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RESEARCH ARTICLE

Occurrence of diazepam and its metabolites in wastewater and surface waters in Beijing

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Abstract Occurrence of diazepam and its metabolites, nordiazepam, temazepam, and oxazepam in the water environment in Beijing was investigated. Samples were collected from four rivers flowing through the city and from all the thirteen sewage treatment plants in the urban area. Average influent concentrations of diazepman, temazepam, and oxazepam in 2013 summer ranged from 0.9 to 7.1, 1.5 to 3.4, and 2.9 to 12.4 ng L^{-1}, respectively, whereas nordiazepam concentrations were below quantification limit on the majority of sampling dates. No significant seasonal variation in influent concentrations was observed. Removal during treatment was low for diazepman (<50%), temazepam (<20%), and oxazepam (<20%), consistent with previous findings reported in the literature. Wastewater-based epidemiology approach was applied to back-calculate population size-normalized diazepam consumption (using temazepam as biomarker) in Beijing, which was found to be at least 3.8 times more of the national average. Diazepam, temazepam, and oxazepam were widely detected in surface waters, with concentrations greater than concentrations in sewage influents at many sampling points, strongly indicating direct discharge of wastewater of high diazepam concentrations into the surface waters in the city.

Keywords Diazepam · Metabolites · Occurrence · Consumption · Sewage-based epidemiology

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Introduction

Pharmaceuticals are a large and diverse group of organic compounds used in huge quantities around the globe (Daughton and Ternes 1999; Jones et al. 2005). Most pharmaceuticals are not completely metabolized by human bodies and are excreted either unchanged or as metabolites or conjugates (Heberer 2002). The excreted parent compounds and metabolites typically enter sewage and undergo wastewater treatment processes. For many pharmaceuticals and metabolites, complete removal cannot be achieved during treatment. Thus, their residues may be released into the environment via effluent discharge or land application of sludge. The released residues may pose adverse effects to humans and wildlife at very low concentrations (Henry and Black 2007; Jones et al. 2005). For this reason, pharmaceuticals and their metabolites have been widely considered as a group of emerging contaminants. Numerous studies have been performed to examine their occurrence, fate, and ecological effects in the environment (Kolpin et al. 2002; Pomati et al. 2007; Pomati et al. 2008).

Diazepam (DZP) is a well-known benzodiazepine-type drug that is commonly used to treat a range of symptoms including anxiety, insomnia, epileptics, and convulsion. In human bodies, it is metabolized into nordiazepam (NZP), temazepam (TZP), and oxazepam (OZP). TZP and OZP are

also pharmacologically active and used as anxiolytic drugs in some countries. Like many other pharmaceuticals, DZP and its metabolites have been widely detected in wastewater (Ternes et al. 2001), surface water (Calamari et al. 2003; Ternes 2001), and drinking water (Zuccato et al. 2000). Concentrations of these compounds found in waters range from a few nanograms per liter to a few hundred nanograms per liter (Calisto and Esteves 2009). Removal of DZP and its metabolites during sewage treatment was typically low (<50%) (Kosjek et al. 2012), which explains the wide detection of these compounds in surface waters.

While sewage treatment plays an important role in controlling the release of pharmaceuticals into the environments, it also provides an opportunity to estimate the consumption of pharmaceuticals. Drug consumption estimation can be done by the sewage-based epidemiology that is proposed by Daughton and Ternes (2001) and first implemented in 2004 by Zuccato et al. (2005). This approach involves collecting influents from sewage treatment plants (STPs) and measuring the concentrations of the residues of drugs or their metabolites in the samples. The drug consumption in the communities served by the sampled STPs are then back-calculated by taking account of the flow rates of STPs, populations of the communities, as well as the excretion rates of the drugs and their metabolites (Zuccato et al. 2008; Khan and Nicell 2012). This approach has the advantage that it can generate results in near real time and allows comparison of drug uses between different communities and at different time periods. In the past decade, the approach was extensively applied to estimate illicit drug use in Europe (Zuccato et al. 2008; Kasprzyk-Hordern et al. 2009; Nuijs et al. 2009), North America (Chiaia et al. 2008; Yargeau et al. 2014), Australia (Irvine et al. 2011; Lai et al. 2013a), and China (Lai et al. 2013b; Li et al. 2014; Du et al. 2015; Li et al. 2016). In addition, this approach has also been applied to other substances such as alcohol (Hall 2016; van Wel et al. 2016; Ryu et al. 2016; Gatidou et al. 2016), nicotine, caffeine (Gao et al. 2016), and more recently, psychoactive drugs (Gao et al. 2017; van Nuijs et al. 2015; González-Mariño et al. 2016). However, to the best of our knowledge, there are only two reports in the literature that employed wastewater-based epidemiology to estimate consumptions of benzodiazepines (including DZP, NZP, TZP, and OZP) and antidepressants (Baker et al. 2014; Mackulak et al. 2016).

DZP is the most widely used sedative-hypnotic drug in China. It is also a drug of abuse and is even used in crimes such as hijacking and robbery. However, studies on fate and occurrence of DZP and metabolites in the environment have been very scarce in China. Shao et al. (2009) reported that DZP concentrations ranged from below method detection limit (MDL) to 16 ng L^{-1} in slaughterhouse wastewater and from below MDL to 5 ng L^{-1} in the Nansha River in suburban Beijing. Yuan et al. (2013) found that OZP was below MDL in influents of three STPs in Beijing. Sun et al. (2014) reported that DZP concentrations were below 10 ng L^{-1} in influents of a STP in Xiamen. None of the above authors examined the occurrence of both DZP and its metabolites in wastewater, nor did they apply wastewater-based epidemiology to estimate DZP consumption.

