附件二　　被提名：✓　自荐：____

青年人才托举工程
提 名 书

被托举人姓名 杜鹏

工作单位 北京师范大学

提名机构 中国地理学会学术工作委员会

提名专家姓名 _____

中国地理学会　制

2019 年 12 月

基本情况

1.个人基本信息

姓　名	杜鹏	性别	男	出生年月	1987 年 7 月
民 族	汉	职务	无	学　位	博士
身份证号	230903198707010817		会员证号	S110013814M	
专业方向	环境地理	专业技术职称		讲师	
工作单位	北京师范大学				
通讯地址	北京市 海淀区 新街口外大街 19 号			邮　编	100875
单位电话	010-62205905	手 机		18600562956	
Email	dup@bnu.edu.cn				

主要教育经历（从大学填起，包括国外学历）

起止年月	学校（院）及系名称	专　业	学　位
2011 年 9 月至 2016 年 7 月	北京大学 城市与环境学院	环境地理	博士
2007 年 9 月至 2011 年 7 月	东北农业大学 资源与环境学院	环境科学	学士

主要工作经历（毕业以后从事科技第一线工作的经历）

起止年月	工作单位及部门	职务/职称
2018 年 7 月 至今	北京师范大学/水科学研究院	讲师
2016 年 7 月至 2018 年 6 月	北京大学/城市与环境学院	博士后

在国内外学术组织任职、后备情况

中国地理学会会员

主要科研项目介绍（限 800 字以内）

- 国家自然科学基金青年科学基金项目，41701543，典型城市生活污水和地表水中常见新精神活性物质的时空演变特征，2018.01-2019.12，主持
- 第二次青藏高原综合科学考察研究，任务三，土壤质量变化及其对生态系统的影响专题（2019QZKK0306），子子专题：青藏高原典型区域水蚀、风蚀区土壤重金属背景调查，2019.11-2022.10，主持
- 国家自然科学基金面上项目，41371442，典型违禁药物在城市生活污水和地表水中残留浓度的分析和污水流行病学研究，2014.01-2017.12，主要参加人
- 中国禁毒基金会污水验毒试点项目，通过污水检测毒情方法学研究-以中山市为例，2018.01-2018.12，主要参加人

被托举人主要从事环境地理相关研究，具体开展新型污染物排放、环境行为、归趋及污水流行病学等方向的相关工作。建立了环境中多种超低含量新型污染物（如：毒品、新精神活性物质等）及其代谢产物的分析方法；通过对全国范围地表水和生活污水的分析测定，研究了毒品等新型污染物在中国水环境中的赋存与归趋，并对其造成的生态风险进行评估；同时结合污水流行病学方法对全国范围毒品滥用水平进行估算，分析了我国毒品滥用的时空分异特征及影响因素。拓展了国内环境地理领域研究的新方向，研究成果被国际知名学术期刊《Nature》以新闻形式报道[1]。与此同时，建立并验证基于地表水和污水分析的大尺度区域人群新型污染物暴露估算及健康风险评价的法。

在科研成果转化方面，与公安部及广东省禁毒局合作，在广东省珠三角地区主要城市（广州、佛山、珠海和中山）建立了基于污水分析的毒情监测试点（污水验毒试点），为禁毒部门提供精准的毒情预测，并辅助当地公安部门破获多起毒品犯罪案件（含跨省案件）。基于试点工作前期取得成果，污水验毒工作已在广东省外多个城市（如：北京，上海等）开展，并成为缉毒工作的重要技术手段，所取得成果被中央电视台和地方媒体广为报道（详见证明材料）。目前，公安部充分肯定了污水验毒的可行性和必要性，已开始在全国范围推广污水验毒工作（首期 160 个地级市）。被托举人的研究成果为建立全国范围基于污水分析的毒情监测网络打下坚实基础。

参考文献：

1. Cyranoski, D., 2018. Chinese cities scan sewers for signs of illegal drug use. *Nature* 559, 310-311.

2.学术成果情况（以下信息请附有关证明材料复印件）

发表论文、专著的情况（限填有代表性的论文和著作），不超过 8 项

序号	论文、论著名称	年份	排名	发表刊物或出版社名称	是否被SCI/EI/SCOPUS检索收录	被引用次数
1	Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology	2015	1	Water Research	SCI	73
2	A revised excretion factor for estimating ketamine consumption by wastewater-based epidemiology – Utilising wastewater and seizure data	2020	1	Environment International	SCI	0

3	Using wastewater-based epidemiology to estimate consumption of alcohol and nicotine in major cities of China in 2014 and 2016	2020	5 (共同通讯作者)	Environment International	SCI	0
4	Estimating population exposure to phthalate esters in major Chinese cities through wastewater-based epidemiology	2018	1	Science of the Total Environment	SCI	14
5	Estimating heroin abuse in major Chinese cities through wastewater-based epidemiology	2017	1	Science of the Total Environment	SCI	19
6	Monitoring consumption of methadone and heroin in major Chinese cities by wastewater-based epidemiology	2019	1	Drug and Alcohol Dependence	SCI	4
7	Occurrence and Fate of Heavy Metals in Municipal Wastewater in Heilongjiang Province	2020	1	Water	SCI	0
8	污水中新精神活性物质的分析方法优化及验证	2018	2	环境科学	SCOPUS	2

获奖情况，不超过 3 项				
序号	获准时间	奖项名称	奖励名称/等级	排名情况
1	2017 年	北京大学优秀博士后	校级	1
专利情况，不超过 5 项				
序号	专利名称	专利号/专利状态	排名情况	
1	复合型纳米零价铁颗粒	ZL201510176103.8	3	

2019-2021 年青年人才托举工程重点评审内容

托举工程实施内容

重点介绍 2019-2021 年度科研工作设想与创新性、可行性以及需要“托举”的迫切性（800 字以内）。

1. 科研工作设想与创新性

在新冠肺炎全球大流行的背景下，严防境外输入病例和严防国内疫情二次爆发成为我国“抗疫战争”的首要任务。因此，如何对疫情进行有效预警不仅是我国防疫工作的刚性需求，也是未来全球防疫的核心工作之一。然而，目前世界范围内，仍缺乏能够对疫情进行有效预测的有效手段。近期，诸如《Nature》等[1,2]国际知名学术期刊均发文对基于污水分析的流行病学调查（污水流行病学）预警疫情爆发进行展望，一致认为该方法在疫情预警和公共健康监控领域具有发展潜力，并对其可行性表示了充分肯定。因此，本项目拟基于污水流行病学方法，研究如何建立具有时空展示功能的疫情预警及公共健康监测网络，为防疫工作提供有力支持。

2. 可行性分析

污水流行病学的原理为：能够特异性表征人体健康状况的生物标志物（如：细菌或病毒 DNA/RNA、寄生虫、药物及其代谢产物等）通过尿液排出体外，经市政排水管网汇集到生活污水处理厂，通过分析测定污水厂进水中生物标志物的浓度水平，根据污水厂服务区域人口、生物标志物排泄率及稳定性等参数，对污水厂服务区域疫情进行早期预警并评价人群健康状况。该方法从原理上完全可行，并可通过对多个污水厂实施连续监测，建立具有时空展示功能的疫情预警及公共健康监测网络。

此外，本人长期从事环境地理学与污水流行病学相关研究工作，发表相关 SCI 论文及著作 20 余篇，前期在基于污水流行病学方法建立毒品滥用监测网络上积累大量经验，为本研究的顺利实施奠定了坚实的基础。

3. 托举的迫切性

首先，从新冠肺炎疫情预警角度分析，本研究为国内外疫情防控急需突破的技术瓶颈，为长期疫情防控打基础；其次，从未来全民公共健康监控角度分析，本研究可为避免全球性公卫突发事件提供技术保障；最后，国外一些团队已经陆续开展污水流行病学预警疫情的相

关研究，我国更应紧跟国际脚步，走在疫情防控的最前沿。

参考文献：

1. Mallapaty, S., 2020. HOW SEWAGE COULD REVEAL TRUE SCALE OF CORONAVIRUS OUTBREAK. *Nature* 580, 176-177.
2. Sims, N., Kasprzyk-Hordern, B., 2020. Future Perspectives of Wastewater-Based Epidemiology: Monitoring Infectious Disease Spread and Resistance to the Community Level, *Environment International*. Doi: https://doi.org/10.1016/j.envint.2020.105689.

科研和职业生涯三年规划

（须明确在托举周期中每一年度的主要规划目标和完成计划，说明主要科研方向、个人成长规划、参加国内外学术交流活动计划、托举方向、专家指导和帮扶需求等。 800 字以内）

第一年度：

- 参加技术培训，提升个人专业能力；
- 完成基础资料收集工作，细化研究方案，选定拟研究的生物标志物；
- 建立目标生物标志的的分析测定方法；
- 选定目标污水处理厂，完成第一年度样品采集工作，并完成样品分析测定工作；
- 研究生物标志物在污水管网中的稳定性；
- 参加 1-2 次国内外学术会议，并邀请国外知名专家来国家指导交流；

第二年度：

- 完成第二年度样品采集工作，并完成样品分析测定工作；
- 步分析污水中生物标志物与疫情及常见疾病之间的关系，建立基于污水流行病学的疫情预警及公共健康评价方法；
- 参加国内学术会议，与领域内专家交流讨论；
- 发表学术论文 1-2 篇；
- 邀请专家对研究工作进行指导；
- 参加中国地理学会实施青年人才托举工程组织的有关活，并向专家汇报研究成果及个人成长情况；

第三年度：

- 参加实验室管理培训，提高自身科研能力的同时，加强自身的管理能力；
- 组织第三年度样品采集工作，并完成样品分析测定；
- 根据前两年研究成果，选定试点城市，构建基于污水流行病学的疫情与公共健康监测网络；
- 整理三年的研究成果，并将其向防疫等有关部门汇报，为防疫等工作提供参考建议；
- 参加国内外学术会议，与领域内专家交流讨论，并邀请国外知名专家来国家指导交流；提高自身学术水平；
- 发表学术论文 1-2 篇；
- 参加中国地理学会实施青年人才托举工程组织的有关活，并向专家汇报研究成果及个人成长情况；

托举经费使用情况

须明确在托举周期中每一年度的资金用途和预算计划，如有自筹资金资助须明确经费来源。

第一年度（15.0 万元）：

培训费：参加液相色谱-串联质谱联用仪（LC-MS/MS）使用高级培训，培训费 1.0 万元；
国际交流、合作费：邀请国外知名专家来国内指导交流 1 人次，每人次 2.0 万元，计 2.0 万元；参加国际学术会议 1 人次，每人次 1.5 万元，计 1.5 万元；
材料费：液相色谱柱 3 根，每根 1.0 万元，计 3.0 万元；分析标准品 20 支，每支 0.1 万元，计 2.0 万元；
咨询费：邀请专家指导教学 5 人次，每人次 0.2 万元，计 1.0 万元；
测试费：RT-PCR 检测费，每个样品 500 元，40 个样品，计 2.0 万元；
资料费：购买相关书籍，查阅文献等费用计 0.5 万元；

第二年度（15.0 万元）：

差旅费：污水样品采集差旅费，每人次 0.4 万元，共 5 人次，计 2.0 万元；
材料费：固相萃取小柱 10 盒，每盒 0.3 万元，计 3.0 万元；移液枪头、样品瓶、一次性手套、玻璃器皿等实验室耗材 2.0 万元；
测试费：LC-MS/MS 检测费，每个样品 200 元，共 200 个样品，计 4.0 万元；
会议费：参加国内学术会议 2 人次，每人次 0.5 万元，计 1.0 万元；
专利、出版费：发表论文 2 篇，每篇发表费 0.5 万元，计 1.0 万元；
咨询费：邀请专家指导教学 5 人次，每人次 0.2 万元，计 1.0 万元；
中国地理学会实施青年人才托举工程组织的有关活动费用：1.0 万元；

第三年度（15.0 万元）：

培训费：参加实验室管理培训，培训费 1.0 万元；
国际交流、合作费：邀请国外知名专家来国内指导交流 2 人次，每人次 2.0 万元，计 4.0 万元；参加国际学术会议 1 人次，每人次 1.5 万元，计 1.5 万元；
测试费：RT-PCR 检测费，每个样品 500 元，40 个样品，计 2.0 万元；
会议费：参加国内学术会议 2 人次，每人次 0.5 万元，计 1.0 万元；
专利、出版费：发表论文 2 篇，每篇发表费 0.5 万元，计 1.0 万元；
咨询费：邀请专家指导教学 5 人次，每人次 0.2 万元，计 1.0 万元；
中国地理学会实施青年人才托举工程组织的有关活动费用：2.0 万元；
资料费：购买相关书籍，查阅文献等费用计 0.5 万元；

