50). Stock solutions of CAP, OTC and TC (100 mg L^{-1}) were prepared by dissolving 1 mg of each compound in 10 mL of methanol. Working standards were prepared from the stock solutions freshly on the day of use.

Oasis hydrophilic-lipophilic balance (HLB) polymer cartridges (60 mg/3 mL) were purchased from Waters Corporation (UK), and strong anion exchanger (SAX) cartridges (500 mg/3 mL) were purchased from Agilent Technologies (USA).

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Glass fiber filters (GF/F, 0.7 μm) were purchased from Whatman Corporation (UK).

2.2 Site description and sampling

A field study was conducted of municipal sewage and Nanming River, at Guiyang City, southwestern China (Fig. 1). The Nanming River flows through agricultural areas and the urban area of Guiyang City, and then joins the Qingshuijiang River, a tributary of the Wujiang River. The total drainage area of Nanming River is 1422 km², and the annual average flow is *ca.* 12.4 m³ s⁻¹ (at site 11). Over its upper reach, the Nanming River travels through agricultural landscape and receives inputs from agricultural land, fish farms and small towns or villages. Also, many small livestock operations are distributed on the outskirts of Guiyang City. As the river flows through Guiyang City, it receives municipal sewage discharges. Guiyang City has a population of about 1.2 million, and it is estimated that 0.45 million tons of sewage water (including some wastewater discharged from factories) is produced each day. More than 80% of the sewage water is introduced in two ditches along two banks of the river, and then is discharged into the Nanming River through two outfalls between sites 10 and 11 without any treatment.

Sewage samples were collected monthly at two outfalls of municipal sewage over a period of 1 year from Nov. 2006 to Oct. 2007. Regarding the Nanming River, water and sediment samples were collected at fifteen locations (including one tributary location) in Jan. and Aug., 2007. Site 5 represents a tributary from Aha Lake—the drinking water source of Guiyang City. To obtain representative samples, river water was collected from the center of the river at a depth of *ca.* 20 cm below the water surface. The top part of sediment (0–5 cm) was collected in the center of the stream. Three replicates of sediment were collected in a range of 10 m and then combined to a composite sample. Although sediment was collected where water flow was as slow as possible, sediment at site 11 had to be collected where water flow was high due to the problem of access. This sediment sample (site 11) was mostly composed of sandy grains. Besides, sediment was not available at sites 10 and 15 owing to sediment dredge and fast water flow, respectively.

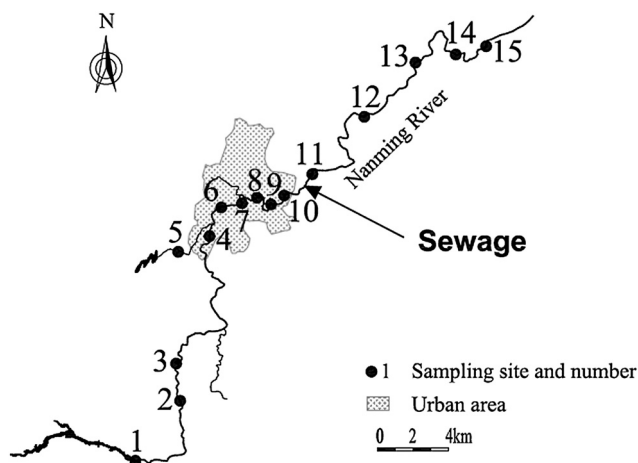


Fig. 1 Sampling locations along the Nanming River.

Polyethylene bottles were used to collect sewage and river water samples. Each bottle had been thoroughly rinsed with de-ionized water prior to sample collection. Sediment samples were collected in polyethylene bags. Temperature of the sewage, river water and sediment was determined immediately. Water and sediment samples were immediately transported to the lab and stored at 4 °C.

2.3 Sample extraction and chemical analysis

2.3.1 Extraction and chromatographic optimization. In the extraction of antibiotics from environmental samples, such as water and sediment, it is usual that interference occurs from sorption to metal ions in the sample matrices,^{3,13} which can result in a poor recovery. TCs are known to form chelate complexes with a range of metal ions and organic molecules.¹⁴ Therefore, the combination of citric acid and EDTA can be adapted to suppress the interference of metal ions and organic molecules. Compared to TCs, CAP is less strongly adsorbed to solid material,¹⁵ so the extraction of CAP is considered less difficult. In this study, EDTA–McIlvaine buffer (pH = 4) was employed to extract TCs and CAP simultaneously from water and sediment samples.

Natural organic matter (NOM), which is predominantly composed of humic and fulvic acids,¹⁶ extensively exists in environment samples. In the sample pretreatment, NOM will be retained on the HLB cartridge, and then be co-eluted when sample elution is carried out using methanol. During the HPLC determination, NOM often results in suppression or abnormal enhancement of the analyte signal.^{17,18} In case a high level of NOM exists in samples, a clean-up procedure is required. When the sample passes through an SAX cartridge in tandem with an HLB cartridge, a majority of humic material can be retained by the SAX cartridge.¹³ In addition, mixing acetone with methanol will increase the elution of target compounds while the elution of humic acids is still suppressed. Therefore, both the SAX cartridge in tandem with the HLB cartridge and use of 90% acetone / 10% methanol as the eluant were employed in this study to reduce the interference of NOM.