The overall objective of this work was to examine the occurrence of DZP, NDZP, TZP, and OZP in the water environment in the urban area of Beijing. All 13 STPs in the urban area were sampled, each during two campaigns, to examine spatial and seasonal variations in concentrations, as well as the removal efficiencies of these compounds. Four rivers flowing through the city were sampled to examine the occurrence of these compounds in surface water. DZP consumption was estimated using wastewater-based epidemiology and compared to national average and consumption in other countries.

Materials and methods

Wastewater sample collection

All the 13 domestic STPs (namely, Gabobeidian (GBD), Qinghe (QH), Xiaohongmen (XHM), Beixiaohe (BXH), Jiuxianqiao (JXQ), Fangzhuang (FZ), Wujiacun (WJC), Lugouqiao (LGQ), Tongzhou Bishui (TZBS), Xiaojiahe (XJH), Beiyuan (BY), Yongfeng (YF), and Wenquan (WQ)) located in the Beijing urban area (Fig. 1) were sampled. Sample collection was conducted in both summer (between 30th of June and 6th of August) and early winter (between 14th of Nov. and 24th of Nov.) in 2013. Each STP was sampled using autosamplers for 2 to 4 days by collecting consecutive 24-h composite samples in the summer season and for 2 days in the winter season. Details of sampling information (flow rates, population served, and sampling dates) are provided in Table S1 (Supporting Material). Wastewater samples were acidified to pH = 2 right upon collection, brought back to laboratory (typically within 4 h), and stored at −20 °C for 1–6 months until analysis.

Surface water sample collection

To examine occurrence of DZP, NZP, TZP, and OZP in surface waters, grab surface water samples were collected from four rivers (Qinghe, Liangshui, South Moat, and Tonghui) that flow through Beijing urban area in May 2014. The Qinghe River starts from the Fragrance Hill in the Haidian district, flows through Chaoyang and Changping district, and converges into the Wenyu River in Shunyi district. The 23.6-km-long river is the most important drainage river in the north part of the city. The Liangshui River, 68-km long, originates from Shijinshan district, flows through Fengtai, Daxing, and Tongzhou districts, and converges into the North Canal. The

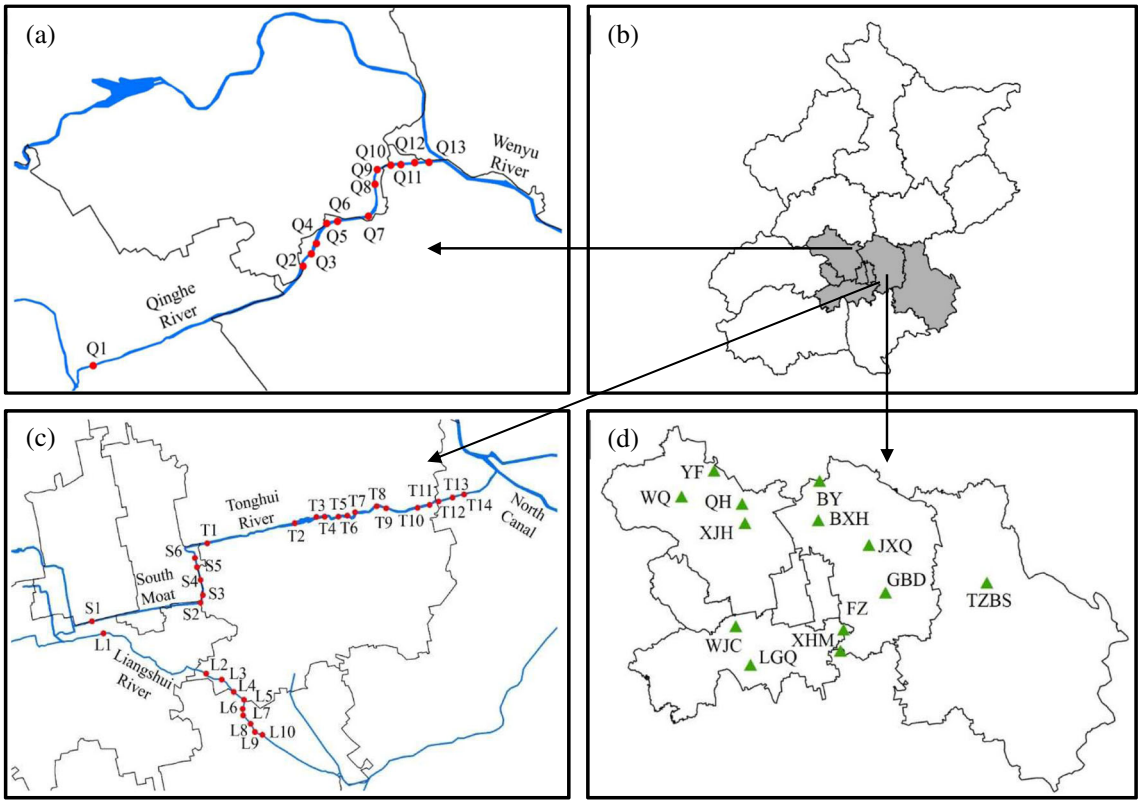

Fig. 1 Location of sampling points at four rivers (**a**, **c**) and 13 STPs (**d**) in Beijing. *Gray area* in (**b**) represents the urban area of the city

15.5-km-long South Moat flows through Xuanwu and Chongwen districts and is the upstream of the Tonghui River. The Tonghui River is 20 km long and converges into the North Canal in Tongzhou district. Samples (each 2 L in volume) were collected at 13 points at the Qinghe River (denoted as Q1–13), 9 points at the Liangshui River (L1–9), 6 points at the South Moat (S1–6), and 14 points at the Tonghui River (T1–14), respectively (Fig. 1). Strong rainfall was avoided during sampling. Samples were collected at points that were at least 2 m from riverbanks. Samples were then acidified to pH = 2 using HCl on the spot in pre-cleaned glass bottles, carried back to laboratory with ice, and stored at −20 °C for less than 2 months until analysis.