托举导师团队意向（3 位研究员或教授级专家）

姓名	职务、职称	单位	手机号	邮箱
宋长青	执行部长、教授	北京师范大学	13911085190	songcq@bnu.edu.cn
程红光	院长、教授	北京师范大学	13911828720	chg@bnu.edu.cn
李喜青	研究员	北京大学	18611196985	xli@urban.pku.edu.cn

所在单位在三年内对被托举人选的培养支持情况和培养目标
本栏目是被托举人所在单位在其托举过程中提供的科研条件、人才培养的支持计划。 北京师范大学水科学研究院、地表过程与资源生态国家重点实验室及水环境模拟国家重点实验室将会为托举人（杜鹏）提供独立实验室及所需实验条件，并为其购买一台液相色谱-串联质谱联用仪（280 万元）作为其专用仪器用于研究；本单位在扶持托举人完成其科研项目的同时，将其列为学院重点培养对象，使其成长为学院及地理科学领域领军人才重要后备力量。
被托举候选人声明
本人对以上内容及全部附件材料进行了审查，对其客观性和真实性负责。 被托举候选人签名：　　年　月　日
工作单位意见
我单位自愿申报“青年人才托举工程”项目。同意　杜鹏　参评“青年人才托举工程”，保证申报材料真实、合法、有效。承诺若被评上，将给予相应支持，愿与中国地理学会共同做好“青年人才托举工程”，并承担相应责任。 单 位 盖 章： 年　月　日
提名机构或提名人意见
我（机构）同意提名　杜鹏　参评“青年人才托举工程”。保证提名材料真实、合法、有效。 提名机构（个人）签 章： 年　月　日

Water Research 84 (2015) 76–84

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology

Peng Du [a], Kaiyang Li [a], Jing Li [a], Zeqiong Xu [a], Xiaofang Fu [a], Jun Yang [b], Huafang Zhang [b], Xiqing Li [a, *]

[a] Laboratory of Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, 100871 Beijing, China
[b] Beijing Urban Drainage Monitoring Center Co. Ltd., 100012 Beijing, China

ARTICLE INFO

Article history:
Received 28 April 2015
Received in revised form
13 July 2015
Accepted 15 July 2015
Available online 17 July 2015

Keywords:
Methamphetamine
Ketamine
Sewage-based epidemiology
Use
China

ABSTRACT

Sewage-based epidemiology was applied to examine geographic variations in methamphetamine (METH) and ketamine (KET) use in China. Influent and effluent wastewater samples were collected from 36 sewage treatment plants (STPs) in 18 major cities that cover all the geographic regions of the country. Mean METH loads of the cities ranged from 12.5 ± 14.9 to 181.2 ± 6.5 mg/1000 inh/d, whereas mean KET loads ranged from <0.2 to 89.6 ± 27.4 mg/1000 inh/d. No clear geographical pattern was observed in METH use, although slightly lower use in north and east China relative to other regions can be suggested. In contrast, an overall increasing trend from the north to the south was evident for KET loads. Apparent METH removal was greater than 80% at most STPs, whereas KET removal was less than 50% at most STPs and was even negative (i.e., measured effluent concentrations were greater than influent concentrations) at a significant number of STPs. Results in Beijing, Shanghai, and Shenzhen indicate that METH use in China may have increased substantially since 2012, whereas KET use did not significantly change, if not decreased. Comparison between seizures and estimated consumptions reveals that seizures in most Chinese provinces are far less than consumptions. In several provinces (e.g., Guangdong and Yunan), however, seizures were found to exceed consumptions, indicating that a significant fraction of METH and KET seized in these provinces is destined for consumption in other places.

1. Introduction

China is a country that has witnessed rapid increases in illicit drug use in the past quarter of a century. The number of registered drug users has increased at an annual rate of at least 15% in the past decade (in some years over 18%), much greater than the annual growth rates of gross domestic production in the same period (Office of China National Narcotic Control Commission, 2014). The number of registered users has amounted to nearly 2.5 million by the end of 2013, which is more than twice the number of 2008. In addition, the registered users only account for a small fraction of the entire population of drug abuse, as only drug users caught by police are registered in China. However, the exact number of drug users in the country cannot be estimated at this point.

Drug seizures have also increased rapidly in China. The total seizure of common illicit drugs (heroin, opium, cannabis and cannabis resin, amphetamine-like drugs, ketamine, ecstasy, and cocaine) has nearly doubled from 2011 (28.8 tons, without correction by purity) to 2014 (52.3 tons) (Office of China National Narcotic Control Commission, 2012, 2015). The increase in total seizure arose primarily from the seizures of synthetic drugs: the increase of methamphetamine and ketamine seizures accounted for nearly 80% of the total increase. While drug seizure increases in the past few years concur with the significant increase in registered drug users, drug seizure by no means reflects the actual drug consumption in China. Thus, even rough estimates on prevalence and consumption of illicit drugs in China are lacking.

In the past decade, a novel approach called sewage-based epidemiology was developed to estimate drug consumption by a particular population. This approach collects influent wastewater samples from sewage treatment plants (STPs) and measures the concentrations of drug residues or its metabolites in the samples. The drug loads and consumptions of the communities served by the

* Corresponding author.
E-mail address: xli@urban.pku.edu.cn (X. Li).

http://dx.doi.org/10.1016/j.watres.2015.07.025
0043-1354/

sampled STPs are then back calculated by applying flow rates of STPs, populations of the communities, as well as correction factors that account for excretion rates and in-sewer stabilities of the drugs (Zuccato et al., 2008). This approach can yield results in near real time as sample collection and analysis can be completed within one or two days. It holds the promise to serve as a good complement to the existing drug monitoring approaches.

Due to the strong interest to monitor drug abuse and measure the effectiveness of drug control efforts, as well as the relative easiness to implement sewage-based epidemiology, dozens of research groups worldwide have started to apply and refine this methodology. Since its first application in Italy by Zuccato et al. (2005), 135 articles related to sewage-based epidemiology and illicit drug use were identified on PUBMED, among which 122 were published between 2008 and 2013 (Prichard et al., 2014). Sewage-based epidemiology studies were predominantly carried out in Europe. It has been applied in Italy (Zuccato et al., 2005, 2008; Castiglioni et al., 2013; Repice et al., 2013), England(Bones et al., 2007; Kasprzyk-Hordern et al., 2009; Baker et al., 2014), Belgium(van Nuijs et al., 2009a; van Nuijs et al., 2011a,b), France(-Karolak et al., 2010), Spain(Postigo et al., 2008; Boleda et al., 2009; Postigo et al., 2010; Martínez Bueno et al., 2011; Andrés-Costa et al., 2014), Norway(Harman et al., 2011; Bramness et al., 2015), Finland(Kankaanpää et al., 2014; Vuori et al., 2014), Sweden(Östman et al., 2014), Slovakia(Mackul'ak et al., 2014), Croatia(Terzic et al., 2010), and across Europe (Thomas et al., 2012; Ort et al., 2014). Sewage-based epidemiology was also extensively applied in US (Chiaia et al., 2008; Loganathan et al., 2009; Bartelt-Hunt et al., 2009; Bisceglia et al., 2010; Banta-Green et al., 2009; Chiaia-Hernandez et al., 2011; Brewer et al., 2012; Heuett et al., 2015) and Canada(Metcalfe et al., 2010; Yargeau et al., 2014). In contrast, sewage-based epidemiology studies were scarce in regions other than Europe and North America. Australia is the country that first saw application of this methodology outside these regions (Irvine et al., 2011; Lai et al., 2013a).

Most of the sewage-based epidemiology studies so far were performed in/during specific settings or events, such as prisons (e.g., van Dyken et al., 2014), schools (Panawennage et al., 2011), and music festivals (Lai et al., 2013a), or at local community and city level (e.g., van Nuijs et al., 2011b; Terzic et al., 2010; Yargeau et al., 2014). Nationwide studies have been very limited. van Nuijs et al. (2009b) estimated cocaine consumption across Belgium by sampling 37 STPs that in total served about 40% of the Belgium population. Mackul'ak et al. (2014) collected wastewater samples from seven major cities in Slovakia and estimated drug consumption rates in these cities. In these nationwide studies, only use of traditional drugs (amphetamine, methamphetamine, cocaine, 3,4-methylenedioxymethamphetamine, heroin, and cannabis) was estimated. Occurrence and consumption of new psychoactive substances such as ketamine was not examined.

Sewage-based epidemiology studies have been scarce in Asia (Kim et al., 2015). China is the first Asian country to carry out such studies. Lai et al. (2013b) applied sewage-based epidemiology for the first time in China to estimate drug use in Hong Kong. They found that ketamine is the predominant drug of abuse, followed by methamphetamine and cocaine. Khan et al. (2014) performed a snapshot study by collecting and analyzing wastewater samples from four megacities, Beijing, Shanghai, Guangzhou, and Shenzhen. Methamphetamine and ketamine were found to be the major drugs of abuse in China. This finding is consistent with the fact that seizures of these two drugs ranked first and second, respectively, in the country (Office of China National Narcotic Control Commission, 2014). Li et al. (2014) sampled all the STPs in the urban area of Beijing to examine the consumption pattern of methamphetamine and amphetamine in the city. While these studies yielded useful insights on drug abuse in China, they did not provide information on drug use at the national level.

The objective of this work was to examine the geographic pattern of methamphetamine and ketamine use by urban inhabitants across China. Influent wastewater samples were collected from 36 STPs of 18 provincial capitals or equivalent cities that cover all the geographic regions of the country. Effluent samples were also collected from selected STPs to derive removal rates during wastewater treatment. Temporal variations in the use of these two drugs in a few megacities were examined by comparing drug loads obtained in 2014 (this study) and by Khan et al. (2014) in 2012. Comparing estimated drug consumptions and seizures in the sampled cities and provinces yielded interesting insights on drug abuse and trafficking in the country.

2. Materials and methods

2.1. Sample collection

Wastewater samples were collected from Beijing (BJ), Guiyang (GY), Haerbin (HRB), Hangzhou (HZ), Jinan (JN), Kunming (KM), Lanzhou (LZ), Luoyang (LY), Nanjing (NJ), Nanning (NN), Shanghai (SH), Shenyang (SY), Shenzhen (SZ), Shijiazhuang (SJZ),Wuhan (WH), Xi'an (XA), Xiamen (XM), Yinchuan (YC). These eighteen cities belong to all the eight geographic regions of the nation: northeast (HRB and SY); north China (BJ and SJZ); northwest (LZ, XA, and YC); central China (LY and WH), east China (JN, NJ, and SH); southeast (XM, HZ), southwest (KM and GY), south China (NN and SZ) (Fig. 1). The majority (15 of total 18) of the cities are provincial capitals or are under direct administration by the central government (BJ and SH). The other three cities (LY, XM, and SZ) are equivalent to provincial capitals in terms of economic development and population sizes. According to the recent census data, the population sizes of the cities range from 2.08 million of YC to 25 million of SH. The sum of the population of all the cities is 163.3 million, representing about 12% of entire population of China.

In total, wastewater samples were collected from 36 STPs in the above mentioned cities. In most cities, two or more STPs were selected for wastewater sample collection. In HRB, JN, LZ, and NN, however, only one STP in each city was sampled. Most sampled STPs treat wastewater from the urban centers of the cities. All the STPs are secondary types and involve activated sludge processes. The population served by the STPs totals 34.1 million, representing about 21% of the total population of the cities and 2.5% of the population of the entire country. The STPs are named as BJ-1 (first STP of Beijing), KM-2 (second STP of Kunming), etc.