As TCs can form chelate complexes with metal ions and sorb on the silanol group in a reversed-phase column,¹⁹ peak tailing can occur in the HPLC separation of TCs, so oxalic acid is usually added in the eluant to eliminate peak tailing.^{20,21} In this study, when a mixture of acetonitrile and 0.01M oxalic acid (40 : 60) was used as the mobile phase, the peak tailing of TCs was avoided.

2.3.2 Sample preparation, extraction and analysis. Water samples were filtered using glass fiber filters (GF/F, Whatman). Then, 500 mL of filtered water sample and 50 mL of extraction buffer were thoroughly mixed and passed through SAX and HLB cartridges, which were set up in tandem and pre-conditioned with 2 mL methanol followed by 2 mL de-ionized water. The SAX cartridge was then removed, and the HLB cartridge was washed with 2 mL 15% methanol (in water) and then dried in argon flow. Following this, 2 mL (1 mL \times 2) 10% methanol (in acetone) was added to the HLB cartridge and the eluant was collected. The eluant was blown to dryness under a gentle argon stream, and re-dissolved in 250 μL methanol, and then analyzed

by HPLC (HP1100, Agilent, Germany) with a UV detector. Isocratic separation of CAP, OTC and TC was achieved using a Hypersil ODS column (200 mm × 4.6 mm, 5 μm). The flow rate of the mobile phase, composed of acetonitrile and 0.01M oxalic acid (40 : 60), was 1 mL min⁻¹ throughout, and the target compounds were eluted within 7 min. The injection volume was 20 μL. A wavelength of 278 nm was selected for detection.

Sediment samples were freeze-dried, and then pulverized to smaller than 100 mesh (0.15 mm). Four grams of sediment sample was accurately weighed into a 150 mL conical flask and 20 mL of extraction buffer was added. The flasks were placed on a shaker for 30 min and then centrifuged for 10 min. The supernatant was then decanted into a 100 mL glass bottle. The residue was extracted twice more, and the supernatant was combined and then filtered through a glass fiber filter. The filtrate was added in SAX-HLB cartridges for clean up, and then treated the same as the water sample. All data are expressed as dry weight of sediment.

2.3.3 Method validation. To ensure the quality of the sample pretreatment and analysis, an evaluation of recovery and limit of detection (LOD) was carried out. Recoveries were evaluated by spiking standard solution into river water and sediment at varying levels (20, 50, and 100 μg L⁻¹ for water, and 20, 50, and 100 μg kg⁻¹ for sediment), and then treated the same as real samples. The recovery of CAP, OTC and TC was 85–94%, 79–89% and 85–98% for water matrix, and 47–83%, 46–54% and 42–62% for sediment matrix. The results of sample analysis was not adjusted by the percentage of recovery. The linearity was evaluated by examining the spiked mixture of antibiotics at 10, 20, 50, 100, and 200 μg L⁻¹ in river water, and 10, 20, 50, 100, and 200 μg kg⁻¹ in sediment, respectively. The linearity was good for three compounds in water ($r^2 > 0.95$) and sediment ($r^2 > 0.92$). LOD was determined as the concentration giving a signal three times the standard deviation (six analyses) of the blank sample. The LOD was 0.02–0.03 μg L⁻¹ for water and 0.5–1.0 μg kg⁻¹ for sediment.

For each run of samples, a spiked standard mixture of antibiotics in water or sediment and a method blank were treated the same as real samples, so as to monitor the method performance. For water samples, method blanks were made from deionized water subject to the same sample processing. For sediment, method blanks were conducted by adding no sample in the extraction followed by the same treatment as real samples. None of method blanks were found to contain antibiotics higher than LOD.

3. Results

The occurrence and monthly variation of antibiotics in the municipal sewage of Guiyang City are shown in Fig. 2. In the sewage from the northern outfall, the mean concentrations of CAP, OTC and TC are 27.0, 2.3 and 11.0 μg L⁻¹, and in the sewage from the southern outfall, these concentrations are 21.2, 2.1 and 9.5 μg L⁻¹. In comparison, OTC and TC concentrations are lower than those in the influent of wastewater treatment plants of Wisconsin, USA (OTC 47 μg L⁻¹, and TC 48 μg L⁻¹),⁵ and TC concentration is similar to the value (4 μg L⁻¹) of untreated municipal sewage.²²

