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Profile and behavior of antiviral drugs in aquatic environments of the Pearl River Delta, China

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HIGHLIGHTS

- Acyclovir was the only antiviral detected in the wastewater.
- Acyclovir was not completely removed in the wastewater in the STP.
- Acyclovir was widely detected in the recipient rivers and reservoirs.
- No antivirals were detected in the wells in the vicinity of the municipal landfills.

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ABSTRACT

Occurrence and behavior of six antiviral pharmaceuticals (acyclovir, ganciclovir, oseltamivir, ribavirin, stavudine and zidovudine) and one active metabolite oseltamivir carboxylate were investigated in wastewater, landfill leachate, river water, reservoir and well water in the vicinity of municipal landfills in the Pearl River Delta, China. Acyclovir was the only antiviral detected in the wastewater at 177–406 (mean = 238) and 114–205 (mean = 154) ng L^{-1} in the influent and final effluent, respectively. Aerobic biodegradation appeared to be the main process for the elimination of acyclovir in the wastewater. Acyclovir was also the only antiviral quantitatively detected in the Pearl River and its tributaries, with a maximum concentration up to 113 ng L^{-1}. Treated wastewater was a major source of acyclovir in the rivers. The highest concentration of acyclovir was observed in winter in the river water and the dilution effect by precipitation was suggested to be the dominant factor impacting the seasonal pattern of acyclovir in the rivers. No antivirals were quantitatively detected in the well water whereas acyclovir was frequently detected in the reservoirs at a maximal concentration of 33.6 ng L^{-1} in the vicinity of the municipal landfills. However, source identification and fate of acyclovir in the reservoirs pend on further research.

1. Introduction

Antiviral drugs are widely used in treatment and prophylaxis of various viral infections including influenza, hepatitis, herpes and HIV (De Clercq and Field, 2006; Kim et al., 2011; Kiso et al., 2004; Olsen et al., 2006; Singer et al., 2007). As many other pharmaceuticals, antiviral drugs may not be completely metabolized by treated human and animals and are subsequently excreted and discharged into wastewaters (Fick et al., 2007; Ghosh et al., 2010a,b; Renner, 2007; Soderstrom et al., 2009). As a result, antiviral pharmaceuticals may find their way to the environment if they are not effectively eliminated during wastewater treatment (Accinelli et al., 2010a,b; Fick et al., 2007; Ellis, 2010; Singer et al., 2007, 2008).

Studies about environmental toxicity of antiviral pharmaceuticals are so far mostly limited to oseltamivir and its active metabolite oseltamivir carboxylate (Singer et al., 2007, 2008, 2011). Research reported rather high chronic no-observed effect concentration for oseltamivir and oseltamivir carboxylate (≥1 mg/L) based on ecotoxic tests using traditional aquatic organisms (Hutchinson et al., 2009; Straub, 2009). Acute toxicity of oseltamivir and its carboxylate in traditional aquatic microorganisms was also shown to be less than predicted (Escher et al., 2010). On the other hand, antivirals were ranked as the eighth predicted most toxic drugs to typical aquatic organisms (Sanderson et al., 2004). Besides, antivirals may produce resistance strains of pathogens in human and animals, which are of particular concerns (Hauser et al., 2011; Kiso et al., 2004; Olsen et al., 2006). It has been reported that Japan has a high rate of emerging resistance to oseltamivir, probably because it is the country where oseltamivir is used most (Ghosh et al., 2009; Soderstrom et al., 2009). In addition, oseltamivir in the environment may also impact the bacterial community structure (Caracciolo et al., 2010).

There have been reports about occurrence of oseltamivir and oseltamivir carboxylate in wastewater and river water of Japan and

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Europe at tens of ng L^{-1} to low μg L^{-1} levels (Azuma et al., 2012; Ellis, 2010; Fick et al., 2007; Ghosh et al., 2009; Leknes et al., 2012; Prasse et al., 2010; Soderstrom et al., 2009; Takanami et al., 2010). Prasse et al. (2010, 2011) have also reported the presence of other antivirals, including acyclovir and its metabolite carboxy-acyclovir, zidovudine, lamivudine, abacavir, and penciclovir in wastewater, river water, and even groundwater in Germany. Zidovudine, lamivudine, and nevirapine were detected in rivers in Kenya at μg L^{-1} level (Koreje et al., 2012). Biodegradation, photodegradation, and ozonation of oseltamivir and its carboxylates have been studied via bench-scale experiments as well as investigations in sewage treatment plants (Accinelli et al., 2007, 2010a,b; Bartels and von Tumpling, 2008; Ghosh et al., 2010a,b; Goncalves et al., 2011; Mestankova et al., 2012; Sacca et al., 2009). Recently, Prasse et al. (2011) revealed rapid biotransformation of acyclovir and penciclovir in activated sludge, whereas the transformation product of acyclovir, carboxy-acyclovir was found to be persistent and was detected in drinking water, groundwater, and surface water from 40 ng L^{-1} to 3.2 μg L^{-1}. In addition, N-(4-carbamoyl-2-imino-5-oxoimidazolidin)-formamido-N-methoxyacetic acid, an ozonation product of acyclovir was detected in finished drinking water of a German waterworks (Prasse et al., 2012). After all, data about distribution and behavior of antivirals in the environment is still limited considering their wide and large amount of usage.