The sampling campaign started in early July and ended in early October of 2014, with the majority of the sampling carried out between early August and mid-September. Each STP was sampled for two days (one weekend day and one weekday) by collecting 24-h composite samples at the sewage inlets using autosamplers, such as FC-9624 (GRASP Science & Technology Co., Ltd, Beijing), ISCO 3000, 3700, 4700 (Teledyne Technologies Inc., Lincoln, NE, USA), and GD-24A (Jinpeng Huanyi Technology Co., Ltd, Beijing). Heavy precipitation days were avoided for sampling. STPs were asked to program the autosamplers to imbibe 100 mL of influent at an interval of 1 h. A few STPs (SY-2, LZ-1, LY-1 and 2, XA-1 and 2) did not have autosamplers and denied entry of our own autosampler. For these STPs, 200 mL of wastewater was collected manually every 2 or 4 h throughout the day and was combined to form a composite sample. Effluent samples were also collected at about half of the STPs, in the same manner and at the same time as for the influents. Following collection, the samples were

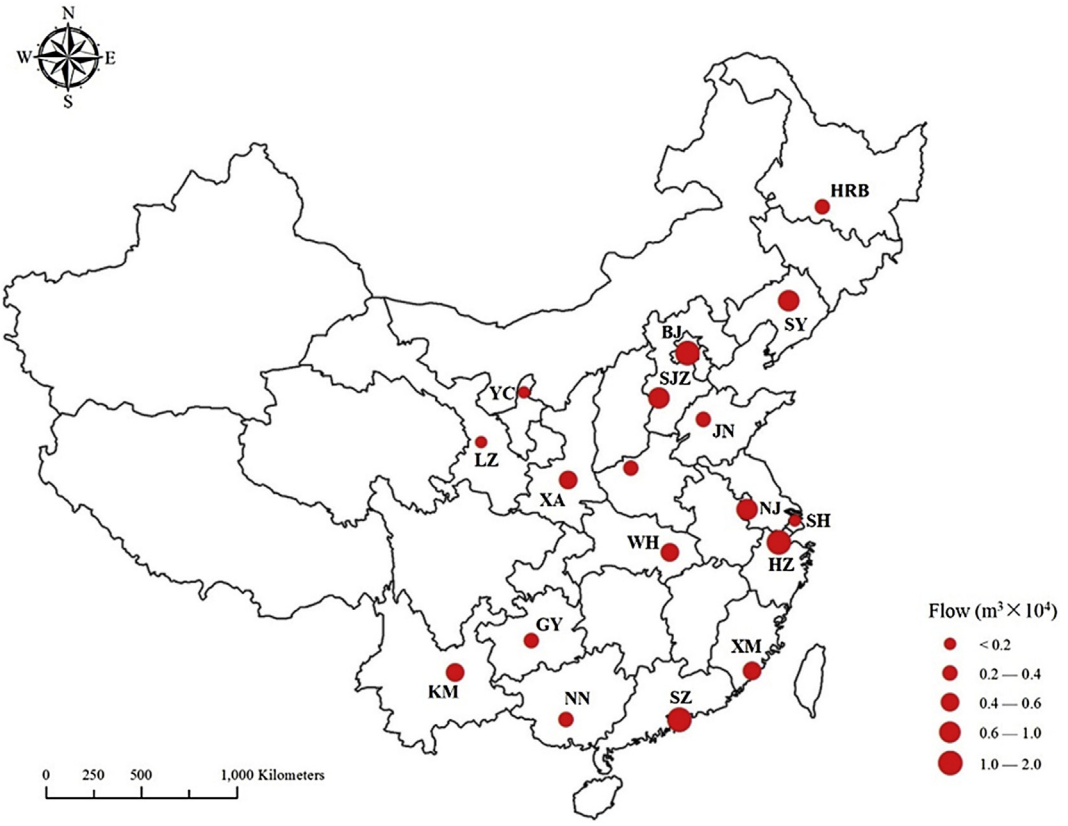


Fig. 1. Locations of cities where wastewater samples were collected. The sizes of the circles represent the total flow rates of the STPs sampled in the cities.

immediately acidified to pH = 2 using HCl, frozen at STPs, and were then carried back to laboratory on ice and stored at −20 °C until analysis. Details of STPs (served populations, flow rates) and sampling information are provided in Table S1.

2.2. Analysis

Standards of METH and KET, as well as their metabolites amphetamine (AMP) and norketamine (NK) were purchased from Cerilliant (Round Rock, TX, USA). The deuterated internal standards (METH-d_8, AMP-d_8, KET-d_4, and NK-d_4) were also purchased from Cerilliant. Sample pretreatment followed the procedure described in a previous paper (Li et al., 2014), with minor modifications. Briefly, 50 mL wastewater was filtered using glass fiber membranes 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 added with internal standards was then loaded to the cartridge at a flow rate of 1–2 mL/min. After the cartridge was dried under vacuum, it was 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 further cleaning step was performed using a 0.22 μm centrifugal filter (VWR International, Radnor, PA, USA). Concentrations of METH and KET associated with suspended particulate matter in wastewater samples were not examined, as sorption of the two drugs to particulate matter was found to be minor (Baker et al., 2012).

Compound separation was carried out using a UFLCXR-LC system (Shimadzu, Japan) with a Phenomenex Gemini C_{18} column (100 mm × 2 mm, 3 μm) and an injection volume of 5 μL. The mobile phase was composed of 30 mM ammonium formate in ultrapure water with pH adjusted to 3 using formic acid (98% in water) (A) and 0.1% formic acid in AcN (B). The flow rate of the mobile phase was controlled at 0.3 mL/min. The elution gradient was as follows: 0–0.1 min: 5% B; 0.1–3.0 min: 30% B; 3.0–5.0 min: 80% B; 5.0–7.5 min: 90% B; 7.5–7.6 min: 5% B; 7.6–13.0 min: 5% B. Concentrations were determined using an API 4000 triple quadrupole mass spectrometer (AB SCIEX, USA) equipped with an electrospray interface operating in positive ionization mode. The quantification of MS system was operated in multiple reaction monitoring (MRM) mode. Details of MS parameters and LOQs of the target compounds were provided in Table S2.

To validate the analytical methods, recovery was determined by spiking ultrapure water with target compounds at three concentrations (4, 10, and 20 ng to 50 mL water) and following the same pretreatment procedures. Matrix effects were determined by spiking the low-concentration influent wastewater that had gone through solid phase extraction. 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 81.8 ± 1.6 to 105.5 ± 2.8% and from −1.1 ± 1.2 to −16.2 ± 0.9% (with the overwhelming majority below −10%), respectively (Table S3). Intra- and inter-day repeatability of instrument and method ranged from 0.7 to 2.2% and from 2.0 to 7.9%, respectively. A procedure blank using ultrapure was included in every 20 wastewater samples and followed the same pretreatment steps. All the target compounds were below detection limit in blanks.

2.3. Mass load estimation and removal rate calculation

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

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

Influent flows on each day of sampling were provided by each respective STP. Populations served by STPs were either obtained from the STPs or based on the most recent census data of the service areas. Uncertainties involved in load estimation were discussed elsewhere (Li et al., 2014). Removal rates were derived by dividing the difference between the influent and effluent concentrations at a STP by the influent concentrations.

3. Results and discussion

3.1. Influent concentrations of METH, AMP, KET and NK

METH concentrations were above LOQ in all the samples collected, indicating omnipresence of this drug in wastewater in China (Table 1, Table S4). The lowest and highest mean METH concentrations were found at NJ-1 (17.0 ± 1.7 ng L^{-1}) and YC-1 (684.6 ± 136.6 ng L^{-1}), respectively. AMP concentrations were below LOQ at BJ-1, NJ-1. The highest mean AMP concentration was observed at XM-2 (603.4 ± 123.0 ng L^{-1}). Strong positive correlation was found between AMP and METH concentrations. The concentration ratios between AMP and METH were less than 0.1 at most STPs, with the overwhelming majority of which having AMP/METH ratio less than 0.07. The exceptions included BJ-2 (0.22 ± 0.11), BJ-2 (0.13 ± 0.05), SJZ-1 (0.15 ± 0.04), HZ-1 (0.16 ± 0.01) and XM-2 (1.74 ± 0.22). METH is partly metabolized into AMP. AMP in wastewater with concentrations less than 10% of METH concentrations is considered to arise from METH metabolism (instead of AMP use itself) (Zuccato et al., 2008). Thus, AMP in sewage of the overwhelming majority of cities in China comes from METH use, indicating that AMP use in China is in overall negligible compared to METH use. This contrasts the situation in Europe where more AMP relative to METH use was observed in many countries (e.g., Vuori et al., 2014; Östman et al., 2014; Andrés-Costa et al., 2014). The exceedingly high AMP/METH ratio at XM-2 indicates that there might be high use or even direct dumping of AMP in the communities the plant serves.

KET concentrations were higher than LOQ at all STPs but SJC-1 and 2, also indicating widespread presence of this compound in wastewater in China. The highest mean KET concentration (376.6 ± 132.7 ng L^{-1}) was found at SZ-1. NK concentrations were above LOQ in merely two out of fourteen STPs (BJ-4 and YC-2) in northeast (HRB and SY), north (BJ and SJZ), and northwest (LZ, XA, YC) China. NK concentrations could not be quantified either at two STPs (JN-1 and NJ-1) in east China. At STPs in south, southwest, southeast, and central China, NK concentrations were above LOQ, with the highest concentrations (74.6 ± 25.7 ng L^{-1}) found at NN-1. Strong positive correlation was also found between NK and KET concentrations. KET/NK ratios ranged between 3.3 ± 1.3 at GY-2 and 14.4 ± 1.6 at XM-2. The mean KET/NK of all the STPs where this ratio could be determined (i.e., both KET and NK concentrations were quantifiable) was 6.3 (±3.3).

3.2. Effluent concentrations and removal rates

Effluent samples were collected from STPs in six cities, namely, BJ, SH, NJ, XM, KM, and YC. AMP concentrations were below either detection limit or LOQ in all the cities but XM, where effluent AMP concentrations were around 4 ng L^{-1}. Average AMP removal ranged from 49.8 ± 10.1 at YC-2 and >99% at XM-2. Effluent METH concentrations were above LOQ in all STPs where effluent samples were collected. At most STPs, effluent METH concentrations were several to several tens ng L^{-1}. SH-1 and XM-2 were two exceptions, where effluent concentrations were greater than 100 and 200 ng L^{-1}, respectively (Table S4). Average METH removal ranged from 24.9 ± 8.5% at XM-1 and >99% at BJ-4. The majority of STPs sampled had removal rates over 80% (Fig. S1), consistent with previous studies (e.g., Li et al., 2014; Loganathan et al., 2009).

Effluent NK concentrations were quantifiable at 10 out of the 17 STPs where effluent samples were collected. The highest effluent NK concentration, 26.0 ± 1.6 ng L^{-1}, was observed at YC-1. At a

Table 1
Mean influent concentrations of METH, AMP, KET, NK (ng L^{-1}). Method LOQ for METH, AMP, KET, NK were 0.8, 4.0, 0.8, 4.0 ng L^{-1}, respectively.