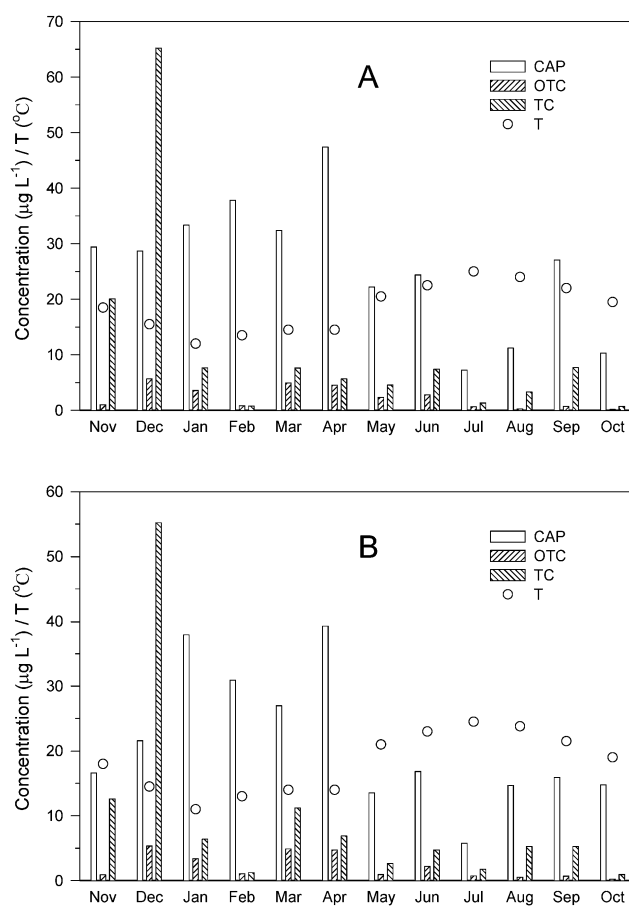


Fig. 2 Monthly variation of antibiotic concentrations in municipal sewage of Guiyang City (A: northern outfall; B: southern outfall).

The variation of the concentrations of CAP, OTC and TC in Nanming River water is shown in Fig. 3. The antibiotic concentrations in river water show both temporal and spatial variations. During winter, downstream waters show the highest level of CAP (mean: 11.2 μg L⁻¹), which is markedly higher than that of the upstream and urban waters (mean: 0.6 and 2.8 μg L⁻¹, respectively). The highest CAP concentration (19.0 μg L⁻¹) appears at site 11, the first sampling site downstream of the outfall of the municipal sewage. The distribution of OTC and TC is similar to that of CAP. The mean OTC concentrations of the upper, urban and downstream waters are 0.1, 0.1 and 1.5 μg L⁻¹, and the mean TC concentrations are 0.6, 0.7 and 3.8 μg L⁻¹. Similarly, the highest OTC concentration (3.0 μg L⁻¹) and TC concentration (6.8 μg L⁻¹) also appear at site 11. Compared to winter, CAP, OTC and TC concentrations of the river waters in summer are obviously lower, with CAP < 1.3 μg L⁻¹, OTC < 0.6 μg L⁻¹ and TC < 0.3 μg L⁻¹ (Fig. 3B).

Antibiotic concentrations of river water in this study were compared to those reported by previous studies. Generally, Nanming River has a higher level of antibiotics in water compared to other study areas. In Pearl River, Guangzhou, China, CAP in water was reported at 41 ng L⁻¹ at high flow season, and 127 ng L⁻¹ at low flow season.⁴ In Poudre River, Colorado, USA, TCs in water were found in the range of 0.08–0.30 μg L⁻¹.²³ In Suwannee River, Washington, USA, OTC in water was reported at only 0.34 μg L⁻¹.²⁴

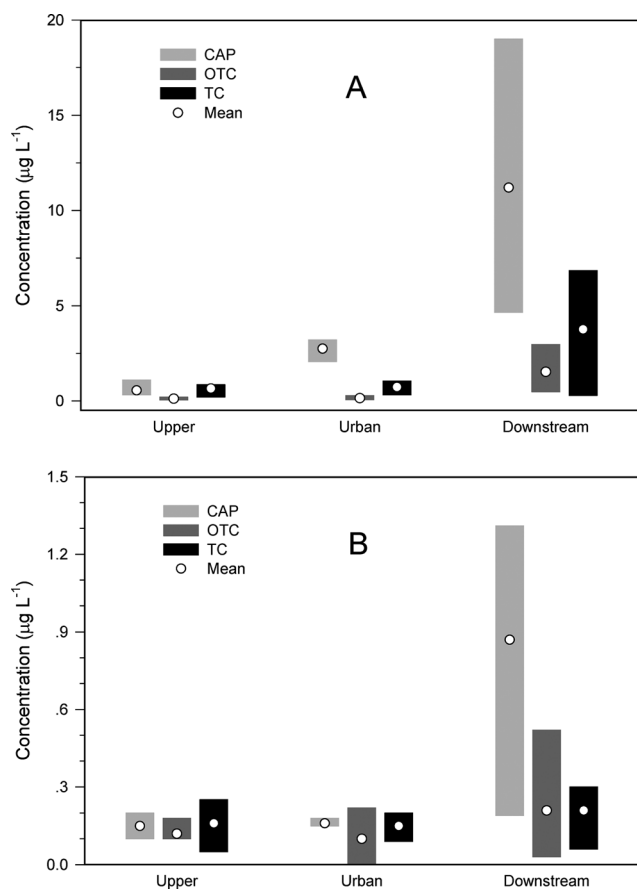


Fig. 3 Variation of antibiotic concentrations in Nanming River water (A: winter; B: summer).