As the country of the biggest population in the world, China has a huge consumption of antivirals and the amount keeps increasing in recent years. The most frequently administered are nucleoside agents such as acyclovir, ganciclovir, ribavirin, and lamivudine (Wang, 2012). The Pearl River Delta (PRD), located in the south of China, is one of the most densely populated areas in the country. PRD may be one of the areas that consume the most antivirals in China due to the humid monsoon subtropical climate that may favor propagation and spread of viruses and germs. In addition, PRD was also one of the areas that have suffered pandemic outbreaks of avian influenza (e.g. H5N1) in China (Chinese Center for Disease Control and Prevention, 2013). Thus, the release of antivirals to the environment of this area is expected. However, there is so far no report about antivirals in the environment of the PRD and even throughout China.

The aims of this work are (1) to investigate occurrence of commonly prescribed antiviral drugs in municipal wastewater and landfill leachate in the PRD since they are important sources of pharmaceuticals, including antivirals in the environment, (2) to study the behavior of the antivirals in wastewater by sampling influent and effluents at the outlets of major treatment units in a typical sewage treatment plant, (3) to delineate distribution of the antivirals in the potentially impacted surface water and groundwater of the PRD, and (4) to reveal seasonal effects on the profile and fate of the antivirals in the aquatic environments. The targets of this work included acyclovir and ganciclovir that are most frequently used for treatment of herpes and viral infections of respiratory tract, ribavirin that is used to treat encephalitis B and hepatitis, and oseltamivir for treatment of avian influenza. In addition, stavudine, and zidovudine that are primarily used to treat HIV were also included.

2. Material and methods

2.1. Chemicals and reagents

Acyclovir (purity > 99%), ganciclovir (purity > 99%), ribavirin (purity > 97%), stavudine (purity > 98%), and zidovudine (purity = 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Oseltamivir (free base, purity = 96%) and oseltamivir carboxylate were bought from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Toronto Research Chemicals (Toronto, ON, Canada), respectively. Isotope-labeled standards ganciclovir-d_5, oseltamivir-d_3 phosphate, ribavirin-$^{13}C_5$, and zidovudine-d_3 were purchased from Toronto Research Chemicals (North York, Ontario. Canada) and acyclovir-d_4 was bought from Campro Scientific (Veenendaal, The Netherlands). All standards were obtained in solid form.

HPLC-grade methanol, *n*-hexane, formic acid, ammonium acetate, and ammonium hydroxide were purchased from Merck (Darmstadt, Germany). Ultra-pure water was generated by a Milli-Q ultra-pure water system (Millipore, Billerica, MA, USA). Analytical grade acetone was bought from Kaixin Chemical (Tianjin, China) and re-distilled prior to use. Sodium azide (NaN_3) was obtained from Fuchen Chemical (Tianjin, China) and washed with methanol for three times prior to use.

2.2. Study area and sampling

As the third largest river of China, the Pearl River flows through the PRD and finally merges into the South China Sea via the Pearl River Estuary (Fig. 1). The annual municipal and industrial wastewater productions of the PRD are around 6000 and 1800 million tons in which 78% and 100%, respectively of them are treated prior to discharge to the environment. The treated wastewater and the rest of untreated wastewater are all finally discharged into the Pearl River and its tributaries.

2.2.1. Wastewater samples

The STP is located in Guangzhou, the biggest city of the PRD (Fig. 1). Detailed description of the STP was provided previously (Yu et al., 2011). Briefly, it has three parallel treatment lines with a total capacity of 550 000 m^3 d^{-1}, serving a population of about 1.5 million. The first and second lines treat predominantly domestic wastewater (~90%) and use identical treatment composing of a screen, a grit chamber, a bioreactor consisting successively of anaerobic, anoxic, and oxic processes and a secondary clarifier. The third line has a bioreactor comprising successively anoxic, anaerobic, and oxic processes. Besides, the third line also receives a certain amount of industrial wastewater and municipal landfill leachate. The hydraulic retention time is 11.5 h for all the treatment lines and the solid retention time is about 10 days. The effluent is disinfected by chlorination prior to discharge into the Pearl River.