STP	METH	AMP	KET	NK
HRB-1	498.2 ± 17.8	38.9 ± 1.4	0.9 ± 0.2	<LOQ
SY-1	229.4 ± 42.1	15.2 ± 2.5	1.4 ± 0.1	<LOQ
SY-2	156.0 ± 14.1	11.5 ± 2.1	1.0 ± 1.4	<LOQ
YC-1	684.6 ± 136.6	45.5 ± 5.7	108.8 ± 16.4	22.4 ± 0.1
YC-2	68.1 ± 18.8	4.1 ± 0.8	3.1 ± 1.3	<LOQ
XA-1	200.8	158	39	<LOQ
XA-2	172.8 ± 63.4	10.6 ± 6.4	12.4 ± 4.1	<LOQ
LZ-1	115.6 ± 35.6	9.1 ± 3.5	4.0 ± 0.7	<LOQ
BJ-1	60.0 ± 15.8	<LOQ	1.8 ± 1.6	<LOQ
BJ-2	156.6 ± 39.7	31.0 ± 8.8	4.3 ± 1.4	<LOQ
BJ-3	120.8 ± 27.2	10.0 ± 2.0	10.5 ± 1.0	<LOQ
BJ-4	185.3 ± 35.5	24.0 ± 10.6	15.2 ± 3.5	2.9 ± 1.1
BJ-5	380.6 ± 67.1	26.0 ± 4.6	7.7 ± 8.4	<LOQ
SJZ-1	168.0 ± 18.7	25.6 ± 3.5	<LOQ	<LOQ
SJZ-2	112.2 ± 4.0	6.4 ± 0.4	<LOQ	<LOQ
SJZ-3	152.6 ± 7.6	9.3 ± 1.2	1.0 ± 0.1	<LOQ
WH-1	108.0 ± 68.5	6.9 ± 3.5	97.5 ± 49.4	27.5 ± 17.6
WH-2	307.2 ± 171.4	21.2 ± 10.2	206.0 ± 155.0	49.7 ± 30.3
LY-1	281.4 ± 113.4	15.6 ± 5.4	47.2 ± 18.9	3.5 ± 2.1
LY-2	428.8 ± 7.4	19.5 ± 0.4	50.0 ± 3.9	8.0 ± 2.7
JN-1	50.8 ± 12.7	4.0 ± 2.8	1.7 ± 0.3	<LOQ
NJ-1	17.0 ± 1.7	<LOQ	2.4 ± 1.3	<LOQ
NJ-2	151.0 ± 13.9	9.5 ± 0.5	57.6 ± 4.2	11.4 ± 0.8
SH-1	455.0 ± 269.0	25.7 ± 10.5	34.5 ± 34.8	6.8 ± 6.8
SH-2	341.4 ± 28.6	22.7 ± 8.1	39.2 ± 12.4	8.0 ± 0.5
HZ-1	309.4 ± 12.7	50.0 ± 3.7	56.0 ± 8.9	6.1 ± 1.5
HZ-2	166.6 ± 15.0	9.6 ± 0.8	87.4 ± 22.3	12.2 ± 1.6
XM-1	331.0 ± 30.3	10.5 ± 1.1	121.2 ± 1.1	23.1 ± 3.8
XM-2	345.2 ± 26.6	603.4 ± 123.0	208.2 ± 41.6	14.4 ± 1.2
NN-1	48.8 ± 58.3	2.5 ± 3.5	350.8 ± 107.5	74.6 ± 25.7
SZ-1	620.0 ± 30.5	28.7 ± 4.8	376.6 ± 132.7	52.3 ± 5.4
SZ-2	207.9 ± 22.8	13.3 ± 0.7	106.7 ± 7.9	26.3 ± 6.0
KM-1	185.6 ± 128.4	13.0 ± 9.3	26.2 ± 5.1	5.9 ± 3.9
KM-2	188.8 ± 92.2	12.2 ± 5.9	85.0 ± 45.6	7.1 ± 4.6
GY-1	199.2 ± 73.0	7.1 ± 0.5	14.3 ± 0.3	3.6 ± 0.6
GY-2	48.7 ± 20.7	1.0 ± 1.4	21.7 ± 3.8	6.9 ± 1.5

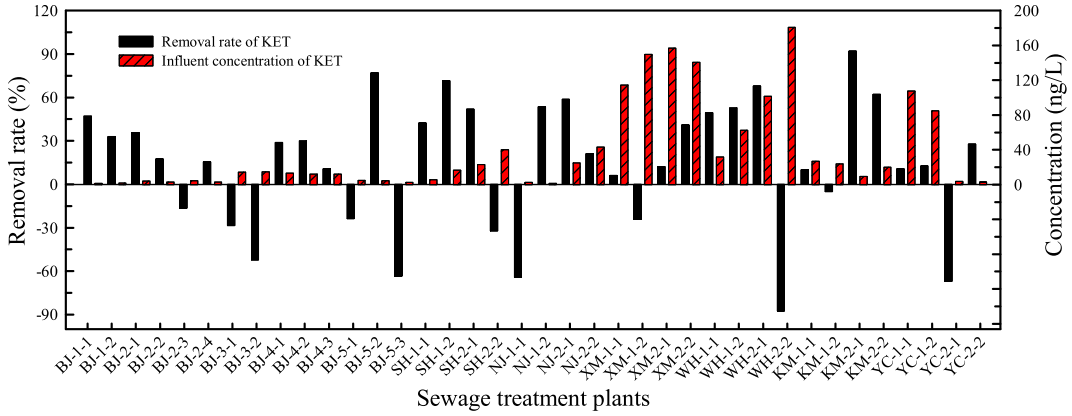


Fig. 2. Apparent removal rates and influent concentrations of ketamine at 17 STPs where both influent and effluent samples were collected.

number of STPs (e.g., YC-1; WH-2), effluent NK concentrations were greater than influent concentrations. Effluent KET concentrations were above LOQ for at least one day at all STPs where effluent samples were collected. High effluent KET concentrations (>100 ng L^{-1}) were observed at XM-1, XM-2, WH-2, and YC-1. At the overwhelming majority of STPs, apparent KET removal rates were below 50% (Fig. 2), in striking contrast to the high removal rates of METH. This observation indicates that more KET would enter receiving waters than METH if the two drugs have same influent concentrations. Furthermore, at 9 out of the 17 STPs, negative apparent KET removal rates were observed.

Negative removal rates were also reported for KET (Yargeau et al., 2014; Andrés-Costa et al., 2014; Baker and Kasprzyk-Hordern, 2013) and other illicit drugs (e.g., Subedi and Kannan, 2014) in the literature. Plausible explanations for negative removal rates of illicit drugs include residence time, enhanced transformation of parent/precursor compounds, and/or desorption from particulate matter during wastewater treatment processes (Subedi and Kannan, 2014; Andrés-Costa et al., 2014). Baker et al. (2012) reported that sorption of ketamine to particulate matter was minor, precluding ketamine desorption from particulate matter as the main cause of negative removal. In this work, influent and effluent samples at a particular STP were collected at same time. However, Chinese STPs using activated sludge process typically have a hydraulic retention time of 8 h. In other words, there is an 8-h mismatch between the influent and effluent samples. If removal of a compound by wastewater treatment process at a STP is poor and there is a pulse of high concentration during the mismatch, apparent negative removal can be expected. This could especially happen when influent concentrations are low. Indeed, negative removal rates observed in this work concurred with low influent concentrations in most cases (Fig. 2), indicating that residence time (or mismatch between influent and effluent samples) is the primary cause of apparent negative ketamine removal.

3.3. Loads of METH, KET

The loads of METH derived from back calculation using equation (1) ranged from 4.9 ± 0.5 mg/1000 inh/d (NJ-1) to 181.2 ± 6.5 mg/1000 inh/d (HRB-1) (Fig. 3, Table S5). Among the 15 cities where two or more STPs were sampled, METH loads derived from STPs in a same city varied by less than a factor of two in 10 cities (Table 1, Fig. 2). In BJ, NJ, and YC, maximum variations in loads (highest vs. lowest loads) within a city were greater than a factor of 10. This is because in these cities, there are STPs that treat wastewater from suburban districts (BJ-1 and YC-2) or from a recently developed district (NJ-2). Relatively much lower METH use in suburban areas has been observed previously (Li et al., 2014). Similar pattern in within-city variations was also observed for KET loads. These observations indicate that spatial variations in METH and KET loads could be significant in Chinese large cities. This can be explained by the fact that these cities typically have several STPs serving districts with significantly different levels of economic development and entertainment activities.

The highest mean METH load was found in HRB (181.2 ± 6.5 mg/1000 inh/d), followed by YC (148.0 ± 145.2 mg/1000 inh/d) (mean of loads at YC-1 and YC-2) (Fig. 3, Table S5). The lowest mean METH loads were observed in NN (12.5 ± 14.9 mg/1000 inh/d) and GY (12.8 ± 6.1 mg/1000 inh/d). Other than HRB and YC, SH and LY also saw relatively high METH loads, 99.4 ± 46.4 and 99.5 ± 31.5 mg/1000 inh/d, respectively. The majority of the cities had mean METH loads between 30.8 ± 9.5 mg/1000 inh/d (LZ) and 89.5 ± 23.5 mg/1000 inh/d (SZ). These results indicate that METH was widely used across the country and no clear geographical trend was observed, although it appears that lower use in north and east China relative to other regions can be suggested (Fig. 3). Khan et al. (2014) reported that a north-south trend was evident in the use of METH in China. Apparently, that conclusion needs to be revised, as their study did not include STPs in northeast and northwest China, where high METH loads were observed.

The mean METH load in STPs of the investigated cities in China was 67.8 (±45.2) mg/1000 inh/d. This load is more than twice the mean load of cities across Europe (Ort et al., 2014). It is much higher than the loads reported in most European countries, such as Belgium (van Nuijs et al., 2011b, 2014), Netherlands (Thomas et al., 2012), Italy (Castiglioni et al., 2006; Zuccato et al., 2008, 2011; Repice et al., 2013), UK (Zuccato et al., 2008), Spain (Postigo et al., 2010; Andrés-Costa et al., 2014) Sweden (Östman et al., 2014), higher than those in Finland (Kankaanpää et al., 2014; Vuori et al., 2014), similar with those in Slovakia (Mackul'ak et al., 2014), and lower than levels reported in Norway and Czech Republic (Thomas et al., 2012; Ort et al., 2014). The mean load in China is lower than those reported in Australia (Lai et al., 2013a) and some American cities (Chiaia et al., 2008), similar with those reported in Hong Kong (Lai et al., 2013b) and large cities in Canada (Metcalfe et al., 2010; Yargeau et al., 2014), and much higher than loads in South Korea (Kim et al., 2015).

The loads of KET ranged from <0.2 mg/1000 inh/d (SJZ-1, 2) to 89.6 ± 27.4 mg/1000 inh/d (NN-1) (Fig. 3, Table S5). The highest mean KET load was found in NN (89.6 ± 27.4 mg/1000 inh/d), followed by WH (61.9 ± 33.8 mg/1000 inh/d) and SZ (53.0 ± 21.9 mg/1000 inh/d). Medium level of KET loads were observed in XM

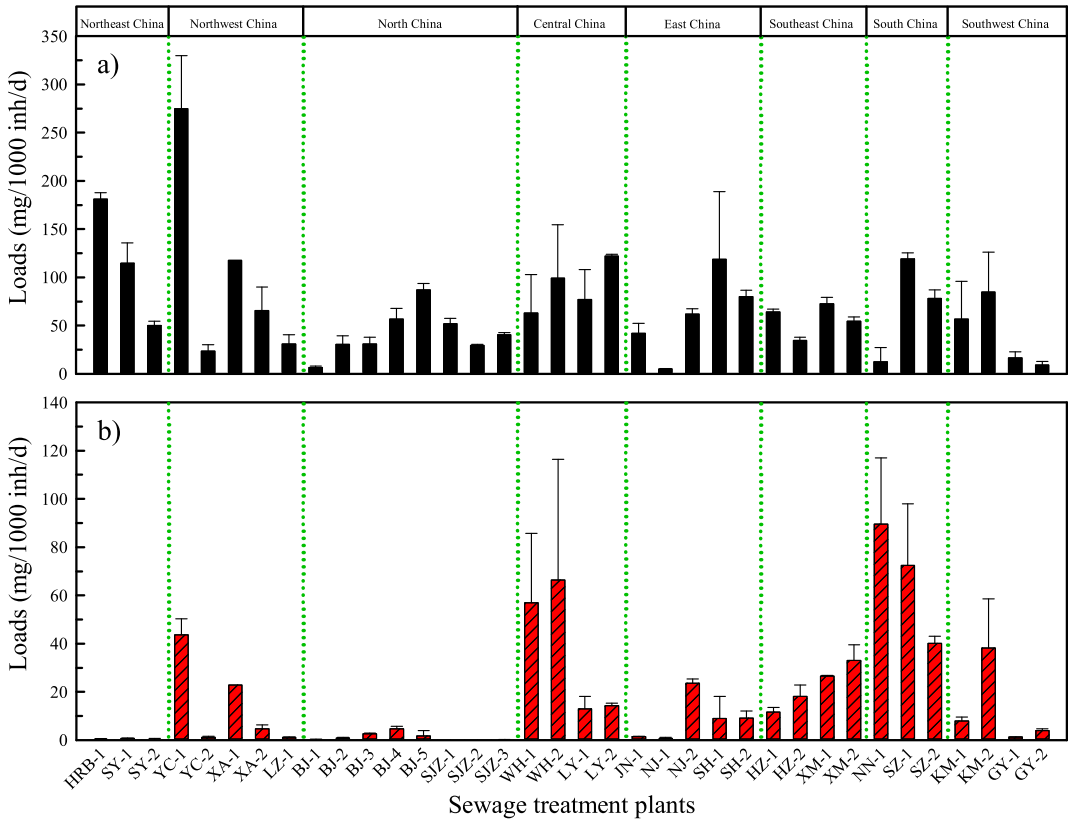


Fig. 3. Average loads of METH (a) and KET (b) at the STPs.