The variation of CAP, OTC and TC in the sediment of Nanming River is shown in Fig. 4. During winter, CAP concentration in sediment shows a drastic variation. At the upper and urban reaches CAP concentrations are at a low level (mean: 2.7 and 2.1 $\mu\text{g kg}^{-1}$), whereas at the downstream reach CAP concentration is at a much higher level (mean: 475 $\mu\text{g kg}^{-1}$), with the highest value (1138 $\mu\text{g kg}^{-1}$) appearing at site 12. During summer, CAP concentrations of all the sampling sites are in the range of 33.3–734 $\mu\text{g kg}^{-1}$, with the highest level of CAP appearing at the urban reach (mean: 390 $\mu\text{g kg}^{-1}$). In winter, OTC in sediment shows a similar trend with CAP. The mean OTC concentration of the downstream reach is 202 $\mu\text{g kg}^{-1}$, much higher than the values of the upper and urban reaches (mean: 9.0 and 31.9 $\mu\text{g kg}^{-1}$). Similarly, the highest OTC concentration (335 $\mu\text{g kg}^{-1}$) also appears at site 12. In summer, OTC concentrations of all the sampling sites are in the range of 8.9–190 $\mu\text{g kg}^{-1}$. In winter, the distribution of TC is different than that observed for CAP and OTC. At the upper and urban reaches, TC concentrations are at a higher level (mean: 134 and 39.9 $\mu\text{g kg}^{-1}$) compared to CAP and OTC. At the downstream sites TC concentration (mean: 208 $\mu\text{g kg}^{-1}$) is also elevated, but to a much less extent than CAP and OTC. The highest TC concentration (312 $\mu\text{g kg}^{-1}$) in winter also appears at site 12. In summer, TC concentrations of all the sampling sites are at a lower level of 0.1–158 $\mu\text{g kg}^{-1}$.

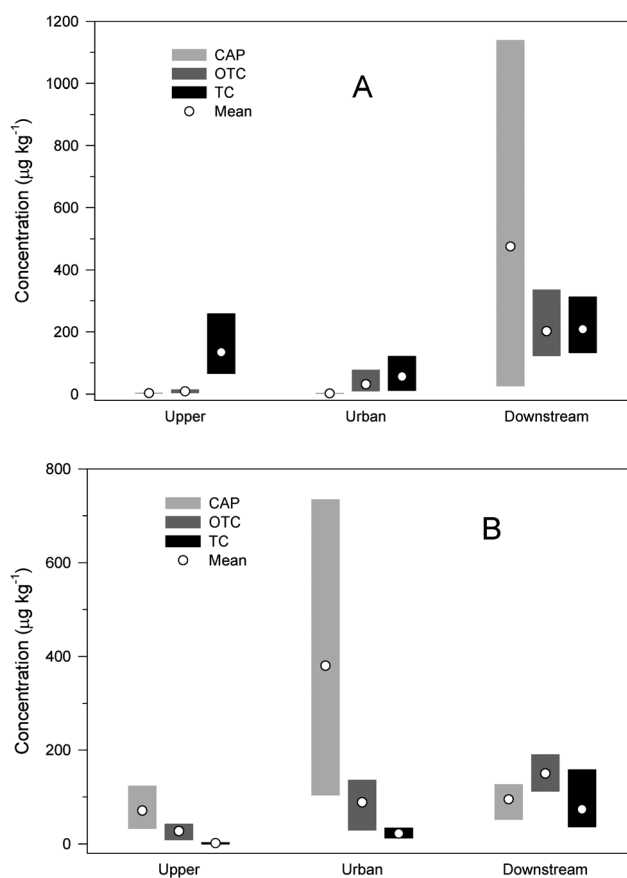


Fig. 4 Variation of antibiotic concentrations in the sediment of Nanming River (A: winter; B: summer).

4. Discussion

4.1 Occurrence of CAP and TCs in municipal sewage

The sewage has an apparently higher CAP concentration than OTC and TC, indicating the predominance of CAP in the municipal sewage. Antibiotics in municipal sewage can originate from both medicine and food ingested by humans. Because of its toxic effects such as agranulocytosis and aplastic anaemia,²⁵ CAP is limited to use only in exceptional cases like meningitis.⁷ Thus, the high CAP concentration in sewage may imply that most CAP is contributed from food of animal origin rather than from human medicines. Although the use of CAP has been banned in food production in many countries^{1,26} as well as in China,²⁷ the illegal use of CAP in livestock or aquaculture as feed additives can still be found due to its efficiency as an antibiotic, easy availability and low cost.²⁸ Furthermore, CAP is highly effective when used in veterinary practice because it inhibits a variety of aerobic and anaerobic microorganisms,²⁹ so it is still used to control outbreaks of diseases in aquaculture, especially in larval hatcheries and rearings.^{30–32} The illegal use of CAP as feed additive can also be confirmed by other research. In southeast Asia, CAP residues have been detected in aquacultural products.³³ In fact, we found that CAP in the feed of a fish farm in Guiyang City was up to 30.1 mg kg^{-1} , and Lv *et al.*³⁴ reported that CAP concentration in the sediment of a fish farm in Guangzhou City was 172 $\mu\text{g kg}^{-1}$.