The influent and final effluent samples were collected along the first and third treatment lines of the STP in July 2010 (summer) to obtain a general profile of the antivirals in the wastewater. In order to gain an insight of fate of the antivirals in the wastewater, influent and effluents at the outlets of anaerobic tank, anoxic tank, secondary clarifier, and the final effluent were sampled along the first line in February 2011 (winter). Furthermore, daily variation was investigated through everyday sampling over a period of one week from February 15 to 21, 2011. Wastewater samples were collected hourly from 8:00 am to 12:00 pm (10 L/h) on a weekday to build a 40 L composite sample. Dewatered sludge was collected as grab samples during winter sampling.

2.2.2. Sampling in wells and reservoirs

Landfill leachates were collected from two municipal landfills in Guangzhou (Fig. 1). LF1 was put into operation in 1989 and closed in 2002, with a total filled waste of about 5 million tons. Adverse impact of its leachate on the groundwater was evidenced by increased levels of NH_3 and NH_4^+ in the well water (Guangzhou Environmental Protection Bureau, 2012). As for the largest landfill in Guangzhou, LF2 was operated in 2002 and is currently accepting municipal refuse of 7000–9000 tons/day. Raw leachates were sampled to get a profile of the antivirals in municipal landfill leachate. There are several villages in the vicinity of the landfills. Many villagers have wells at home that were used for water supplies. However, the well water is mostly used only for washing rather than drinking and cooking due to concerns of contamination by leakage of leachate from the landfills. Fourteen and 13 wells were sampled in the villages that are potentially impacted by the leachate from LF1 and LF2, respectively. The wells have depth of less than 10 m (shallow aquifers) and are with distance of 582–3178 m from the landfills. Most of the wells have covers and