(29.7 ± 6.6 mg/1000 inh/d), KM (23.1 ± 21.3 mg/1000 inh/d), and YC (22.2 ± 24.7 mg/1000 inh/d). Mean KET loads were greater than 10 but less than 20 mg/1000 inh/d in NJ, HZ, LY, XA, greater than 1 but less than 10 mg/1000 inh·d in SH, JN, LZ, GY, BJ, and less than 1 mg/1000 inh/d in HRB, SJZ, and SY. It is clear that variation in KET loads among the cities (by a factor of >100) was much greater than that in METH loads (by a factor of <15). In addition, KET loads display a clear geographic trend: KET loads are much higher in the cities south of Yangtze River (WH, SH, HZ, XM, SZ, NN, KM, NJ), with GY as an exception; KET loads were lowest in north (BJ, SJZ), northeast (HRB, SY) and some east cities (JN); Northwest (YC, XA) and some central cities (LY) had KET loads falling in between. In general, KET loads increased from the north to the south of the country, although there are a couple of exceptions (YC in the North and GY in the South).

Concentrations and load data for ketamine were reported in much less studies in the literature. Previous studies have shown that KET concentrations and loads were low in most European and American cities (Chiaia et al., 2008; van Nuijs et al., 2014; Martínez Bueno et al., 2011; Huerta-Fontela et al., 2008; Bijlsma et al., 2012), and high in metropolitan Asian cities (Lin et al., 2010, 2014; Lai et al., 2013b). It is worth noting, however, some STPs in United Kingdom and Canada were reported to have moderately high KET concentrations (Baker and Kasprzyk-Hordern, 2011; Baker et al., 2014). Recently, Baker et al. (2014) reported that daily loads at a large STP in England (with a population of 3,400,000) ranged from 176 to 327 g d^{-1} (which translate into loads ranging from 51.8 to 96.2 mg/1000 inh/d). Yargeau et al. (2014) reported an average KET concentration of 40 ng L^{-1} at a large STP in Canada (population 1.5 M, flow 2 M m^3), which translates to a load of 53.3 mg/1000 inh/d. These loads are similar with the highest loads observed at WH, SZ, and NN in this study. KET loads in Hong Kong were much higher that the highest loads found in southern mainland cities (Lai et al., 2013b). Load data was not available in Taiwan. However, KET concentrations (147–298 ng L^{-1}) in STP influents in Taipei were at the upper end of the KET concentration range observed in this study (Lin et al., 2010). More recently, Lin et al. (2014) reported that KET concentrations at two STPs in Taipei and Kaohsiung were several times higher than the highest KET concentrations found in this study. Jiang et al. (2015) reported that median KET concentrations at Nanwan and Kenting STPs in southern Taiwan were 18,633 and 84,666 ng L^{-1}, respectively. Thus, KET loads in STPs of major cities in Taiwan are likely much higher than or at least similar to the highest loads observed in this study.

3.4. Comparison between loads in 2012 and 2014 at selected STPs

Among the 36 STPs sampled in this study, 6 plants, BJ-1, 2, 5, SH-1, SZ-1, 2 were also sampled in a campaign in 2012. These plants correspond to Xiaojiahe, Xiaohongmen, Fangzhuang STPs of Beijing, Quyang STP of Shanghai, and Binhe and Nanshan STPs of Shenzhen in the 2012 campaign, respectively (Khan et al., 2014). Student t-test indicates that METHs loads at BJ-1 in 2012 and 2014 were not statistically different. However, METH loads were significantly higher in 2014 than in 2012 at the other STPs (Fig. 4). At these STPs, loads in 2014 were at least 20% more than those in 2012. At SH-1 and SZ-2, the differences were even greater by a factor of 3.0 and 3.6, respectively. These results indicate that METH use significantly increased in the three megacities in the past two years.

In contrast, KET loads of 2014 in Shenzhen were either significantly lower than (SZ-1) or roughly the same (SZ-2) as those of 2012 (Fig. 4). The average KET load at SH-1 in 2014 (9.0 mg/1000 inh/d) was much higher than that in 2012 (3.1 ± 0.3 mg/1000 inh/d. However, the daily variation in KET load in 2014 was

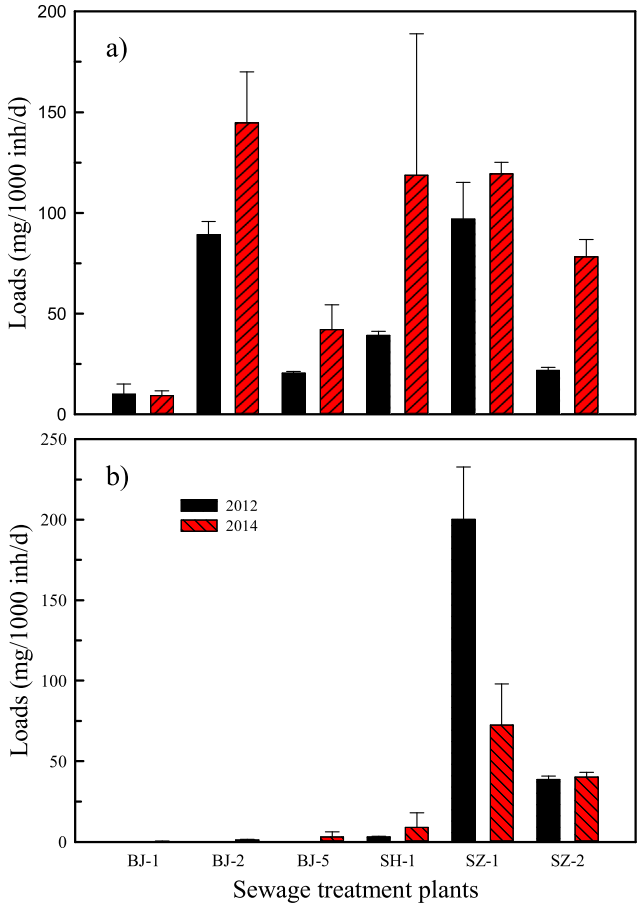


Fig. 4. METH (a) and KET (b) loads in 2012 and 2014 at selected STPs in Beijing, Shanghai, and Shenzhen.

large (from 2.3 to 15.7 mg/1000 inh/d) at this plant. Thus it is hard to conclude that load at SH-1 was consistently higher in 2014 relative to 2012. The limit of quantification of KET in the 2012 study was 5 ng L^{-1}, which is higher than most KET concentrations detected in STPs in Beijing in 2014. This means that it is not clear whether KET loads in Beijing were higher in 2014 relative to 2012. These results indicate that in the past two years KET use in the three cities did not significantly increase, if not decreased. The temporal trends of METH and KET use observed here is consistent with the fact that the number of newly registered METH users increased, whereas the number of newly registered KET users decreased in China in the recent years (Bureau of Drug Control, Ministry of Public Security, personal communication).

3.5. Comparison between estimated consumption and seizures

METH in STPs may also come from the metabolism of selegiline, a drug of clinical use in China. However, licit contribution to METH loads from selegiline was estimated to be around 0.03 mg/1000 inh/d only (Khan et al., 2014; Li et al., 2014). This contribution is negligible, even compared to the lowest load observed at NJ-1. Ketamine are also legally used in China, as an anesthetic in surgeries for human and animals. However, ketamine was below LOQ at a number of STPs (BJ-1, SH-2, SJC-1, 2) in north and northeast China whose influents are known to include wastewater from hospitals. Considering that significant regional variation of per capita medical use of ketamine in the same country is unlikely, low ketamine concentrations at these STPs indicate that contribution of medical use to ketamine loads in STPs in China is also negligible. Hence, both METH and KET loads in Chinese STPs can be considered to be exclusively from illegal use of the two drugs.

Drug consumptions can be derived from loads by multiplying correction factors that take into account drug excretion rates. A correction factor of 2.3 (corresponding to an excretion rate of 43%) is widely accepted for METH (Zuccato et al., 2008; van Nuijs et al., 2011a). However, dramatically different excretion rates were used in the literature. Andrés-Costa et al. (2014) adopted an excretion rate of 2.3%, whereas Yargeau et al. (2014) used a value of 30%. A correction factor of 43.5 (based on an excretion rate of 2.3%) would yield KET consumption much higher (by over one order of magnitude) than METH consumption in most Chinese cities. This does not seem reasonable as the number of registered METH users is always much greater than that of registered KET users, even in southern cities where KET are much more popular than in other regions of the country (Bureau of Drug Control, Ministry of Public Security, personal communication). Hence, a correction factor of 3.3 (based on an excretion rate of 30%) was used to estimate KET consumption.

METH and KET consumptions at the urban areas of the cities were estimated by multiplying the average loads by the correction factors and the urban populations of the cities. Consumptions of the provinces were then extrapolated assuming the per capita consumption of a province is the same as that of the city. Consumptions of the provinces derived this way represent the maximum possible consumptions, as the cities sampled are all political, economic, and cultural centers of the provinces and drug prevalence in the urban areas of these cities are expected to be much higher than the province averages. METH consumptions at urban areas ranged from 18 kg y^{-1} (NN) to 1149.5 kg y^{-1} (SH), whereas KET consumption ranged from 0.3 kg y^{-1} (SJZ) to 425.5 kg y^{-1} (WH) (Table 2). Estimated METH consumptions at the urban areas of BJ, HRB, SH, LY, LZ, XA, and YC were greater than the seizures of the corresponding provinces (only seizure data at provincial level were found in this study) (Table 2, bold values). Estimated KET consumptions at the urban areas of the cities were greater than the seizures of the corresponding provinces except Heilongjiang, Fujiang, Guangxi, Guangdong, and Guizhou (Table 2). These results indicate that seizures in these provinces are far less than the actual consumptions, as urban populations of the cities only account for a small fraction of the populations of these provinces.

In contrast, in Fujian, Guangdong, Guangxi, Yunnan, Guizhou for METH and Fujian and Guangdong for KET, the ratios of estimated consumptions of the cities to the seizure of the corresponding provinces were very small (<0.1). Furthermore, the seizures in these provinces were even greater than or close to the maximum possible consumptions of the provinces (extrapolated based on consumptions of the cities) (Table 2, bold values). Hence, a significant portion of METH and KET seized in these provinces must be destined for consumption in other provinces or even other countries. Thus, this study demonstrates that sewage-based epidemiology can not only yield estimates of illicit drug consumptions but also provide insights of drug trafficking between different regions.

4. Conclusions

A nationwide reconnaissance was performed to examine the METH and KET use in major Chinese cities. METH was found to be widely used across the country and no clear geographical trend was observed. In contrast, KET use in south China was in overall much greater than in north China. Removal of KET was much lower than METH removal. Significant increases in METH use in China have occurred since 2012, whereas KET use remained stable or even decreased. Seizures were found to be much less than consumptions in most provinces but exceed consumptions in some provinces,

Table 2
Estimated consumptions and seizures of METH and KET in provinces or cities where wastewater samples were collected.