Since antibiotics are being increasingly used in farm animal production as mentioned above, the residue of antibiotics is very prevalent in food of animal origin.³⁵ In addition, antibiotics in food can still remain after heating in food preparation.^{35–37} Furthermore, after ingestion by humans, antibiotics such as TCs are poorly absorbed in the digestive tract with 50–80% excreted unmetabolized.³⁸ Consequently, a large amount of antibiotics used as animal feed additives can enter domestic sewage.

Antibiotic concentration in sewage can vary in different seasons.⁵ The seasonal variation of antibiotic concentrations in the sewage of Guiyang City shows a generally negative correlation with temperature (Fig. 2), *i.e.*, warm seasons (summer and fall) have a lower level of antibiotics than cold seasons (winter and spring). This is most evident for CAP, for which the correlation coefficient is -0.84 (12 samples). Regarding OTC and TC, a relatively weak relationship with temperature can also be observed. The factors contributing to the variation of antibiotic concentrations may include different water consumption, medicine consumption pattern and temperature in warm and cold seasons. Warm seasons usually show higher water consumption rates than cold seasons, resulting in a dilution of antibiotic concentration in the domestic sewage. It has been shown that the medicine consumption pattern can vary with the characteristics of diseases in different seasons, with more prescriptions issued in the winter and fewer in the summer.³⁹ Furthermore, temperature is an important factor affecting the decomposition and degradation of antibiotics,^{40,41} so a higher temperature in summer will enhance the attenuation of antibiotics. As a consequence, a higher level of antibiotics has been observed in the sewage during cold seasons.

The correlation coefficient for each antibiotic between two outfalls was also calculated to compare the features of each antibiotic in the two regions of Guiyang City divided by the Nanming River. The correlation coefficients for CAP, OTC and TC are 0.86, 0.97 and 0.99 (12 samples), respectively. The high correlation coefficients for OTC and TC may indicate a very similar consumption pattern of OTC and TC in the two regions. The correlation coefficient for CAP is a little lower, indicating a less similar consumption pattern of CAP in the two regions.

4.2 Occurrence and distribution of CAP and TCs in the Nanming River

The spatial and temporal distribution of antibiotics in the river was evaluated. The mean antibiotic concentrations in upstream and urban river waters are generally at a low level ($<0.7 \mu\text{g L}^{-1}$), except that CAP concentration in winter is at the slightly higher level of $2.8 \mu\text{g L}^{-1}$. However, the downstream waters show seriously elevated CAP, OTC and TC concentrations. When compared to the urban reaches, the mean CAP, OTC and TC concentrations in the downstream waters are 4.0, 15.3 and 5.4 times higher in winter, and 5.4, 2.1 and 1.4 times higher in summer. In the upstream area antibiotics can originate from agricultural land, fish farms and livestock waste in the countryside, while in the urban area antibiotics can also originate from some surface waste of the city. The serious elevation of antibiotic concentrations downstream from the sewage discharge outfalls is contributed from municipal sewage. Previous studies also

demonstrate that municipal sewage is an important source of antibiotics to the receiving water.^{12,42,43}

Regarding the temporal variation, the antibiotic concentration of river waters in summer is much lower than in winter. The significant difference of antibiotic concentrations from season to season is considered to be caused primarily by the water flow conditions and the antibiotic concentrations of the municipal sewage. The water flow of the Nanming River in summer can be 10 times higher than in winter, resulting in a great dilution of antibiotic concentration. Moreover, the average water temperature was only $8 \text{ }^\circ\text{C}$ in January in comparison with $24 \text{ }^\circ\text{C}$ in August. The low temperature in winter may also contribute to the high antibiotic concentrations, since biodegradation is a significant natural attenuation mechanism and degradation of antibiotics will be slower in cold conditions.^{40,41} Photolysis is usually regarded as a significant factor affecting the fate of antibiotics such as TCs,⁴² amoxicillin,⁴⁴ and carbamazepine *etc.*⁴⁵ Especially, the surface water in Guiyang City (carbonate area) has typically a slightly alkaline pH of 7.1–8.2,⁴⁶ which would favor the photolysis of antibiotics.⁴⁴ Therefore, the difference in the antibiotic concentration in summer and winter can also be attributed to photolysis.

The temporal variation of antibiotic concentrations in sediment showed significant differences. Compared to winter, TC concentration of the sediment in summer is lower, whereas CAP and OTC concentrations are higher in the upper and urban reaches (Fig. 4). In addition, the downstream reach usually shows the highest antibiotic concentrations in both water and sediment due to the impact of municipal sewage (Fig. 3; 4A). However, for CAP in the sediment in summer, the highest concentration was observed in the urban area (Fig. 4B).