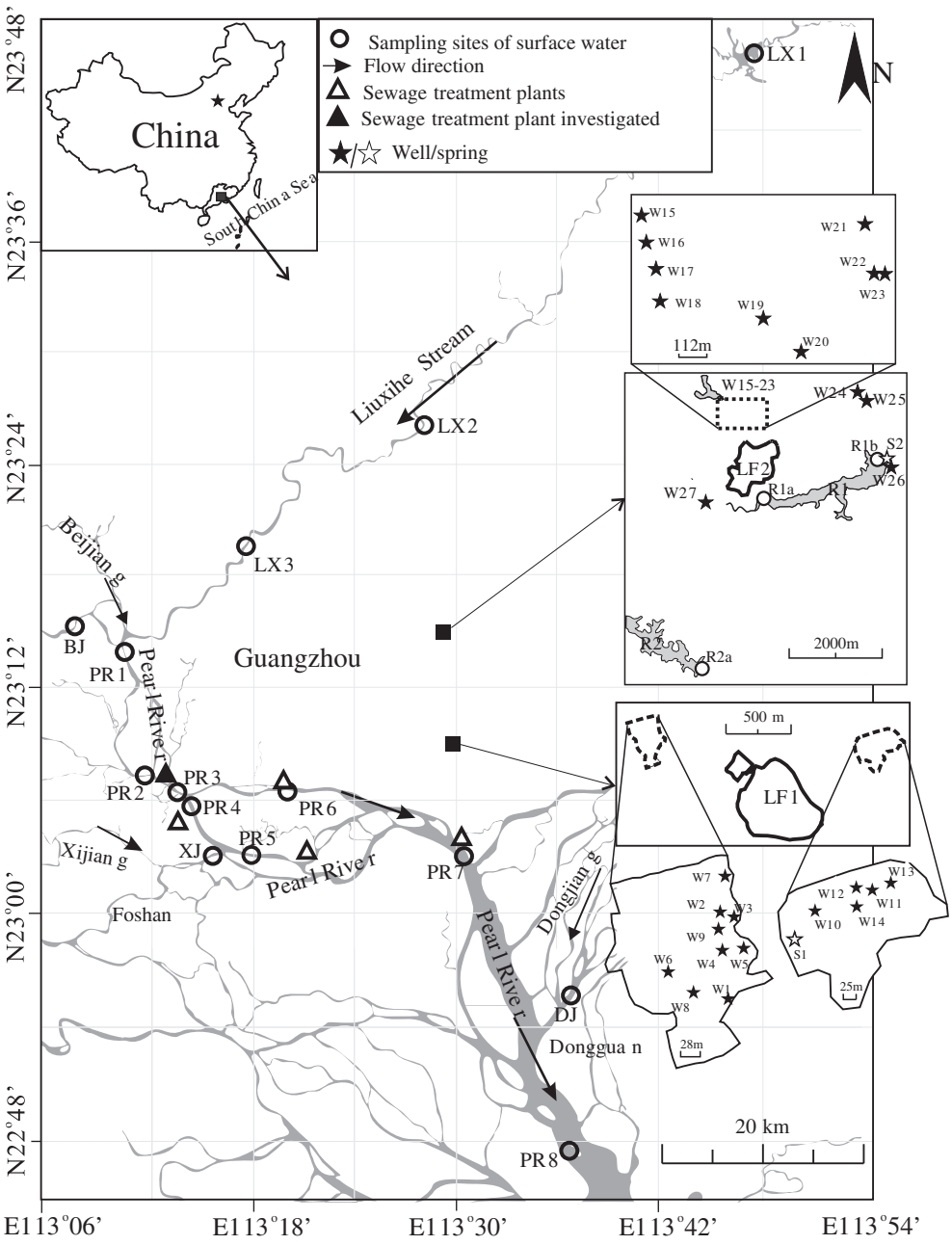

Fig. 1. Sketch map of the studied area and sampling sites.

are therefore not significantly impacted by rain and sunlight. A spring water sample was also collected in the vicinity of each landfill. Besides, there are 2 reservoirs, named R1 and R2 located on the south of landfill LF2. Water samples were collected in the upstream and downstream of R1 at a distance of 943 (R1a) and 2846 m (R1b) from LF2, respectively. R2 was also sampled at a distance of 4683 m from LF2 (Fig. 1). Samplings were conducted in March–April (spring), June (summer), October (fall) 2012 and January 2013 (winter).

2.2.3. Sampling of river water

River water samples were collected in the Pearl River and the tributaries (Fig. 1). Fourteen sampling sites were set, 3 in Liuxi River (LX1 to LX3), 8 in the mainstream Pearl River (PR1 to PR8), and each one in Beijiang (BJ), Xijiang (XJ), and Dongjiang (DJ) at the confluences with the mainstream Pearl River, respectively, covering rural (LX1 and LX2), suburban (LX3, BJ, and PR1, PR8, DJ), and urban sections (PR2–PR7, XJ). Samplings were performed in July 2010 (summer), December 2010 (winter), and October 2011 (fall). Samples were always collected during ebbing time to minimize dilution by intruding seawater.

Samplings were always conducted on non-rainy days. All water samples were collected in amber glass bottles without headspace. Sodium azide was added (0.5 g L^{-1}) immediately after sampling to suppress potential bioactivity. Samples were kept in icepacks after sampling and during transport to the laboratory where the water samples were stored at 4 °C in the dark until treatment within 48 h and the sludge was stored at −20 °C in a freezer.

2.3. Sample preparation and analysis

Sample treatment followed the procedure described by Prasse et al. (2010). Briefly, water samples were filtered with 0.7 μm glass

fiber filters (GF/F, Whatman, Maidstone, England). An aliquot of the filtrate (100 mL for raw wastewater/landfill leachate, 200 mL for treated wastewater and river water, and 500 mL for well water and reservoir water) was spiked with 100 ng of each isotope-labeled internal standard (Table 1) and adjusted to pH 8 with ammonium hydroxide. Sample extraction/concentration was performed on a 500 mg Isolute ENV+ cartridge (Biotage, Uppsala, Sweden) using a Syncore Polyvap system with a solid phase extraction (SPE) unit (Buchi, Switzerland). The cartridge was preconditioned successively with 2 mL of *n*-hexane, 2 mL of acetone, 3 × 2 mL of methanol and 4 × 2 mL of pure water (pH 8). After sample loading, the cartridge was vacuum dried for 10 min. The analytes were then eluted with 5 × 2 mL of methanol–acetone (50/50, v/v) containing 0.2% formic acid. The eluate was concentrated to 100 μL and then reconstituted to 1 mL with 5 mM ammonium acetate buffer prior to instrumental analysis.

The suspended particulate matter of the wastewater samples retained on GF/F filters and sludge samples were lyophilized and homogenized prior to being treated by ultrasonic-assisted extraction using methanol. The extracts were concentrated and diluted with pure water to bring the methanol content to <2%. The diluted extracts were then further concentrated and purified using SPE as described above.

The antivirals were determined on an Agilent 1200 liquid chromatography system coupled with an Agilent 6410 triple quadrupole mass spectrometer (LC–MS/MS) using electrospray ionization in positive mode. A Phenomenex Synergi Hydro-RP (50 × 2 mm, 2.5 μm particle size) column preceded by a 4 × 3 mm C18 guard column (Phenomenex, Torrance, CA, USA) was used for separation at a flow rate of 0.25 mL min^{-1} and at 25 °C. The mobile phase consisted of pure water with 5 mM ammonium acetate (A) and methanol (B). Separation was achieved with a gradient elution as follows: 0–1 min, 0% B; 2 min, 70% B; 4 min, 90% B; 4.1 min, 0% B. A post-time of 5 min was set for column equilibration before next injection. The total run time was 15 min.