Geographic region	Province	Population (million)	City	Population (urban area) (million)	Average loads (mg/1000 inh/d)		Consumption-city (kg/y)		Consumption-city/Seizure[b]		Consumption-province[c]/Seizure	
					METH	KET	METH	KET	METH	KET	METH	KET
Northeast	Liaoning	43.9	Shenyang	4.60	82.4	0.5	322.3	2.9	0.7	**1.2**	6.4	11.8
	Heilongjiang	38.3	Haerbin	3.15	181.2	0.3	485.3	1.31	**4.7**	0.4	56.9	5.4
North	Beijing[a]	21.1	Beijing	10.2	44.2	2.2	383.5	27.2	**4.3**	**27.2**	8.9	53.7
	Hebei	73.3	Shijiazhuang	2.63	40.6	0.09	90.9	0.3	0.8	**9.4**	21.5	263.0
Northwest	Shanxi	37.6	Xi-An	4.50	83.0	10.8	317.7	58.3	**4.5**	**3.5**	37.4	28.9
	Gansu	25.8	Lanzou	1.75	30.8	1.1	45.8	2.2	**2.6**	**223.7**	38.7	3300.2
	Ningxia	6.54	Yingchuan	0.80	148.0	22.2	100.7	21.4	**1.4**	**6.9**	11.5	56.1
East	Shanghai[a]	24.1	Shanghai	13.60	99.4	9.1	1149.5	149.0	**2.0**	**26.1**	3.5	46.4
	Jiangsu	79.4	Nanjing	5.25	33.4	12.2	149.3	76.9	0.4	**2.5**	5.4	37.0
	Shandong	97.3	Jinan	3.00	42.0	1.4	107.0	5.0	0.3	**3.8**	8.6	122.6
Southeast	Zhejiang	55.0	Hangzhou	4.40	49.5	14.9	185.0	79.0	0.6	**2.1**	7.8	26.
	Fujian	37.7	Xiamen	1.55	63.6	29.7	83.8	55.5	0.1	0.05	**1.9**	**1.1**
Central	Henan	94.1	Luoyang	1.80	99.5	13.6	152.3	29.4	**2.2**	**14.8**	112.8	773.6
	Hubei	58.0	Wuhan	5.70	81.1	61.9	392.9	425.0	0.5	**3.1**	4.7	31.1
South	Guangdong	106.4	Shenzhen	2.95	89.5	43.7	224.6	155.3	0.03	0.02	**1.1**	**0.7**
	Guangxi	47.2	Nanning	1.70	12.5	89.6	18.0	183.4	0.05	0.1	**1.4**	3.1
Southwest	Guizhou	35.0	Guiyang	1.85	12.8	2.6	20.1	5.8	0.07	0.2	**1.3**	3.5
	Yunnan	46.9	Kunming	2.25	70.7	23.1	135.3	62.5	0.01	**480.9**	**0.3**	10017.5

Bold values represent either consumptions in cities are larger than seizures of the provinces or seizures of provinces are larger than maximum possible consumptions of the provinces.

[a] Beijing and Shanghai are municipalities under direct administration of the central government of China. The two cities both govern a dozen of counties and have the same status as provinces.

[b] Seizure data are not purity corrected.

[c] Consumption of a province was based on extrapolation using consumption in the urban area of the corresponding city and thus represents the maximum possible consumption of the province.

indicating that there were net outflows of METH and KET from these provinces.

Acknowledgments

This work is based on work funded by the National Science Foundation of China (Grant No. 40772147 and 41401566). The authors wish to thank all the personnel at the 36 sewage treatment plants who assisted in wastewater sampling. We also thank two anonymous reviewers whose comments improved the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.07.025.

References

Andrés-Costa, M.J., Rubio-Lopez, N., Morales Suarez-Varela, M., Pico, Y., 2014. Occurrence and removal of drugs of abuse in wastewater treatment plants of Valencia (Spain). Environ. Pollut. 194, 152–162.

Baker, D.R., Barron, L., Kasprzyk-Hordern, B., 2014. Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A: chemical analysis and drug use estimates. Sci. Total Environ. 487, 629–641.

Baker, D.R., Kasprzyk-Hordern, B., 2011. Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography–positive electrospray ionisation tandem mass spectrometry. J. Chromatogr. A 1218 (12), 1620–1631.

Baker, D.R., Očenášková, V., Kvicalova, M., Kasprzyk-Hordern, B., 2012. Drugs of abuse in wastewater and suspended particulate matter—further developments in sewage epidemiology. Environ. Int. 48, 28–38.

Baker, D.R., Kasprzyk-Hordern, B., 2013. Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: new developments. Sci. Total Environ. 454, 442–456.

Bartelt-Hunt, S.L., Snow, D.D., Damon, T., Shockley, J., Hoagland, K., 2009. The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. Environ. Pollut. 157 (3), 786–791.

Banta-Green, C.J., Field, J.A., Chiaia, A.C., Sudakin, D.L., Power, L., de Montigny, L., 2009. The spatial epidemiology of cocaine, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) use: a demonstration using a population measure of community drug load derived from municipal wastewater. Addiction 104 (11), 1874–1880.

Bijlsma, L., Emke, E., Hernández, F., De Voogt, P., 2012. Investigation of drugs of abuse and relevant metabolites in Dutch sewage water by liquid chromatography coupled to high resolution mass spectrometry. Chemosphere 89 (11), 1399–1406.

Bisceglia, K.J., Roberts, A.L., Schantz, M.M., Lippa, K.A., 2010. Quantification of drugs of abuse in municipal wastewater via SPE and direct injection liquid chromatography mass spectrometry. Anal. Bioanal. Chem. 398 (6), 2701–2712.

Boleda, M.A., Galceran, M.A., Ventura, F., 2009. Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. Water Res. 43 (4), 1126–1136.

Bones, J., Thomas, K.V., Paull, B., 2007. Using environmental analytical data to estimate levels of community consumption of illicit drugs and abused pharmaceuticals. J. Environ. Monit. 9 (7), 701–707.

Bramness, J.G., Reid, M.J., Solvik, K.F., Vindenes, V., 2015. Recent trends in the availability and use of amphetamine and methamphetamine in Norway. Forensic Sci. Int. 246, 92–97.

Brewer, A.J., Ort, C., Banta-Green, C.J., Berset, J.-D., Field, J.A., 2012. Normalized diurnal and between-day trends in illicit and legal drug loads that account for changes in population. Environ. Sci. Technol. 46 (15), 8305–8314.

Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernandez, F.I., Reid, M., Ort, C., Thomas, K.V., Van Nuijs, A.L., De Voogt, P., 2013. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environ. Sci. Technol. 47 (3), 1452–1460.

Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R., Bagnati, R., 2006. Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry. Anal. Chem. 78 (24), 8421–8429.

Chiaia-Hernandez, A.C., Banta-Green, C.J., Field, J.A., 2011. Interpreting methamphetamine levels in a high-use community. Environ. Sci. Pollut. Res. 18 (9), 1471–1477.

Chiaia, A.C., Banta-Green, C., Field, J., 2008. Eliminating solid phase extraction with large-volume injection LC/MS/MS: analysis of illicit and legal drugs and human urine indicators in US wastewaters. Environ. Sci. Technol. 42 (23), 8841–8848.

Harman, C., Reid, M., Thomas, K.V., 2011. In situ calibration of a passive sampling device for selected illicit drugs and their metabolites in wastewater, and subsequent year-long assessment of community drug usage. Environ. Sci. Technol. 45 (13), 5676–5682.

Heuett, N.V., Ramirez, C.E., Fernandez, A., Gardinali, P.R., 2015. Analysis of drugs of abuse by online SPE-LC high resolution mass spectrometry: communal assessment of consumption. Sci. Total Environ. 511, 319–330.

Huerta-Fontela, M., Galceran, M.T., Martin-Alonso, J., Ventura, F., 2008. Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain. Sci. Total Environ. 397 (1), 31–40.

Irvine, R.J., Kostakis, C., Felgate, P.D., Jaehne, E.J., Chen, C., White, J.M., 2011. Population drug use in Australia: a wastewater analysis. Forensic Sci. Int. 210 (1), 69–73.

Jiang, J., Lee, C., Fang, M., Tu, B., Liang, Y., 2015. Impacts of emerging contaminants on surrounding aquatic environment from a Youth festival. Environ. Sci. Technol. 49, 792–799.
Kankaanpää, A., Ariniemi, K., Heinonen, M., Kuoppasalmi, K., Gunnar, T., 2014. Use of illicit stimulant drugs in Finland: a wastewater study in ten major cities. Sci. Total Environ. 487, 696–702.
Karolak, S., Nefau, T., Bailly, E., Solgadi, A., Levi, Y., 2010. Estimation of illicit drugs consumption by wastewater analysis in Paris area (France). Forensic Sci. Int. 200 (1–3), 153–160.
Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. Illicit drugs and pharmaceuticals in the environment–forensic applications of environmental data. Part 1: estimation of the usage of drugs in local communities. Environ. Pollut. 157 (6), 1773–1777.
Khan, U., van Nuijs, A.L., Li, J., Maho, W., Du, P., Li, K., Hou, L., Zhang, J., Meng, X., Li, X., Covaci, A., 2014. Application of a sewage-based approach to assess the use of ten illicit drugs in four Chinese megacities. Sci. Total Environ. 487, 710–721.
Kim, K.Y., Lai, F.Y., Kim, H., Thai, P.K., Mueller, J.F., Oh, J., 2015. The first application of wastewater-based drug epidemiology in five South Korean cities. Sci. Total Environ. 524–525, 440–446.
Lai, F.Y., Bruno, R., Hall, W., Gartner, C., Ort, C., Kirkbride, P., Prichard, J., Thai, P.K., Carter, S., Mueller, J.F., 2013a. Profiles of illicit drug use during annual key holiday and control periods in Australia: wastewater analysis in an urban, a semi-rural and a vacation area. Addiction 108 (3), 556–565.
Lai, F.Y., Bruno, R., Leung, H.W., Thai, P.K., Ort, C., Carter, S., Thompson, K., Lam, P.K., Mueller, J.F., 2013b. Estimating daily and diurnal variations of illicit drug use in Hong Kong: a pilot study of using wastewater analysis in an Asian metropolitan city. Forensic Sci. Int. 233 (1), 126–132.
Li, J., Hou, L., Du, P., Yang, J., Li, K., Xu, Z., Wang, C., Zhang, H., Li, X., 2014. Estimation of amphetamine and methamphetamine uses in Beijing through sewage-based analysis. Sci. Total Environ. 490, 724–732.
Lin, A.Y.-C., Wang, X.-H., Lin, C.-F., 2010. Impact of wastewaters and hospital effluents on the occurrence of controlled substances in surface waters. Chemosphere 81 (5), 562–570.
Lin, A.Y., Lee, W.N., Wang, X.H., 2014. Ketamine and the metabolite norketamine: persistence and phototransformation toxicity in hospital wastewater and surface water. Water Res. 53, 351–360.
Loganathan, B., Phillips, M., Mowery, H., Jones-Lepp, T.L., 2009. Contamination profiles and mass loadings of macrolide antibiotics and illicit drugs from a small urban wastewater treatment plant. Chemosphere 75 (1), 70–77.
Mackul'ak, T., Škubak, J., Grabic, R., Ryba, J., Birosova, L., Fedorova, G., Špalkova, V., Bodik, I., 2014. National study of illicit drug use in Slovakia based on wastewater analysis. Sci. Total Environ. 494–495, 158–165.
Martínez Bueno, M.J., Ucles, S., Hernando, M.D., Fernandez-Alba, A.R., 2011. Development of a solvent-free method for the simultaneous identification/quantification of drugs of abuse and their metabolites in environmental water by LC-MS/MS. Talanta 85 (1), 157–166.
Metcalfe, C., Tindale, K., Li, H., Rodayan, A., Yargeau, V., 2010. Illicit drugs in Canadian municipal wastewater and estimates of community drug use. Environ. Pollut. 158 (10), 3179–3185.
Office of China National Narcotic Control Commission, 2012. Annual Report on Drug Control in China (Beijing, China).
Office of China National Narcotic Control Commission, 2014. Annual Report on Drug Control in China (Beijing, China).
Office of China National Narcotic Control Commission, 2015. Annual Report on Drug Control in China (Beijing, China).
Ort, C., van Nuijs2, A.L.N., Berset, J., Bijlsma, L., Castiglioni, S., Covaci, A., de Voogt, P., Emke, E., Fatta-Kassinos, D., Griffiths, P., Hernández, F., González-Mariño, I., Grabic, R., Kasprzyk-Hordern, B., Mastroianni, N., Meierjohann, A., Nefau, T., Östman, M., Pico, Y., Racamonde, I., Reid, M., Slobodnik, J., Terzic, S., Thomaidis, N., Thomas, K.V., 2014. Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. Addiction 109, 1338–1352.
Östman, M., Fick, J., Nasstrom, E., Lindberg, R.H., 2014. A snapshot of illicit drug use in Sweden acquired through sewage water analysis. Sci. Total Environ. 472, 862–871.
Panawennage, D., Castiglioni, S., Zuccato, E., Davoli, E., Chiarelli, M.P., 2011. Measurement of illicitdrug consumption in small populations: prognosis for noninvasive drug testing of student populations. In: Castiglioni, S., Zuccato, E., Fanelli, R. (Eds.), Illicit Drugs in the Environment: Occurrence, Analysis, and Fate Using Mass Spectrometry. John Wiley &Sons, Hoboken, New Jersey.
Postigo, C., de Alda, M.J.L., Barceló, D., 2010. Drugs of abuse and their metabolites in the Ebro River basin: occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. Environ. Int. 36 (1), 75–84.
Postigo, C., Lopez de Alda, M.J., Barceló, D., 2008. Analysis of drugs of abuse and their human metabolites in water by LC-MS/MS: a non-intrusive tool for drug abuse estimation at the community level. TrAC Trends Anal. Chem. 27 (11), 1053–1069.
Prichard, J., Hall, W., de Voogt, P., Zuccato, E., 2014. Sewage epidemiology and illicit drug research: the development of ethical research guidelines. Sci. Total Environ. 472, 550–555.
Repice, C., Dal Grande, M., Maggi, R., Pedrazzani, R., 2013. Licit and illicit drugs in a wastewater treatment plant in Verona, Italy. Sci. Total Environ. 463–464, 27–34.
Subedi, B., Kannan, K., 2014. Mass loading and removal of select illicit drugs in two wastewater treatment plants in New York State and estimation of illicit drug usage in communities through wastewater analysis. Environ. Sci. Technol. 48 (12), 6661–6670.
Terzic, S., Senta, I., Ahel, M., 2010. Illicit drugs in wastewater of the city of Zagreb (Croatia)–estimation of drug abuse in a transition country. Environ. Pollut. 158 (8), 2686–2693.
Thomas, K.V., Bijlsma, L., Castiglioni, S., Covaci, A., Emke, E., Grabic, R., Hernández, F., Karolak, S., Kasprzyk-Hordern, B., Lindberg, R.H., 2012. Comparing illicit drug use in 19 European cities through sewage analysis. Sci. Total Environ. 432, 432–439.
van Dyken, E., Thai, P., Lai, F.Y., Ort, C., Prichard, J., Bruno, R., Hall, W., Kirkbride, K.P., Mueller, J.F., 2014. Monitoring substance use in prisons: assessing the potential value of wastewater analysis. Sci. Justice 54 (5), 338–345.
van Nuijs, A.L., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Neels, H., Covaci, A., 2009a. Cocaine and metabolites in waste and surface water across Belgium. Environ. Pollut. 157 (1), 123–129.
van Nuijs, A.L., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2009b. Analysis of drugs of abuse in wastewater by hydrophilic interaction liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 395 (3), 819–828.
van Nuijs, A.L., Mougel, J.-F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2011a. A one year investigation of the occurrence of illicit drugs in wastewater from Brussels, Belgium. J. Environ. Monit. 13 (4), 1008–1016.
van Nuijs, A.L., Mougel, J.-F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2011b. Sewage epidemiology—a real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. Environ. Int. 37 (3), 612–621.
van Nuijs, A.L., Gheorghe, A., Jorens, P.G., Maudens, K., Neels, H., Covaci, A., 2014. Optimization, validation, and the application of liquid chromatography-tandem mass spectrometry for the analysis of new drugs of abuse in wastewater. Drug Test. Anal. 6 (7–8), 861–867.
Vuori, E., Happonen, M., Gergov, M., Nenonen, T., Jarvinen, A., Ketola, R.A., Vahala, R., 2014. Wastewater analysis reveals regional variability in exposure to abused drugs and opioids in Finland. Sci. Total Environ. 487, 688–695.
Yargeau, V., Taylor, B., Li, H., Rodayan, A., Metcalfe, C.D., 2014. Analysis of drugs of abuse in wastewater from two Canadian cities. Sci. Total Environ. 487, 722–730.
Zuccato, E., Castiglioni, S., Tettamanti, M., Olandese, R., Bagnati, R., Melis, M., Fanelli, R., 2011. Changes in illicit drug consumption patterns in 2009 detected by wastewater analysis. Drug Alcohol Depend. 118 (2), 464–469.
Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., Fanelli, R., 2008. Estimating community drug abuse by wastewater analysis. Environ. Health Perspect. 116 (8), 1027–1032.
Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., 2005. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. Environ. Health 4, 14.