Analysis

Standards of DZP, NZP, TZP, OZP, and deuterated internal standards (DZP-d_5, NZP-d_5, TZP-d_5, OZP-d_5) were purchased from Sigma-Aldrich. Prior to solid phase extraction, 50 mL wastewater or 200 mL river water was filtered to remove solid particles, followed by adding deuterated internal standards for quantification. An Oasis MCX cartridge was conditioned in sequence with 6 mL methanol (MeOH), 4 mL deionized water, and 4 mL deionized water at pH = 2. The wastewater or river water was then loaded to the cartridge at a flow rate of 1 mL min^{-1}. The cartridge was then dried under vacuum and eluted with 4 mL of MeOH and 4 mL of 5% NH_3 in MeOH. The eluate was evaporated to dryness and redissolved in 400 μL acetonitrile (AcN)/water (5/95, *v*/*v*). A final cleaning step was performed using a 0.45-μm centrifugal filter (VWR International, Radnor, PA, USA).

A liquid chromatography system ($UFLC_{XR,}$ Shimadzu, Japan) with a Phenomenex Gemini C_{18} column (50 mm × 2 mm, 3 μm) was used to separate the compounds. The injection volume was 20 μL. The mobile phase was 5 mM ammonium formate in ultrapure water (A) and AcN (B). The elution gradient was as follows: 0–0.5 min: 95% A, 5% B; 0.5–2.0 min: 70% A, 30% B; 2.0–6.5 min: 55% A, 45% B; 6.5–8.5 min: 10% A, 90% B; and 8.5–14.0 min: 95% A, 5% B. The flow rate of mobile phase was 0.5 mL min^{-1}. Concentrations were determined using an API 4000 triple quadrupole mass spectrometer (AB SCIEX, USA) equipped with an electrospray interface operating in a positive ionization mode. The MS system was operated in multiple reaction monitoring (MRM) mode for quantification. Details of MS parameters and method quantification limits (MQLs) of the target compounds are provided in Table S2.

To validate the analytical methods, influent wastewater collected at FZ STP on July 1 of 2013 was spiked with target compounds at two concentrations (200 and 400 ng L^{-1}) and followed the same pretreatment procedures. The concentrations of the spiked and unspiked (as blank) samples were

determined. The differences in concentrations between the spiked and unspiked samples were divided by the spiked concentrations to yield recoveries. Matrix effects were determined by spiking the extracts (from solid phase extraction process) of the influent wastewater (also collected at FZ STP). The differences in concentrations between the spiked and unspiked samples were divided by the spiked concentrations to yield matrix effects. The recoveries and matrix effects of target compounds ranged from 73.9 to 99.9% and from 70.1 to 93.1%, respectively (Table S3).

Stability test

To examine the stability of compounds during storage, raw wastewater was collected from XJH plant on December 10, 2016. Each compound was spiked separately to the wastewater to around 500 ng L^{-1}, with three replicates. An aliquot of 50 mL was taken immediately from the spiked wastewater. The spiked wastewater was left under room temperature for 24 h. Then, another aliquot of 50 mL was sampled. The aliquots were pretreated and analyzed following the procedure described above. The difference between the average concentration right after spiking and the average concentration after 24 h was divided by the concentration right after spiking to yield degradation of a compound during storage. Degradation of DZP, NZP, OZP, and TZP was all less than 10%, indicating that these compounds were quite stable during sample collection. It is worth noting, however, stability of these compounds in sewers may be different from the stability during sample collection as wastewater in sewers may have higher biological activity than the collected wastewater. Future work is warranted to address this limitation.

Load estimation and apparent removal

The daily mass load of each target drug residue per 1000 inhabitants at a specific STP was estimated using the following equation:

$$\text{Load of a residue}\left(\frac{mg}{1000\ \text{in}h\cdot d}\right) = \frac{\text{Residue conc.}\left(\frac{ng}{L}\right)\times \text{influent flow}\left(\frac{L}{d}\right)}{\frac{\text{Population served}}{1000}} \times \frac{1}{10^6}\left(\frac{mg}{ng}\right) \quad (1)$$

Concentrations of the drug residues were determined using the analytical methods described above. Influent flows on each day of sampling were provided by the STPs (Li et al. 2014). Populations served by STPs were either obtained from the STPs or based on the most recent census data of the service areas (Li et al. 2014). Apparent removal rates were derived by dividing the difference between the influent and effluent concentrations at a STP by the influent concentrations:

$$\text{Apparent removal} = \frac{\text{Influent concentration} - \text{Effluent concentration}}{\text{Influent concentration}} \quad (2)$$