MS data acquisition was accomplished in selected reaction mode (SRM) with two most intense and specific ion transitions as quantifier and qualifier for each compound (Table 1). Instrument control, data acquisition and data processing were done with MassHunter Workstation. A 7-point curve was established for each analyte using internal standard method.

2.4. Quality assurance and quality control

Recovery test was performed according to the procedure described elsewhere (Huang et al., 2010). The absolute recoveries ranged from 42% to 77% for the analytes except for ribavirin. However, the relative recoveries were satisfactory (64–117%) due to the use of isotope-labeled analogs of the analytes as surrogate standards. Matrix effect ranged from 24 to 60% except for ribavirin (93%). The limits of quantification (LOQ) estimated based on instrumental quantification limits, recoveries, and enrichment factors were 3–19 ng L^{-1} except for ribavirin with quite high LOQ due to poor absolute recovery (Table 1). Procedural and instrumental blanks and replicate analyses of environmental samples were set to monitor the analysis performance. None of the analytes was quantifiable in the blanks. The relative standard deviation of duplicate analysis of environmental samples was within 17%.

3. Results and discussion

3.1. Distribution and behavior of the antiviral drugs in the wastewater

Acyclovir was ubiquitously detected in the wastewater (Figs. 2 and 3). Ganciclovir and ribavirin were below the quantification limits in the influent, whereas the other antivirals were not detected in the wastewater (Table S1, Supporting material). This result was quite different from previous reports for wastewater in Japan and Europe in which oseltamivir and oseltamivir acid were frequently detected (Fick et al., 2007; Ghosh et al., 2009, 2010a,b; Prasse et al., 2010; Soderstrom et al., 2009; Singer et al., 2008; Slater et al., 2011). In addition, abacavir, lamivudine, nevirapine, penciclovir, stavudine, and zidovudine were detected at several to hundreds of ng L^{-1} in the wastewater of Germany (Prasse et al., 2010) whereas the concentrations of lamivudine and nevirapine were approximately 1 μg L^{-1} in treated wastewater in Kenya (Koreje et al., 2012). This may reflect difference in consumption of antiviral drugs in different countries. Acyclovir was only detected in the suspended particulate matter of the influent at 39 ng g^{-1} dry weight while none of the antivirals was quantitatively detected in the dewatered sludge, which agreed well with their strong hydrophilicity indicated by the low Kow values (Table 1). The following discussion will therefore focus on distribution and fate of acyclovir in the dissolved phase of wastewater (filtrate).

Concentration of acyclovir in the influent samples ranged from 177 to 406 ng L^{-1} (Figs. 2 and 3), significantly lower than that in the wastewater of Germany which was 1780 ng L^{-1} (Prasse et al., 2010). The result demonstrated that the acyclovir concentration in the influent was higher in the third treatment line than in the first line ($p = 0.03$, ANOVA), which might be ascribed to the addition of landfill leachate in the third line. However, the difference was smoothed out ($p = 0.34$, ANOVA) after treatment in the STP as shown by the similar concentration of 114–205 ng L^{-1} in the final effluent (Fig. 2).

Table 1
SRM ion transitions, internal standard (IS), limits of quantification (LOQ, ng L^{-1}), absolute recoveries (AR), and relative recoveries (RR).

Analytes	CAS no.	Kow[a]	MRM ion transition[c]	IS	Recovery (%, mean ± SD, n = 4)		LOQ (ng L^{-1})	
					AR	RR	Wastewater	River water
Acyclovir	59277-89-3	−1.59	**226 → 152** 226 → 135	ACV-d_4	42.1 ± 3.3	102.4 ± 4.7	13	6
Ganciclovir	82410-32-0	−1.7[b]	**256 → 152** 256 → 135	GCV-d_5	52.7 ± 3.3	107.0 ± 29.7	19	9
Oseltamivir	196618-13-0	0.36	**313 → 116** 313 → 137	OSE-d_3	73.8 ± 5.1	116.9 ± 10.9	7	3
Oseltamivir carboxylate	187227-45-8	−2.1	**285 → 138** 285 → 180	OSE-d_3	47.9 ± 4.2	89.1 ± 8.6	11	5
Ribavirin	36791-04-5	−1.85	**245 → 131** 245 → 96	RBV-$^{13}C_5$	6.7 ± 1.0	77.4 ± 33.4	152	74
Stavudine	3056-17-5	−0.47	**225 → 166** 225 → 120	ZDV-d_3	76.8 ± 9.1	96.8 ± 4.7	13	7
Zidovudine	30516-87-1	−0.1	**268 → 127** 268 → 110	ZDV-d_3	73.2 ± 5.4	64.4 ± 7.0	14	7

[a] Prasse et al. (2010).
[b] http://www.drugbank.ca/.
[c] Quantification ion transitions are in boldface.