Environment International 138 (2020) 105645

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

A revised excretion factor for estimating ketamine consumption by wastewater-based epidemiology – Utilising wastewater and seizure data

Peng Du[a,b], Qiuda Zheng[c], Kevin V. Thomas[c], Xiqing Li[b], Phong K. Thai[c,*]

[a] Beijing Key Laboratory of Urban Hydrological Cycle and Sponge City Technology, College of Water Sciences, Beijing Normal University, Beijing 100875, China
[b] Laboratory of Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China
[c] Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, Brisbane, Queensland 4102, Australia

ARTICLE INFO

Handling Editor: Yong-Guan Zhu

Keywords:
Wastewater-based epidemiology
Ketamine
Norketamine
Excretion factor
Metabolite ratio

ABSTRACT

The rate of drug excretion (excretion factor) is a critical parameter for monitoring drug consumption in the population by wastewater-based epidemiology (WBE). Previous studies have refined excretion factors for common illicit drugs, such as cocaine, amphetamine, methamphetamine, heroin, to improve the accuracy and reduce uncertainty in back-calculating consumption. Nevertheless, for ketamine, one of the most prevalent psychoactive substances, a careful review of its excretion factors has not been performed due to limited pharmacokinetic data. Here we review WBE studies and seizure data to refine and validate the excretion factors for ketamine and norketamine. The average ketamine/norketamine ratio in wastewater (5.36) was much higher than that found in urine (0.64), which means that the excretion factors derived only from pharmacokinetics data are not appropriate. Based on the comparison of the ratio between estimated consumptions of ketamine and methamphetamine by WBE with their corresponding ratio in official seizure data, a revised WBE excretion factor of 20% was proposed for ketamine following this review and applied to estimate the ketamine consumption in China. The revised estimates of ketamine consumption corresponded well with drug statistics. This suggests that the revised ketamine excretion factor is appropriate for estimating ketamine consumption by WBE. Systematic review of WBE studies is a suitable approach to refine the excretion factors for substances with inadequate pharmacokinetic data.

1. Introduction

Ketamine (2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone) was developed in the early 1960s and has been widely used as an anesthetic in medical and veterinary procedures (Trujillo et al., 2011). Ketamine can also produce a dissociative state and cause hallucinations, making it a substance of misuse in many countries (Xu and Lei, 2014). The misuse of ketamine has to date been reported in 32 countries (e.g. China, America, UK, etc.) with 70 countries listing ketamine as a controlled substance (WHO, 2015). Ketamine misuse can lead to damage of the psychopathological and central nervous system (Bokor and Anderson, 2014). Furthermore, it can also cause epigastric pain, hepatic dysfunction, impaired gallbladder activity, and kidney damage following long-term misuse (Corazza et al., 2013; Delimbeuf et al., 2014). Monitoring the level of ketamine consumption in communities is therefore essential to provide effectiveness of both drug control and public health policies.

Wastewater-based epidemiology (WBE), an approach that analyses drug residues in wastewater to estimate drug consumption, has become an established method for monitoring population level illicit drug consumption (EMCDDA, 2016; ACIC, 2018; Du et al., 2019), including that of ketamine. High concentrations of ketamine and norketamine (a major metabolite of ketamine) residues have been reported in Hong Kong wastewater (Lai et al., 2013). Du et al. (2015) also quantified ketamine and norketamine in wastewater influents to estimate ketamine consumption in mainland China and found an increasing trend of ketamine misuse from the north to the south across the country. Similar work has been conducted in other countries such as Canada (Yargeau et al., 2014) and the United Kingdom (Baker et al., 2014). Many other WBE studies measured ketamine and norketamine in order to estimate the population consumption of ketamine as shown in Table 1.

To obtain accurate estimates of ketamine consumption by WBE, the excretion factors (EFs) of the ketamine or its metabolites (i.e. norketamine) are essential (Castiglioni et al., 2014; Gracia-Lor et al., 2016). The EF of a drug is the fraction of the drug dosage that is excreted in the urine and feces as unchanged drug or its metabolites after consumption.

* Corresponding author.
E-mail address: p.thai@uq.edu.au (P.K. Thai).

https://doi.org/10.1016/j.envint.2020.105645
Received 18 December 2019; Received in revised form 6 March 2020; Accepted 7 March 2020

Table 1
Summary of wastewater analysis studies that involved the measurements of ketamine (KET), norketamine (NK) and methamphetamine (METH).

Reference	Year of study[a]	Country	WWTP size ($\times 10^4$)[a]	No. of data	Influent concentrations (mean/range) (ng/L)			KET/NK ratio[b]
					KET	NK	METH	
Chiaia et al. (2008)		US	201		4 (< LOD-16)	< LOD	572 (< LOD-2000)	
Baker and Kasprzyk-Hordern, 2011a	2010	UK	21–32.3	7	79.4	26.5	2	3.00
Baker and Kasprzyk-Hordern, 2011b	2011	UK	> 10	12	113.6 (45.7–349.4)	8.8 (< LOQ-20.1)	0.2 (< LOD-0.6)	10.81
Baker et al. (2012)	2011	Czech	> 30	7	< LOD	< LOD	569 (393.0–822.7)	
Baker et al. (2013)		UK	90	79	52.2[c] (4.9–447.3)	10.7[c] (5.0–96.4)	2.3[c] (0.6–70.3)	4.88
Lai et al. (2013)	2011	China	350	48	290[d]	23[d]	62[d]	13[e]
Baker et al. (2014)	2011	UK	340	14	199.9 (145–277)	46.3 (25.2–85.1)	22.9 (18.0–39.8)	4.64
Borova et al. (2014)	2012	Greece	3.815	5	2.3 (< LOD-6.1)	6.9 (< LOD-9.9)	2.8 (< LOD-5.6)	0.87
Khan et al. (2014)	2012	China	> 1000	36	151 (< LOQ-1175)	16 (< LOQ-101)	334 (32–3768)	7.87
Yargeau et al. (2014)	2011	Canada	157.5	4	40		18	
Östman et al. (2014)	2012	Sweden	212.45	33	< LOQ	< LOQ	8.5 (< LOQ-68)	
van Dyken et al. (2014)	2013	Australia	0.0437	7	26 (17–43)	2 (1–4)	130 (60–250)	12.5
Andrés-Costa et al. (2014)	2011–13	Spain	125		18.9 (< LOQ-131.8)		26.1 (1.2–69.1)	
van Nuijs et al. (2014)	2012	Belgium	> 30	48	12 (5–28)	< LOQ		
Castiglioni et al. (2015)	2010–13	Italy	658.52	357	< LOQ-80	< LOD	< LOQ-24	
Mackul'ak et al. (2015)	2013	Slovakia	12.5	1	< 5.4	< 3.4	763	
Du et al. (2015)	2014	China	> 3500	78	61.2 (< LOQ-376.6)	11.2 (< LOQ-74.6)	229.3 (17–684.6)	6.40
Kim et al. (2015)	2012–13	South Korea	> 370	15	< LOD	< LOD	18.8 (< LOQ-49.7)	
Senta et al. (2015)	2015	Croatia		1	5.6	3.7	< LOD	1.51
Mackul'ak et al. (2016)	2013	Slovakia	111	63	< LOD	< LOD		
van Dyken et al. (2016)	2013		0.0437	24	30[d]	no report	25[d]	
Zhang et al. (2016)	2013	China	> 1070	64	4.8 (< LOQ-19.5)	3.4 (< LOQ-6.2)		1.52
Li et al. (2019)	2016	China	> 3000	101	18.5 (< LOD-98.4)	4.4 (< LOD-27.8)	136.7 (14.8–367.5)	4.03
Du et al. (2020)	2017	Malaysia	22	20	273.9 (188.0–354.4)	91.3 (51.2–106.4)	1014.3 (690.4–1640.0)	3.04
Lin et al. (2014)[f]	2012	Taiwan (China)		15	90.4 (11–240)	89.7 (6.1–330)		2.00
Average KET/NK ratio (No. of data weighted)								**5.36 (95% CI[g])**

[a] Empty cell means data not available.
[b] KET/NK ratio was calculated from concentration values (both above LOQ).
[c] Median.
[d] Mass load (mg/1000 inh/d).
[e] Load ratio.
[f] Hospital effluents.
[g] Confidence interval.