Results and discussion

Concentrations of DZP, NZP, TZP, and OZP in influent wastewater

DZP was detected and quantified in on all sampling dates at GBD, QH, XHM, BXH, JXQ, LGQ, XJH, and WQ in 2013 summer (Table 1). DZP concentrations were below method quantification limit (MQL, 1 ng L^{-1}) on one or two sampling dates at YF, FZ, WJC, BY, and TZBS. At the overwhelming majority of the STPs, DZP concentrations were below 5 ng L^{-1}. The exceptions were one sampling date at JXQ (6.0 ng L^{-1}) and two sampling dates at TZBS (19.8 and 6.9 ng L^{-1}). In general, DZP concentrations observed in this work were similar to influent concentrations reported in previous studies at Beijing and Xiamen of China (Shao et al. 2009; Sun et al. 2014), slightly higher than those at STPs in Las Vegas (Vanderford and Snyder 2006), slightly lower than those reported in England (Baker and Kasprzyk-Hordern 2013), and much lower than those reported in Germany (Wolf et al. 2004), Slovenia (Kosjek et al. 2012), and Belgium (Ven et al. 2004).

NZP was below MQL on all sampling dates at GBD, QH, BXH, LGQ, XJH, and WQ and was above MQL but less than 3 ng L^{-1} on some sampling dates at other STPs (Tables 1 and 2). The low frequency in the detection of NZP was probably due to its low excretion rate (Calisto and Esteves 2009) and relatively high MQL (2 ng L^{-1}) (compared to those of DZP, TZP, and OZP). NZP concentrations reported in England were much higher than in this work (Baker and Kasprzyk-Hordern 2011; Baker and Kasprzyk-Hordern 2013). Influent TZP concentrations ranged from below MDL to 4.3 ng L^{-1}, whereas influent OZP concentrations ranged from below MDL to 19.5 ng L^{-1} (Table 1). Influent TZP concentrations observed here were much lower than those in England (Baker and Kasprzyk-Hordern 2011; Baker and Kasprzyk-Hordern 2013) and Netherlands (Aa et al. 2013). OZP concentrations were also much lower than those in England (Baker and Kasprzyk-Hordern 2011; Baker and Kasprzyk-Hordern 2013), Netherlands (Aa et al. 2013), and Germany (effluents) (Hass et al. 2012).

The reported excretion rates of DZP and OZP following DZP ingestion varied significantly in the literature (from <1 to

Table 1 Influent concentrations (ng L^{-1}) of DZP, OZP, NZP, and TZP at STPs other than XMH, BXH, and JXQ

STP	DZP	OZP	NZP	TZP	DZP	OZP	NZP	TZP
	Summer influent				Winter influent			
GBD	3.6	10.6	<MQL	3.1	1.6	16.4	<MQL	4.1
	3.9	9.5	<MQL	2.6	1.5	8.0	<MQL	2.2
Mean	3.7 ± 0.2	10.0 ± 0.8	<MQL	2.8 ± 0.4	1.5 ± 0.1	12.2 ± 5.9	<MQL	3.1 ± 1.3
QH	1.3	13.0	<MQL	2.8	1.1	13.7	<MQL	3.5
	2.2	10.8	<MDL	3.9	<MQL	6.2	<MQL	1.3
	2.2	7.0	<MQL	2.0	–	–	–	–
	2.3	6.7	<MDL	1.9	–	–	–	–
Mean	2.0 ± 0.5	9.4 ± 3.0	<MQL	2.7 ± 0.9	0.8 ± 0.4	9.9 ± 5.3	<MQL	2.4 ± 1.6
FZ	2.0	13.7	<MQL	2.3	<MQL	18.6	2.1	3.4
	1.6	16.2	2.1	4.1	–	–	–	–
	1.3	6.1	<MQL	2.6	–	–	–	–
	<MQL	13.5	<MQL	2.3	–	–	–	–
Mean	1.3 ± 0.6	12.4 ± 4.4	1.3 ± 0.5	2.8 ± 0.9	–	–	–	–
WJC	2.4	10.4	2.1	3.8	2.0	23.9	2.1	3.1
	0.5	4.5	<MQL	1.9	2.2	14.5	<MQL	3.0
Mean	1.5 ± 1.4	7.4 ± 4.2	1.5 ± 0.7	2.8 ± 1.3	2.1 ± 0.1	19.2 ± 6.6	1.6 ± 0.8	3.0 ± 0.1
LGQ	1.5	7.7	<MQL	2.1	2.6	8.2	<MQL	2.8
	2.5	9.9	<MQL	3.2	–	–	–	–
Mean	2.0 ± 0.7	8.8 ± 1.6	<MQL	2.6 ± 0.8	–	–	–	–
TZBS	1.3	9.9	<MQL	2.9	1.1	7.0	<MQL	1.7
	19.8	<MDL	<MDL	<MDL	–	–	–	–
	0.5	6.8	<MQL	2.3	–	–	–	–
	6.9	8.7	2.1	3.3	–	–	–	–
Mean	7.1 ± 8.9	6.3 ± 4.4	1.0 ± 0.9	2.1 ± 1.5	–	–	–	–
XJH	2.5	6.6	<MQL	2.9	4.3	17.3	2.3	3.4
	2.0	6.5	<MQL	2.4	2.4	6.1	<MQL	3.3
	1.1	5.8	<MQL	2.9	–	–	–	–
Mean	1.9 ± 0.7	6.3 ± 0.4	<MQL	2.7 ± 0.3	3.3 ± 1.3	11.7 ± 8.0	1.6 ± 0.9	3.3 ± 0.1
BY	1.4	9.8	2.0	3.2	<MQL	7.3	<MQL	2.0
	3.6	8.9	<MQL	3.5	–	–	–	–
	1.9	5.7	<MQL	1.6	–	–	–	–
	<MQL	7.3	<MQL	2.5	–	–	–	–
Mean	1.8 ± 1.3	7.9 ± 1.8	1.3 ± 0.5	2.7 ± 0.8	–	–	–	–
YF	<MQL	4.5	<MQL	2.4	<MQL	8.8	<MQL	2.0
	<MQL	3.8	<MQL	2.0	–	–	–	–
	1.0	3.7	<MDL	1.3	–	–	–	–
	1.7	19.5	2.8	4.2	–	–	–	–
Mean	0.9 ± 0.6	7.9 ± 7.8	1.2 ± 1.2	2.5 ± 1.2	–	–	–	–
WQ	1.4	2.7	<MDL	<MQL	<MQL	1.4	<MDL	1.0
	2.4	3.4	<MQL	2.5	–	–	–	–
	5.0	2.7	<MQL	1.4	–	–	–	–
Mean	2.9 ± 1.8	2.9 ± 0.4	<MQL	1.5 ± 1.0	–	–	–	–