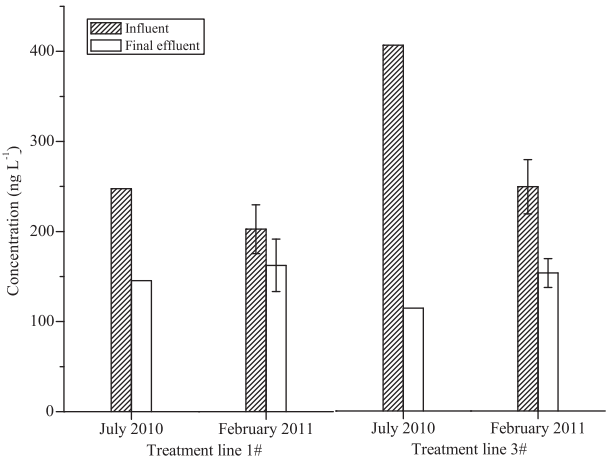

Fig. 2. Seasonal pattern of acyclovir in the wastewater. Error bars represent standard deviations (n = 7) for February. No uncertainty is shown for July as this was only a single day's sampling.

The concentrations of acyclovir were 203 ± 27 and 162 ± 29 ng L^{-1} and 249 ± 30 and 153 ± 16 ng L^{-1} in the influent and effluent from the first and third treatment lines, respectively over a period of one week with a slightly higher concentration on Monday (Fig. 3a, b).

Acyclovir in the wastewater basically transported in the aqueous phase according to the result that more than 99% of acyclovir was in the filtrates of the wastewater samples, which was in agreement with the results of previous research that oseltamivir and its carboxylate were not sorbed to appreciable proportions in sediment in rivers (Prasse et al., 2010). The concentration of acyclovir changed from 259 ng L^{-1} in the influent to 229 and 232 ng L^{-1} in the anaerobic and anoxic effluents, respectively. Following aerobic activated sludge treatment and secondary clarification, the acyclovir concentration was 172 ng L^{-1} in the secondary effluent, whereas after chlorination, the concentration was 199 ng L^{-1} in the final effluent (Fig. 3c). It is well known that pharmaceuticals in wastewater are primarily removed by biodegradation and sorption (Vieno et al., 2005). Previous researches have also reported extensive elimination of acyclovir by biodegradation (98%) in German wastewater (Prasse et al., 2010) and by activated sludge batch systems, with a significant portion of acyclovir being transformed to persistent carboxy-acyclovir rather than being mineralized (Prasse et al., 2011). However, carboxy-acyclovir was not analyzed in this work. Difference in transformation rate of acyclovir between this work and previous work (Prasse et al., 2010, 2011) might be due to several factors such as different treatment techniques, different hydraulic retention times and/or solid retention times, and microbial consortia in the STPs. Plus, previous researches have proved that sampling methodology may significantly affect measured results, which was site- and compound-specific (Ort et al., 2010a,b). However, proportional composite samplings were not adopted in this work because composite samplers were not allowed to be set in the STP. Therefore, the influent and effluents collected during the same period of time were not exactly the same wastewater package considering the hydraulic time of 11.5 h in the STP, which might also lead to biased estimation. Therefore, more accurate sampling protocol needs to be used in order to better elucidate the mass loads and fate of the antivirals in the wastewater. Furthermore, retransformation of carboxy-acyclovir to parent acyclovir cannot be completely excluded during treatment as it has been observed for other pharmaceuticals (Radke et al., 2009). Variations in removal were also observed for oseltamivir, oseltamivir carboxylate, and amantadine in the wastewater of Japan (Azuma et al., 2012). Concalves et al. (2011) and Bartels and von Tumpling (2008) reported half-lives of 15 to 150 days for oseltamivir ester and oseltamivir carboxylate during photodegradation in surface water under natural solar irradiation, whereas under simulated solar irradiation, the half-lives of oseltamivir carboxylate were 48 h in pure water and 12 h in surface water (Concalves et al., 2011). Prasse et al. (2012) observed rapid ozonation of acyclovir and carboxy-acyclovir under typical treatment conditions for both treated wastewater and drinking water in Germany (pH 7–8 and ozone content of dissolved organic carbon of 0.4). However, there is no report so far about transformation of the antivirals during chlorination. Therefore, further discussion or speculation cannot be made based on the limited data available.