The variation of antibiotics in sediment is unexpected. The possible explanation is that the temporal variation of CAP and OTC concentrations may be significantly affected by the varying contribution of antibiotics in different seasons. In the low flow season, antibiotics primarily originate from the adjacent area of the river, whereas in the high flow season, antibiotics can originate from a larger area. In fact, many small livestock farms are dispersed in the outskirts of Guiyang City, and the wastes in these places can be transported to the river by runoff in the high flow season. The high CAP and OTC concentrations in the sediment in summer might be contributed from these sources. To some extent, this can be supported by the CAP and OTC concentrations in the sediment at tributary site 5 that were seriously elevated in summer (52.0 and $35.1 \mu\text{g kg}^{-1}$) compared to winter (0.5 and $1.9 \mu\text{g kg}^{-1}$). A similar result was reported in a previous study of Kim and Carlson.⁶ They observed a significantly higher concentration of tylosin in sediment samples during summer.

As sewage makes a significant contribution of antibiotics to the receiving water, the downstream sediment may also be seriously affected. During winter, a marked elevation of the concentrations of CAP, OTC and TC in sediment could be observed at the downstream reach compared to the urban reach (Fig. 4). During summer, a similar trend for OTC and TC was also observed but to a much lesser extent. This means that the antibiotic concentrations in sediment can be highly influenced when municipal sewage mixes with river water. The impact of sewage on the sediment can occur in two ways. First, a fraction of

Table 1 Pseudo-Partitioning Coefficient of antibiotics ($L\ kg^{-1}$)^a

		CAP		OTC		TC	
		Range	Mean	Range	Mean	Range	Mean
This study	Winter	2.73–125	43.3	55.5–333	146	21.7–76.9	48.5
	Summer	40.3–239	115	366–1600	884	184–608	294
Kim and Carlson ⁶					1267		1051
Tolls ¹⁵				290–1030		1140–1620	
Kim <i>et al.</i> ⁴²							8400
Stuer-Lauridsen <i>et al.</i> ⁴⁸					3020		

^a Note: Data were calculated according to the antibiotic concentrations of sediment and water samples at sites 11–14 (4 samples).

antibiotics in the sewage can be sorbed onto the sediment. Second, a fraction of antibiotics associated with the suspended solids of the sewage will settle into the sediment. Regarding the downstream reach, although site 11 is the first sampling site downstream of the outfalls of the municipal sewage, the highest antibiotic concentrations in sediment usually appear at site 12 as mentioned above. This is consistent with the fact that only sandy grain sediment could be collected at site 11 due to the high flow rate of river water here. In comparison with clay sediment, sandy grain sediment usually has a larger size of grain and shows a smaller surface area for sorption,¹³ and therefore contains a lower level of antibiotics.

In order to better understand the environmental significance of the high load of antibiotics introduced from the sewage to the receiving water, the behavior of antibiotics in the downstream environment was addressed. With regards to the downstream sites (sites 11–14), a pseudo-partitioning coefficient is calculated as the ratio of the antibiotic concentration in sediment to the concentration in water (Table 1). Although the river water and sediment are not at equilibrium, the calculated coefficient can be a valuable indicator of the sorption characteristics of the compounds,⁶ and help to evaluate the environmental impact. The antibiotics have a higher Pseudo-Partitioning Coefficient in summer than winter, most likely due to the dilution of antibiotic concentrations in water by the high flow in summer. Generally, the result shows that OTC is the most strongly sorbed compound followed by TC and CAP. This is consistent with previous research. Kim and Carlson⁶ reported a higher Pseudo-Partitioning Coefficient of OTC than TC in the Cache La Poudre River of northern Colorado, USA, and Figueroa *et al.*⁴⁷ also reported a stronger sorption of OTC than TC in 10 mM Na-montmorillonite solution. Besides, Tolls¹⁵ pointed out that CAP appears to have a weak sorption affinity. It can be concluded that antibiotics such as OTC and TC can accumulate in sediment through sorption, forming a key mechanism accounting for the attenuation of antibiotics in the aqueous phase.

The partitioning trend indicates that OTC and TC in the Nanming River have a lower sorption to the sediment in comparison to literature values (Table 1). The sorption characteristics of antibiotics can be influenced by fundamental factors and chemical composition of the solution. Generally, TC sorption tends to decrease with increasing pH and ionic strength.^{47,49,50} Decrease of OTC sorption on illite or montmorillonite was observed when pH or ionic strength was increased.⁵¹ As reviewed by Sassman and Lee,⁵⁰ at alkaline pH values (pH > 7), TCs can complex with metals including 1 : 1 and 2 : 1 metal-

TC complexes with Ca^{2+} and Mg^{2+} . The formation of metal-TC complexes can lead to enhanced aqueous solubility, and accordingly decrease the sorption of TCs. In the drainage area of the Nanming River, the rock types are basically carbonate rocks that consist mostly of limestone and dolomite. As a consequence of the dissolution of the carbonate minerals such as calcite and dolomite in the rocks, the surface water in this region has a relatively high pH range (7.1–8.2) as well as high concentrations of TDS (263–783 $mg\ L^{-1}$), Ca^{2+} (46–129 $mg\ L^{-1}$) and Mg^{2+} (8.64–30 $mg\ L^{-1}$).⁴⁶ It is possible that the relatively low sorption of OTC and TC to the sediment in the study area can be attributed to the high Ca^{2+} and Mg^{2+} concentrations, ionic strength and pH of the river water. Based on the discussion above, a higher potential for the release of antibiotics to overlying water under favorable conditions can be inferred.