10% for DZP and from 2.8 to 8.7% for OZP), where TZP excretion rates fell into a relatively narrow range (6.4–9.0%) (Calisto and Esteves 2009; Chiba et al. 1995; Arnold 1975). Generally, unchanged DZP was reported to be the minor excretion product compared to other metabolites, especially TZP and OZP (Baker et al. 2014). However, average DZP concentrations were similar to or slightly higher than OZP concentrations at TZBS and WQ. The exceedingly high

Table 2 Influent and effluent concentrations (ng L^{-1}) of DZP, OZP, NZP, and TZP at XMH, BXH, and JXQ STPs

STP	DZP	OZP	NZP	TZP	DZP	OZP	NZP	TZP	DZP	OZP	NZP	TZP
	Summer influent				Summer effluent				Winter influent			
XHM	2.1	9.9	<MQL	3.7	1.2	7.1	0.9	3.4	1.5	13	<MQL	2.6
	2.1	7.7	2.1	2.4	1.6	9.6	1.1	2.1	1.2	7.7	<MQL	1.9
	2.2	7.3	<MDL	2.3	2.7	8.9	1.0	2.4	–	–	–	–
	1.9	10.4	<MQL	3.1	1.2	10.0	0.9	2.3	–	–	–	–
	3.1	14.2	2.4	3.9	–	–	–	–	–	–	–	–
Mean	2.3 ± 0.5	9.9 ± 2.8	1.3 ± 1.0	3.1 ± 0.7	1.7 ± 0.6	8.9 ± 1.1	1.0 ± 0.1	2.5 ± 0.5	1.3 ± 0.2	10.3 ± 3.8	<MQL	2.2 ± 0.5
BXH	2.4	8.6	<MQL	3.4	1.7	6.9	1.2	2.2	1.4	14.2	<MQL	3
	2.1	8.7	<MQL	2.8	1.4	7.7	1.3	2.5	<MQL	4.5	<MQL	2.1
	2.5	9.8	<MQL	4.3	0.8	8.2	1.1	2.1	–	–	–	–
	3.2	12.6	<MQL	3.2	1.3	16.4	2.2	4.0	–	–	–	–
Mean	2.5 ± 0.5	9.9 ± 1.8	<MQL	3.4 ± 0.7	1.3 ± 0.3	9.8 ± 3.8	1.5 ± 0.4	2.7 ± 0.8	0.9 ± 0.6	9.3 ± 6.9	<MQL	2.5 ± 0.6
JXQ	2.4	11	<MQL	3.1	2.2	8.7	1.5	2.2	1.4	9.4	<MQL	3.7
	6	8.2	<MQL	2.2	4.7	9.5	1.4	2.6	–	–	–	–
	2.9	9.3	<MQL	3.5	2.8	7.5	<MDL	2.1	–	–	–	–
	4.9	8.7	2.1	2.7	–	–	–	–	–	–	–	–
Mean	4.0 ± 1.7	9.3 ± 1.2	1.3 ± 0.5	2.9 ± 0.6	3.2 ± 1.1	8.6 ± 0.8	1.4 ± 0.0	2.3 ± 0.2	–	–	–	–

DZP relative to OZP concentrations can only be explained by direct disposal of DZP in the communities served by the two STPs. Excluding TZBS and WQ, the mean ratio of DZP to OZP was 0.25 ± 0.09. The average ratios of NZP to TZP (determined based on NZP concentrations above MQL) was 0.64 ± 0.12. This ratio was slightly higher than that (about 0.5) obtained from urine specimens (Luk et al. 2014). The ratios of NZP to OZP and TZP to OZP were 0.19 ± 0.05 and 0.35 ± 0.07, respectively. These ratios were much lower than the ratios obtained from urine specimens (about 0.3 and 0.7, respectively) (Luk et al. 2014). This can be explained by the fact that in China OZP is also a prescribed drug, whereas NZP and TZP are solely from metabolism of DZP.

Fewer samples were collected during the sampling campaign conducted in early winter in 2013. The detection frequencies of DZP, NZP, TZP, and OZP in the winter season were similar to those in summer of 2013. In addition, the concentration ranges of these compounds were also similar to those observed in the summer season (Tables 1 and 2).