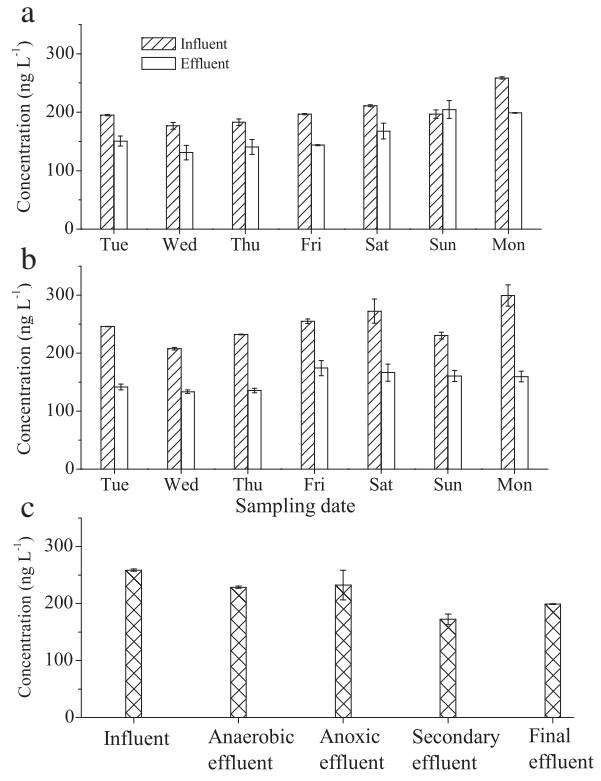

Fig. 3. Daily variation of acyclovir concentration in the wastewater in (a) the first treatment line, (b) the third line, and (c) its behavior in the STP. Error bars represent analytical standard deviations (n = 2).

3.2. Antiviral drugs in the river water

Acyclovir was also the only antiviral pharmaceutical quantified in the river water with a maximum concentration of 113 ng L^{-1} (Fig. 4a and Table S2 in Supporting material), agreeing well with the result of the wastewater mentioned above. This result was quite different from those reported for the river water in other countries. For instance, zidovudine, oseltamivir, and oseltamivir acid were detected in German rivers with maximum concentrations of 170, 15, 24 ng L^{-1}, respectively (Prasse et al., 2010). Oseltamivir and oseltamivir acid were up to 288 ng L^{-1} in river waters of Japan during influenza outbreak seasons (Azuma et al., 2012), and the highest concentration measured for zidovudine was up to 9 μg L^{-1} in Nairobi river water, Kenya, which was ascribed to high HIV/AIDS prevalence in that region (Koreje et al., 2012). In contrast, acyclovir that was widely detected in the Pearl River catchment in this work was not detected in Nairobi river basin. These results suggested different usage of the antivirals in different regions.

Spatially, in Liuxi River (LX1–LX3) that runs through rural and suburban areas, acyclovir was only occasionally detected with low ng L^{-1} concentrations (not detected — 6 ng L^{-1}). On the contrary, throughout the mainstream of the Pearl River (PR1–PR8) and the other three tributary rivers, acyclovir was ubiquitously detected at 9–113 ng L^{-1} (Table S2, Supporting material), comparable to those in the rivers and streams in Hessian Ried (2–190 ng L^{-1}) but slightly higher than those

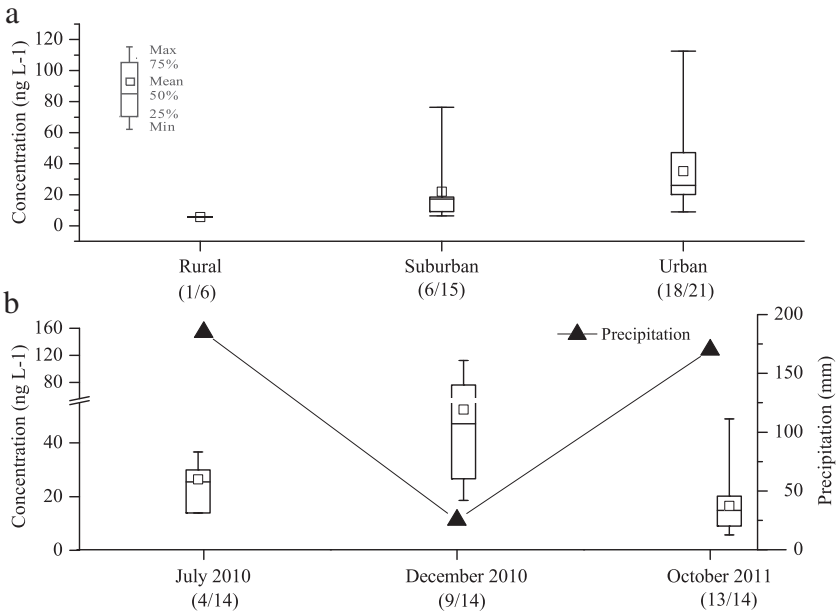

Fig. 4. Spatial distribution (a) and seasonal pattern (b) of acyclovir in the Pearl River and the tributaries. Detection frequency is presented as samples quantified/samples analyzed in the parentheses.
Data of precipitation was obtained from Guangzhou Statistics (2013).