5. Conclusions

The occurrence of antibiotics in the municipal sewage of Guiyang City suggests that some antibiotics in domestic sewage can originate largely from food of animal origin in addition to human medicine. The antibiotic concentrations in the sewage showed a temporal variation and were negatively correlated with temperature. The factors contributing to the variation of antibiotic concentrations in sewage may include different water consumption, medicine consumption pattern and temperature in warm and cold seasons.

Regarding antibiotic concentrations in the Nanming River, low flow season corresponded to a high level of antibiotics in aqueous phase due to the low flow condition and the high antibiotic concentrations in the municipal sewage. During summer, a decrease of antibiotic concentrations in river water was caused by the high flow condition, but a high level of CAP and OTC in sediment was measured, most likely because some wastes with a high level of CAP and OTC were carried into the river by runoffs in the high flow season.

The partitioning of antibiotics between water and sediment indicates that the sorption of TCs to the sediment in the study area, compared to previous research, is weaker to some extent. The reasons for this may include high Ca^{2+} and Mg^{2+} concentrations, ionic strength and pH of the river water, which is caused by the dissolution of carbonate minerals in the study area. It can be inferred that the sorbed antibiotics have a higher potential to release to the overlying water under favorable conditions and adversely affect the aquatic environment.

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References

- 1 A. K. Sarmah, M. T. Meyer and A. B. A. Boxall, *Chemosphere*, 2006, **65**, 725–759.
- 2 R. Hirsch, T. A. Ternes, K. Haberer, A. Mehlich, F. Ballwanz and K.-L. Kratz, *J. Chromatogr., A*, 1998, **815**, 213–223.
- 3 S. Yang, J. Cha and K. Carlson, *J. Chromatogr., A*, 2005, **1097**, 40–53.
- 4 W.-H. Xu, G. Zhang, S.-C. Zou, X.-D. Li and Y.-C. Liu, *Environ. Pollut.*, 2007, **145**, 672–679.
- 5 K. G. Karthikeyan and M. T. Meyer, *Sci. Total Environ.*, 2006, **361**, 196–207.
- 6 S.-C. Kim and K. Carlson, *Environ. Sci. Technol.*, 2007, **41**, 50–57.
- 7 R. Hirsch, T. Ternes, K. Haberer and K.-L. Kratz, *Sci. Total Environ.*, 1999, **225**, 109–118.
- 8 M. Rysz and P. J. J. Alvarez, *Wat. Res.*, 2004, **38**, 3705–3712.
- 9 S. Thiele-Bruhn and I.-C. Beck, *Chemosphere*, 2005, **59**, 457–465.
- 10 A. B. A. Boxall, D. W. Kolpin and J. Tolls, *Environ. Sci. Technol.*, 2003, **37**, 286A–294A.
- 11 N. F. Col and R. W. O'Connor, *Rev. Infect. Dis.*, 1987, **9**(Suppl.3), S232–S243.
- 12 A. L. Batt, I. B. Bruce and D. S. Aga, *Environ. Pollut.*, 2006, **142**, 295–302.
- 13 P. A. Blackwell, H. H. Lützhöft, H.-P. Ma, B. Halling-Sørensen, A. B. A. Boxall and P. Kay, *J. Chromatogr., A*, 2004, **1045**, 111–117.
- 14 C. R. Anderson, H. S. Rupp and W. Wu, *J. Chromatogr., A*, 2005, **1075**, 23–32.
- 15 J. Tolls, *Environ. Sci. Technol.*, 2001, **35**, 3397–3406.
- 16 F. J. Stevenson, *Humus Chemistry: Genesis, Composition, Reactions*, Wiley, New York, 1982.
- 17 B. K. Matuszewski, M. L. Constanzer and C. M. Chavez-Eng, *Anal. Chem.*, 2003, **75**, 3019–3030.
- 18 C. R. Mallet, Z. Lu and J. R. Mazzeo, *Rapid Commun. Mass Spectrom.*, 2004, **18**, 49–58.
- 19 H. Oka, K. Uno, K.-I. Harada and M. Suzuki, *J. Chromatogr., A*, 1984, **284**, 227–234.
- 20 H. Oka, Y. Ito and H. Matsumoto, *J. Chromatogr. A*, 2000, **882**, 109–133.