Loads of DZP, NZP, TZP, and OZP

Average loads of DZP, NZP, TZP, and OZP in the summer season ranged from 0.1 (±0.1) to 2.1 (±0.9), 0.1 (±0.0) to 0.6 (±0.3), 0.2 (±0.0) to 1.5 (±0.3), and 0.7 (±0.0) to 4.7 (±0.6) mg/1000 inh · d, respectively (Table 3). The lowest and highest loads of the four compounds all occurred at BY and JXQ, respectively. The loads at the STPs in the urban center (GBD, XMH, FZ, JXQ, and QH) were in general higher than those at the STPs in the suburban areas (e.g., XJH, BY, and TZBS). There was no clear trend with regard to average loads in the winter season relative to loads in the summer season. The loads of NZP, TZP, and OZP were much lower than those reported at a STP in England (4.7–5.9, 47.6–59.7, and 7.9–9.9 mg/1000 inh · d, respectively, derived based on daily loads to the STP and the population STP served) (Baker et al. 2014).

Back-calculated DZP consumption

Since there might be direct disposal of DZP, and the reported excretion rate of unchanged DZP varies significantly in the literature, DZP is not an appropriate target residue to back-calculate DZP consumption. As NZP concentrations were above MQL only in a small fraction of influent samples, NZP could not be used for DZP consumption calculation either. OZP is also a prescription drug in China, but its prescription or production data is not available. Thus, OZP could not be used to back-calculate DZP consumption either. TZP is not prescribed in China, and thus, it is solely from DZP metabolism. In addition, TZP could be quantified in almost all the influent samples. Thus, DZP consumption in Beijing was back-calculated by dividing the total TZP load by the excretion rate of TZP and by the molecular weight ratio between TZP and DZP:

$$\text{DZP consumption} = \textit{TZP}\ \text{load} \times \frac{\textit{DZP}\ \text{molecular weight}}{\textit{TZP}\ \text{molecular weight}} \frac{1}{\textit{TZP}\ \text{excretion}}$$

TZP excretion following DZP administration was reported to range from 6.4 to 9.0% in the literature (Arnold 1975;

Table 3 Loads (mg/1000 inh · d) of DZP, NZP, TZP, and OZP at the STPs

STP	DZP		NZP		TZP		OZP	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
GBD	1.0 (0.1[a])	0.4 (0.0)	<0.5	<0.5	0.8 (0.1)	0.8 (0.4)	2.7 (0.2)	3.2 (1.6)
QH	0.7 (0.2)	0.3 (0.2)	0.2 (0.2)	<0.7	1.0 (0.3)	0.9 (0.6)	3.4 (1.1)	3.6 (1.9)
XHM	0.4 (0.1)	0.2 (0.0)	0.2 (0.2)	<0.4	0.6 (0.1)	0.4 (0.1)	1.8 (0.5)	1.9 (0.7)
BXH	0.9 (0.2)	0.3 (0.2)	<0.7	<0.7	1.2 (0.2)	0.9 (0.2)	3.5 (0.6)	3.3 (2.4)
JXQ	2.1 (0.9)	0.7	0.6 (0.3)	<1.0	1.5 (0.3)	1.9	4.7 (0.6)	4.8
FZ	0.3 (0.1)	<0.2	0.3 (0.1)	0.5	0.7 (0.2)	0.8	3.0 (1.0)	4.4
WJC	0.5 (0.4)	0.7 (0.0)	0.5 (0.2)	0.5 (0.2)	0.9 (0.4)	0.9 (0.0)	2.3 (1.3)	6.0 (2.1)
LGQ	0.3 (0.1)	0.4	<0.3	<0.3	0.4 (0.1)	0.4	1.4 (0.2)	1.3
TZBS	1.2 (1.5)	0.2	0.2 (0.2)	<0.4	0.4 (0.3)	0.3	1.1 (0.8)	1.2
XJH	0.2 (0.1)	0.4 (0.1)	<0.2	0.2 (0.1)	0.3 (0.0)	0.4 (0.0)	0.7 (0.0)	1.3 (0.9)
BY	0.2 (0.1)	<0.1	0.1 (0.0)	<0.2	0.2 (0.1)	0.2	0.7 (0.2)	0.6
YF	0.2 (0.1)	<0.2	0.3 (0.3)	<0.5	0.6 (0.3)	0.5	1.8 (1.8)	2.0
WQ	1.0 (0.6)	<0.3	<0.7	<0.7	0.5 (0.3)	0.3	1.0 (0.1)	0.5

[a] Standard deviation

Chiba et al. 1995). Based on the reported excretion rate range, DZP consumption in Beijing fell within the range between 7.6 and 10.6 mg/1000 inh · d. This consumption was much lower than that reported in England (28 mg/1000 inh · d) by Baker et al. who used OZP as the biomarker and an excretion rate of 33% (Baker et al. 2014). Based on the DZP production in China in 2013 (CMEIN 2014), the maximum national average consumption (assuming all DZP produced was consumed) was 2.0 mg/1000 inh · d. It is clear that DZP consumption in Beijing was at least 3.8 times of the national average.