in Ruhr and its tributaries (3–31 ng L^{-1}) in Germany (Prasse et al., 2010). Both detection frequency and concentration of acyclovir showed an order of urban sections > suburban section > rural section (Fig. 4a), which was quite different from that in German rivers where acyclovir concentration decreased within the urban area (Prasse et al., 2010). Sites right downstream of large scale STPs (i.e., PR3 and PR6) witnessed higher concentration, implying that treated municipal wastewater was a significant source of acyclovir in the surface waters of the Pearl River catchment, which may be associated with its uncompleted elimination in wastewater during treatment in the STPs.

Previous works have revealed seasonal effects on distributions of pharmaceuticals in the environment (Choi et al., 2008; Vieno et al., 2005; Yu et al., 2011). The concentrations of oseltamivir and oseltamivir carboxylate in two Japanese rivers were found to strongly increase during conventional flu-seasons (Azuma et al., 2012; Soderstrom et al., 2009). In this work, in the Pearl River and the tributaries, both the maximum and average concentrations were the highest in winter (113 ng L^{-1} and 54 ng L^{-1}, respectively), while the highest detection frequency was observed in fall (Fig. 4b, Table S2, Supporting material). This is quite different from the result of the wastewater mentioned above that the acyclovir concentration in the influent was higher in summer than in winter whereas the concentration in the treated effluent was similar in both seasons. Dilution effect by rainfall was thus suggested to be the predominant factor affecting the seasonal pattern of acyclovir in the Pearl River catchment evidenced by the reverse trend of the precipitation to acyclovir concentration (Fig. 4b). Impacts of water flow on distribution of pharmaceuticals in rivers have been reported previously (Kolpin et al., 2004; Yu et al., 2011).

3.3. Antivirals in the reservoir water and well water

Acyclovir and ganciclovir were detected in the landfill leachate at 967–2394 (mean of 1650) and 418–1131 (mean of 794) ng L^{-1}, respectively. In the water of the reservoirs, acyclovir was frequently detected, ranging from non-detectable to 34 ng L^{-1} (mean of 13 ng L^{-1}, Table S3, Supporting material). Spatially, the concentration showed a decreasing trend with an increase of distance from landfill LF2. However, it cannot be confirmed whether acyclovir in the reservoirs sourced from leakage of the landfill leachate due to limited samples of reservoir water as well as landfill leachate. The mean concentration of acyclovir in the reservoir water showed an order of winter (17 ng L^{-1}) > spring (14 ng L^{-1}) > summer (11 ng L^{-1}) > fall (7 ng L^{-1}). However, the seasonal difference was not statistically significant ($p > 0.05$, ANOVA) and more samples are needed to better elucidate the seasonal variation.

None of the antivirals was detected in either the well waters or the spring water, suggesting that municipal landfills not likely cause significant contamination of antivirals in the groundwater in the vicinity. Prasse et al. (2011) also did not observe quantifiable acyclovir in the groundwater in Germany. However, the metabolite carboxy-acyclovir was detected at about 200 ng L^{-1} in the oxic groundwater and non-detectable in the anoxic groundwater in Germany (Prasse et al., 2011). Unfortunately, carboxy-acyclovir was not targeted in this work. Metabolites of antiviral pharmaceuticals should thus be included in the future work to better illustrate the occurrence and fate of antivirals in the environment.

4. Conclusions

Occurrence and fate of commonly prescribed antiviral drugs were investigated in wastewater, landfill leachate, river water, reservoir water, and well water of the Pearl River Delta, South China. Only acyclovir was quantitatively detected in the wastewater, rivers, and reservoirs. Although acyclovir and ganciclovir were detected at rather high concentrations in the landfill leachates, none of the antivirals were detected in the well water in the vicinity of the municipal landfills.

Acyclovir concentration was 238 ± 56 (177–406) and 154 ± 24 (114–205) ng L^{-1} in the influent and final effluent samples, respectively. Aerobic biodegradation appeared as the dominant process for the removal of acyclovir in the wastewater during treatment in the STP. Spatially, urban sections of the Pearl River had both the highest concentration and detection frequency of acyclovir. Treated municipal wastewater was a significant source of acyclovir in the mainstream Pearl River. The highest concentration of acyclovir was observed in winter in the Pearl River and the tributaries and dilution effect by precipitation was supposed as the most significant factor impacting the seasonal pattern in the rivers.

Conflict of interest

The authors declare that there is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years since the beginning of the submitted work that could inappropriately influence, or be perceived to influence this work.

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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.2013.07.062.

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