- 21 A. A. M. Stolker and U. A. T. Brinkman, *J. Chromatogr. A*, 2005, **1067**, 15–53.
- 22 K. G. Karthikeyan and W. F. Bleam, *DNR Project #163 Final Project Report*; Wisconsin Dept. of Natural Resources; Wisconsin Dept. of Agriculture, Trade, and Consumer Protection: Wisconsin, October 2003. <http://www.dnr.state.wi.us/org/water/dwg/gw/research/reports/169.pdf>.
- 23 S. Yang and K. Carlson, *Wat. Res.*, 2003, **37**, 4645–4656.
- 24 M. E. Lindsey, M. Meyer and E. M. Thurman, *Anal. Chem.*, 2001, **73**, 4640–4646.
- 25 S. Fabiansson, T. Nilsson and J. Backstrom, *J. Sci. Food Agric.*, 1976, **27**, 1156–1162.
- 26 H. T. Rønning, K. Einarsen and T. N. Asp, *J. Chromatogr., A*, 2006, **1118**, 226–233.
- 27 X.-F. Wu and L.-H. Yang, *Progress in Veterinary Medicine*, 2004, **25**, 41–43, (in Chinese).
- 28 P. Vinas, N. Balsalobre and M. Hernandez-Cordoba, *Anal. Chim. Acta*, 2006, **558**, 11–15.
- 29 Z. Y. Huang, M. Y. Sun, S. Li and G. L. Huang, *Aquacult. Res.*, 2006, **37**, 1540–1545.
- 30 I. Uriate, A. Farias and J. C. Castilla, *Aquacult. Eng.*, 2001, **25**, 139–147.
- 31 L. Torkildsen and T. Magnesen, *Aquacult. Int.*, 2004, **12**, 489–507.
- 32 A. L. Campa-Córdova, A. Luna-González, F. Ascencio, E. Cortés-Jacinto and C. J. Cáceres-Martínez, *Aquaculture*, 2006, **260**, 145–150.
- 33 G. Huys, K. Bartie, M. Cnockaert, D. T. H. Oanh, N. T. Phuong, T. Somsiri, S. Chinabut, F. Md. Yusoff, M. Shariff, M. Giacomini, A. Teale and J. Swings, *Res. Microbiol.*, 2007, **158**, 228–235.
- 34 X.-W. Lv, M.-S. Liang, Z. Dang and C. Yang, *J. Agro-Environ. Sci.*, 2007, **26**, 1195–1200, (in Chinese).
- 35 M. Al-Ghamdi, Z. Al-Mustafa, F. El-Morsy, A. Al-Faky, I. Haider and H. Essa, *Public Health*, 2000, **114**, 300–304.
- 36 M. Kühne, G. Hamscher, U. Körner, D. Schedl and S. Wenzel, *Food Chem.*, 2001, **74**, 423–429.
- 37 R. J. Shakila, S. A. P. Vyla, R. S. Kumar, G. J. Jeyasekaran and G. I. Jasmine, *Food Microbiol.*, 2006, **23**, 47–51.
- 38 B. Halling-Sørensen, G. Sengeløv and J. Tjørnelund, *Arch. Environ. Contamin. Toxicol.*, 2002, **42**, 263–271.
- 39 X. S. Miao, F. Bishay, M. Chen and C. D. Metcalfe, *Environ. Sci. Technol.*, 2004, **38**, 3533–3541.
- 40 F. Ingerslev and B. Halling-sorensen, *Environ. Toxicol. Chem.*, 2000, **19**, 2467–2473.
- 41 F. Ingerslev, L. Torang, M. L. Loke, B. Halling-Sorensen and N. Nyholm, *Chemosphere*, 2001, **44**, 865–872.
- 42 S. Kim, P. Eichhorn, J. N. Jensen, A. S. Weber and D. S. Aga, *Environ. Sci. Technol.*, 2005, **39**, 5816–5823.
- 43 M. Winker, D. Faika, H. Gulyas and R. Otterpohl, *Sci. Total Environ.*, 2008, **399**, 96–104.
- 44 R. Andreozzi, V. Caprio, C. Ciniglia., M. de Champdore, R. L. Giudice and E. Zuccato, *Environ. Sci. Technol.*, 2004, **38**, 6832–6838.
- 45 R. Andreozzi, M. Raffaele and P. Nicklas, *Chemosphere*, 2003, **50**, 1319–1330.
- 46 Y.-C. Lang, C.-Q. Liu, Z.-Q. Zhao, S.-L. Li and G.-L. Han, *Appl. Geochem.*, 2006, **21**, 887–903.
- 47 R. A. Figueroa, A. Leonard and A. A. Mackay, *Environ. Sci. Technol.*, 2004, **38**, 476–483.
- 48 F. Stuer-Lauridsen, M. Birkved, L. P. Hansen, H.-C. Lützhöft and B. Halling-Sørensen, *Chemosphere*, 2000, **40**, 783–793.
- 49 P. Kulshrestha, R. F. Giese Jr. and D. S. Aga, *Environ. Sci. Technol.*, 2004, **38**, 4097–4105.
- 50 S. A. Sassman and L. S. Lee, *Environ. Sci. Technol.*, 2005, **39**, 7452–7459.
- 51 B. B. Sithole and R. D. Guy, *Wat. Air, Soil Pollut.*, 1987, **32**, 315–321.