Removal of DZP, TZP, and OZP

Effluent samples collected at XMH, BXH, and JXQ in 2013 summer were analyzed to derive apparent removal rates. The effluent concentrations of DZP, NZP, TZP, and OZP are presented in Table 2. Since NZP concentrations were below MQL in most influent and effluent samples, removal of this compound was not calculated. Average removal rate of DZP ranged from −24% at XMH to 46% at BXH, whereas average removal rates of TZP and OZP were below 20% at all the STPs (Fig. 2). Negative apparent removal was observed for all the three compounds at one STP at least. The low and even negative removal rates were consistent with previous studies (Calisto and Esteves 2009; Kosjek et al. 2012; Sun et al. 2014). Negative removal observed in this work may arise from the fact that the influent and effluent samples that were collected at the same time, which leaded to a mismatch between influent and effluent samples due to hydraulic retention. If there is a pulse of higher concentration during the mismatch, apparent negative removal can be expected.

Concentrations in surface water

Among the four rivers, NZP was quantifiable at only one sampling point (T7, 4.2 ng L^{-1}) of the Tonghui River (Fig. 3, Table S4), consistent with the very low detection frequencies observed at STPs throughout Beijing (Tables 1 and 2). TZP concentrations were above MQL at all but one point (Q8), with the maximum concentration (13.1 ng L^{-1}) occurring at Q10. TZP showed similar detection frequencies, equivalent median concentrations, and similar concentration ranges in the four rivers (excluding Q8 and Q10) (Fig. 3, Table S4). TZP concentrations at the majority of the sampling points were greater than 4 ng L^{-1}, whereas influent concentrations at the overwhelming majority of the STPs were less than 4 ng L^{-1}. OZP concentrations were below

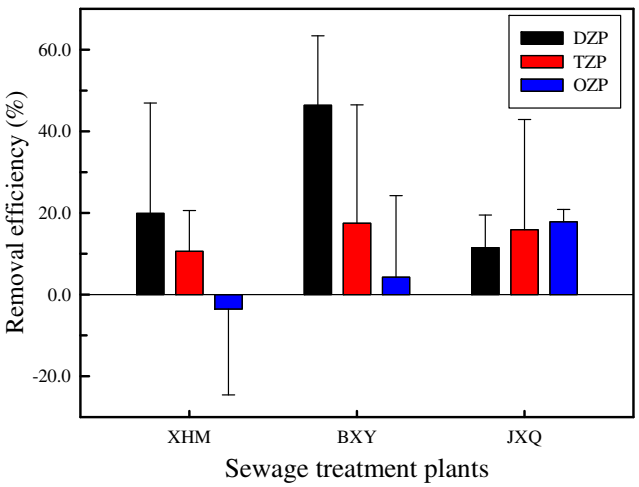

Fig. 2 Removal of DZP, TZP, and OZP at three STPs

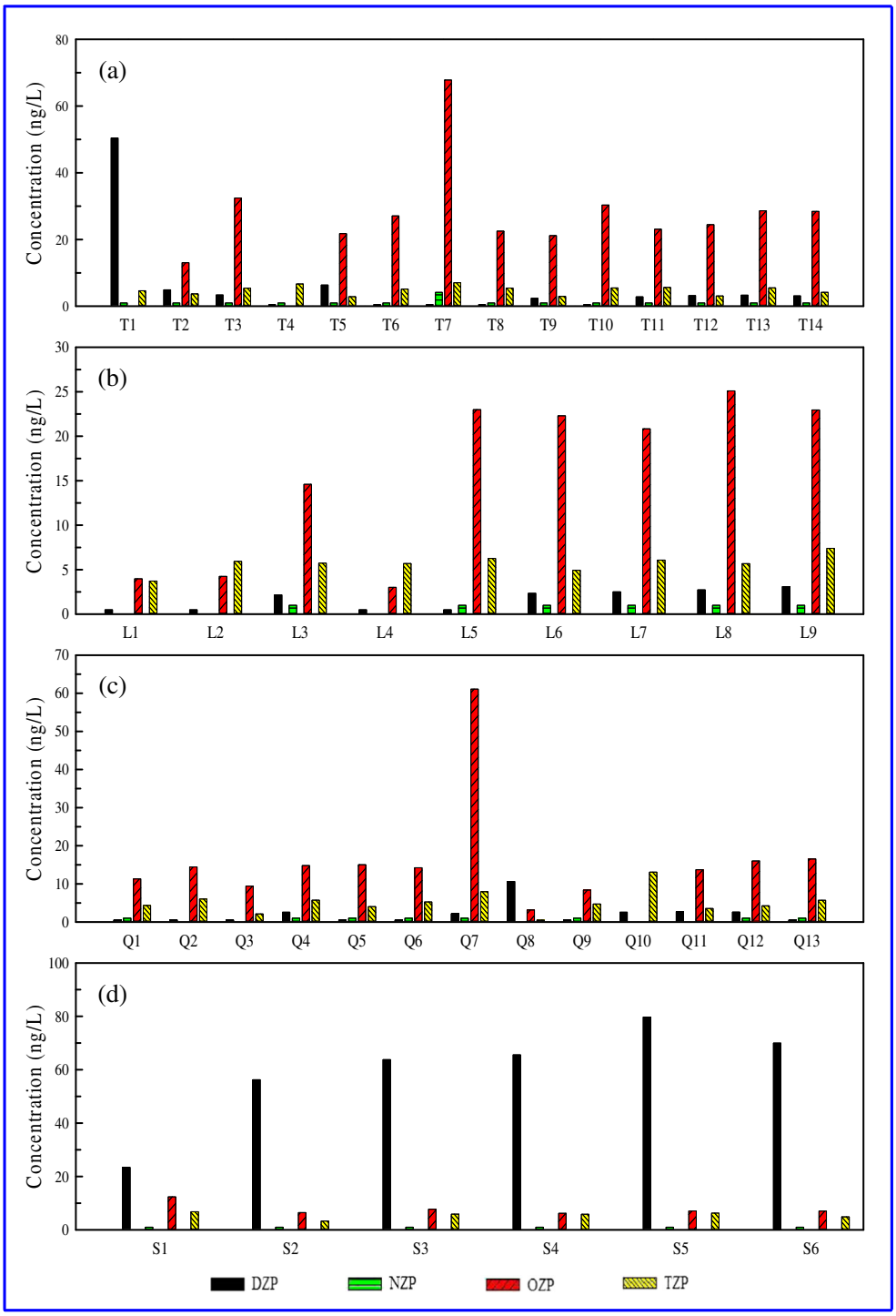

Fig. 3 Concentrations (ng L^{-1}) of DZP, OZP, NZP, and TZP in Tonghui (**a**), Liangshui (**b**), Qinghe River (**c**), and the South Moat (**d**). For sampling points where compounds could not be detected (concentrations below method detection limit), concentrations were assigned with zero and plotted. For sampling points where compounds could be detected but could not be quantified, method quantification limit (MQL) were assigned and plotted

MQL at only three sampling points (Q9, T1, T4), with the maximum concentration occurring at T7 (67.8 ng L^{-1}). OZP concentrations at the majority of points of the Tonghui River and at the downstream points of the Liangshui River (L5–L9) were much greater than the highest average concentrations observed in STP influents (12.4 ± 4.4 ng L^{-1} at FZ). OZP concentrations at the majority of points of the Qinghe River were slightly greater than the highest influent concentration, whereas OZP concentrations at the South Moat fell within the range of influent concentrations. Overall, higher TZP and OZP concentration levels in the Tonghui, Liangshui, and Qinghe rivers relative to sewage influent concentrations indicate that STP effluents were not the only source of TZP and OZP in these rivers. It appears that direct discharge of sewage (without going to STPs) and overflow from

STPs may contribute significantly or even predominantly to the presence of TZP and OZP in the rivers.

Quantification frequencies of DZP were 100% at the South Moat, 64.3% at the Tonghui River, 55.6% at the Liangshui River, and 46.2% at the Qinghe River, respectively. Large fluctuations in DZP (as well as TZP and OZP) concentrations were observed along the Tonghui, Liangshui, and Qinghe rivers. Löffler et al. (2005) demonstrated that DZP degradation in surface water was very slow. Thus, large fluctuations in DZP concentrations indicate dilution and continued discharge along the rivers. The highest DZP concentration, 79.7 ng L^{-1}, was observed at S5. In the Tonghui, Qinghe, and Liangshui rivers, concentrations followed the same overall trend of OZP > TZP > DZP, which was also observed in STP influent. However, DZP concentrations were much higher than TZP and OZP concentrations at all sampling points of the South Moat and T1 of the Tonghui River. DZP concentrations at the South Moat points and T1 were higher than influent concentrations at most STPs by more than one order of magnitude. It is worth noting that a preliminary effort that collected samples at S4 and S6 in November 2013 also detected very high DZP concentrations at these points (43.7 and 37.8 ng L^{-1}, respectively). Extremely high DZP concentrations in South Moat indicate high likelihood of direct discharge of wastewater of high DZP concentrations into the river.

DZP concentrations in the South Moat were higher than most reported values in the literature. The only exception was in surface water in Germany (0.88 μg L^{-1}) (Ternes 1998). The median DZP concentrations in the Tonghui, Liangshui, and Qinghe rivers were higher than the concentrations observed in Lambro and Po rivers in Italy (Calamari et al. 2003) and in England (Baker and Kasprzyk-Hordern 2011), much lower than the concentrations at some points in rivers in Romania and Germany (Ternes et al. 2001; Moldovan 2006; Ternes 1998). NZP concentrations in the four rivers were lower than values reported in rivers in Spain and England (Alonso et al. 2010; Baker and Kasprzyk-Hordern 2011). Mean OZP concentration in a river in England fell within the range of median OZP concentration in the four rivers of this work (Baker and Kasprzyk-Hordern 2011), whereas the median concentration in a river in Spain was higher than the upper end of the range of this work (Alonso et al. 2010). Occurrence of TZP in surface water has been rarely reported in the literature. Baker and Kasprzyk-Hordern (2011) reported a mean TZP concentration of 27.8 ng L^{-1} in a river in England, much higher the maximum concentration in rivers of this study. High TZP and NZP concentrations in sewage influent and river samples observed by Baker and Kasprzyk-Hordern (2011) are understandable, as TZP and NZP are also prescribed drugs in England.

Conclusions

Sewage (both influents and effluents) and surface water samples were collected to examine occurrence of DZP and its metabolites in the water environment in Beijing. Sewage influent concentrations of DZP and metabolites followed the order of OZP > TZP > DZP > NZP and were in general lower than the concentrations reported in European countries. Low and even apparent negative removal rates were observed for DZP, TZP, and OZP during sewage treatment, consistent with findings by previous studies. DZP consumption back-calculated using wastewater-based epidemiology was much higher than the national average estimated using DZP production data in China. DZP, TZP, and OZP concentrations at many points of the sampled rivers were greater than sewage influent concentrations, indicating high likelihood of direct disposal of some of these compounds and direct discharge of sewage into the surface waters in the city.

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