This important parameter of WBE is often deduced from a small number of human pharmacokinetic studies, usually involving small numbers of human subjects (Bruno et al., 2014). The same situation happens for the EFs for ketamine and norketamine, which led to the fact that different EF values were used among studies, from as low as 2.3% (Andrés-Costa et al., 2014) to as high as 30% (Yargeau et al., 2014). The lack of a well-established EF for ketamine and its metabolites result in large uncertainties when consumption is estimated.

Earlier studies have evaluated the EFs of common illicit drugs, such as cocaine, heroin, methamphetamine, amphetamine, Δ9-tetrahydrocannabinol (THC) and 3,4-methylenedioxymethamphetamine (MDMA) based on reviewing available pharmacokinetic data (Khan and Nicell, 2011, 2012; Castiglioni et al., 2011, 2016). But refining the EFs for target drugs is a continued effort in order to increase reliability, and reduce the uncertainties of the final estimates (Gracia-Lor et al., 2016). For this purpose, Thai et al. (2016, 2019) proposed and validated a new approach to refine the EFs of codeine, methadone and EDDP by combining pharmacokinetic data with WBE data to overcome the shortcoming of small human cohort studies. This approach can be applied to identify suitable EFs of ketamine and norketamine from the large range of values mentioned above, in order to estimate its consumption by WBE more accurately.

This study thus aims to review pharmacokinetic and WBE studies and then combine with seizure data to refine the EFs of ketamine and norketamine. The newly derived EFs will be used to estimate ketamine consumption from a real wastewater monitoring data set to assess its applicability.

2. Methods

2.1. Data collection

The Web of Science (http://apps.webofknowledge.com/), China National Knowledge Infrastructure (http://www.cnki.net/) and Google Scholar (http://scholar.google.com/) database were searched for studies related to ketamine and norketamine (only scientific literature was used in this study). The search focused on two main categories as follows: (a) pharmacokinetics of ketamine, namely: ketamine, norketamine, excretion, urine/urinary, percentage; and (b) monitoring ketamine use using WBE, namely: ketamine, norketamine in wastewater/sewage, excretion factor/correction factor. The search was limited to scientific literature published in English and Chinese. After the search, all abstracts were screened to identify whether the studies could be used in this work. We also obtained the seizures data of ketamine and methamphetamine in China from government report as part of the validation data for the new value of the EFs.

2.2. Refining the EFs

After screening, data from the selected studies were summarized to identify current EFs of ketamine and norketamine, the ratio of ketamine/norketamine in wastewater and urine, and other statistics of ketamine use. Then the new EF of ketamine was deducted by matching the WBE estimates with the official statistics as described previously by Thai et al. (2016).

Table 2
Summary of studies involving the measurements of ketamine (KET), norketamine (NK), dehydronorketamine (DHNK) and hydroxynorketamine (HNK) in urine samples.

Reference	Study site[b]	No. of data	Infusion routes	HD[h]	Concentrations (mean/range) (ng/mL)				KET/NK ratio
					KET	NK	DHNK	HNK	
Lua and Lin (2004)	Taiwan	168	SAK[c]	NO	62 (24–86)	175 (77–344)			0.44
Chen et al. (2007)		3	IJ[d] (10 mg/p[e])	NO	38 (5.4–131)	30 (12.5–74.1)	101 (22.8–278.9)		1.31
Kim et al. (2008)		6	SAK	NO	18792 (30–56160)	10480 (nd-29310)			1.76
Lin et al. (2004)	Taiwan	50	SAK	NO	661 (105–7055)	952 (114–3477)	2284 (292–6830)		0.43
	Taiwan	50	SAK	YES	317 (120–866)	1329 (200–3942)	2901 (330–10340)		0.53
Harun et al. (2009)	Malaysia	34	SAK	YES	2255 (22–17260)	4071 (200–10990)			1.44
Huang et al. (2005)	Taiwan	20	SAK	NO	1193 (86–6515)	2499 (291–11490)	16042 (57–79974)		0.84
Moaddel et al. (2010)		1	IF[e] (40 mg/h for 5 days)	NO	3769	136	16,667		27.7
Cheng et al. (2008)	Taiwan	14	SAK	NO	1486(21–5620)	2577(113–9031)			0.41
Wu et al. (2007)	Taiwan	30	IF (10 mg/p)	NO	3.6	5.84	32.33		0.62
de Bairros et al. (2014)	Brazil	1		NO	87.3	5805	8760		0.02
Chou et al. (2004)	Taiwan	4		NO	479 (175–1172)	745(53–1708)			1.56
Fassauer et al. (2017)		3	IF (5 mg/p)	NO	12.9	19.3	45.7	32.5	0.66
Moore et al. (2001)		33	complex-	NO	1083 (6–7744)	1156 (7–7986)	2601 (37–23239)		0.74
Wang et al. (2005)		10		NO	1645 (114–4570)	3040 (453–9805)	8570 (307–33715)	676 (52.1–4099)	0.68
Huang et al. (2007)		30		NO	5156 (585–41700)	19,101 (770–153074)	85,859 (460–1139400)		0.55
Adamowicz et al. (2005)		67	IIF[f] (0.75–1.59 mg/kg)	YES	263 (3–1204)	383 (6–2559)			0.73
Parkin et al. (2008)[a]		6	OA[g] (50 mg/p)	NO	110	721			0.15
Average KET/NK ratio (No. of data weighted)									**0.64 (95% CI)**

[a] Data estimated from figures.
[b] Empty cell means data not available.
[c] Suspected abusing KET.
[d] Injection.
[e] Infusion.
[f] Intravenous infusion.
[g] Oral administration.
[h] Hydrolysis.

As there was no official consumption data of ketamine that could be used for direct deduction of the EF, we had to apply an indirect approach. We assume that there is a positive correlation between the consumption and seizure of drugs as demonstrated previously by many studies (Schifano and Corkery, 2007; Schifano et al., 2005; Baldwin et al., 2018; Goulding et al., 2020). Therefore, we can deduct the EF by comparing the ratio between estimated consumptions of different drugs with their corresponding ratio in official seizure data. Since methamphetamine is a very common drug reported in WBE and seizure data in China, we used the methamphetamine/ketamine ratio for the deduction purposes as below:

$$\frac{Consumption_{METH}}{Consumption_{KET}} = \frac{Seizure_{METH}}{Seizure_{KET}} \quad (1)$$

$$Consumption_{METH} = \frac{C_{METH} \times F}{P} \times \frac{1}{EF_{METH}} \times K \quad (2)$$

$$EF_{KET} = \frac{Massload_{KET}}{Consumption_{KET}} \quad (3)$$

where $Consumption_{KET}$ (mg/1000 inh/d) is the ketamine consumption, and could be calculated by Eq. (1); $Consumption_{METH}$ (mg/1000 inh/d) is the methamphetamine consumption estimates through WBE, and could be calculated by Eq. (2); C_{METH} (ng/L) is the influent concentration of methamphetamine; F is the influent flow rate of the specific wastewater treatment plant (L/d); P is the population served by the wastewater treatment plant; EF_{METH} is the EF of methamphetamine (43%) (Baselt, 2008), unchanged methamphetamine was used as the biomarker for back-estimation in this study; K is a unit conversion coefficient (1.0×10^{-3}); the new EF for ketamine (EF_{KET}) is calculated by Eq. (3).

Data of ketamine and methamphetamine from two sampling campaigns across China in 2014 (Du et al., 2015) and 2016 (Li et al., 2019) were used to deduct the new EFs. National statistical seizure data (ketamine and methamphetamine) were extracted from the China National Narcotic Control Committee. Estimated consumption and official seizure data of methamphetamine was employed as reference values for our deduction.

2.3. Validation of the new EFs by a real WBE data set

As described above, there was no official consumption data that can be used for validating the new EFs values. Therefore, the validation was performed by the same approach by comparing the ratio between estimated consumptions of ketamine and methamphetamine in WBE with their corresponding ratio in official seizure data in a new data set.

For the validation purpose, ketamine and methamphetamine were measured in 190 influent wastewater samples from 23 major Chinese cities in 2017, with adequate quality control/quality assurance measures. The data set were generated from samples from WWTPs serving more than 40 million and in all seven geographical regions of China. Details on the sampling and analysis are described in the Supplementary Information. Eventually, the ratio of methamphetamine and ketamine consumption across China (METH/KET) from wastewater monitoring of 2017 was compared to the ratio derived from seizure data in same year.

3. Results and discussion

3.1. Search results

Twenty-five WBE studies have to date reported the measurement or estimation of ketamine, norketamine and methamphetamine and were used in our review (Table 1). Ketamine and norketamine, the two target biomarkers of ketamine, have been monitored in wastewater in many countries along with other drugs using multi-residue methods. The mean ketamine and norketamine concentration ranged from < LOD to 273.9 ng/L and < LOD to 91.3 ng/L, respectively. But more monitoring campaigns have been conducted in China for the two biomarkers of ketamine because of the high prevalence of ketamine use in the country. For the validation of the refined ketamine and norketamine EFs discussed in Section 3.5, methamphetamine concentrations were also recorded in Table 1. Seventeen publications reported the urine concentrations of ketamine, norketamine, dehydronorketamine and hydroxynorketamine, which could be used to calculate the urinary ketamine/norketamine ratio (Table 2). We also referred to Baselt (2008), which contains a monograph on ketamine metabolism and excretion. Studies focus on animals and human plasma samples were not included in this study (Cohen and Trevor, 1974; Stenberg and Idvall, 1981a,b). Seizure data of ketamine and methamphetamine were obtained from official reports and were used to estimate and validate the new EFs.

3.2. Currently used excretion factors in the literature

In general, ketamine use has been estimated by quantifying norketamine and ketamine in influents. Lai et al. (2013) adopted norketamine, and Andrés-Costa et al. (2014) adopted ketamine (unchanged) as biomarkers for their back-estimation, using 1.6% and 2.3% as their respective EFs. These EFs were taken from pharmacokinetic studies reported by Wieber et al. (1975), and included in monographs of Nutt and Williams (2004), Wills (2005) and Baselt (2008) (Table 3). In other studies, an EF of 30% has been used for ketamine by Yargeau et al. (2014) and Du et al. (2015) (Table 3). This EF was said to be sourced from a website (www.drugs.com), but we could not trace to the specific webpage.

The available pharmacokinetic excretion data for ketamine (2.3%) and for norketamine (1.6%) (ketamine/norketamine ratio ~1.44), have been obtained from studies where the drug was administered intravenously (Wieber et al., 1975) (Table 3). However, the most common route of illicit ketamine administration in humans currently is by snorting the drug (Wills, 2005). Different routes of administration may result in different EFs for ketamine and norketamine (Blonk et al., 2010; Soto et al., 2012), especially when ketamine administered by snorting will be available in the blood immediately without passing through the liver (first pass effect). To the best of our knowledge, excretion data related to nasal administration of ketamine are not available (Baker et al., 2014). Therefore, caution should be exercised when back-estimating the consumption of illicit ketamine because the EFs mentioned above may not be appropriate. Du et al. (2015) found that the application of low EFs (i.e. 2.3% and 1.6%) would yield a level of ketamine consumption one order of magnitude higher than that of methamphetamine in most Chinese cities. Such an estimate does not seem reasonable as the current prevalent profile of drug misuse in China where both the number of methamphetamine users and seized methamphetamine are always much greater than those of ketamine (OCNNCC, 2014, 2016, 2018).

3.3. Ketamine/norketamine ratio in urine and wastewater

From urine analysis studies, the weighted average ratio of ketamine and norketamine in raw urine samples was 0.64 (95% CI) (Table 2). This value was even lower than the ketamine/norketamine ratio derived from the two EF values mentioned above (~1.44). Except the extreme high ratio (27.7) from one patient reported by Moaddel et al. (2010) most studies reported ratios of ketamine/norketamine around 1, which are different from the ratios found in wastewater samples as described below.

Table 1 shows that the weighted average ketamine/norketamine ratio was 5.36 (95% CI) in influent wastewater samples. Ketamine/norketamine ratio in wastewater can be observed to vary markly from the mean value. The lowest ratio value of 0.87 was reported by Borova et al. (2014), whereas ratios higher than 10 were found in studies of Baker and Kasprzyk-Hordern, 2011; Lai et al., 2013; van Dyken et al., 2014. Such deviations are probably because of the high individual variability in ketamine metabolism. Another potential confounding factor is the direct disposal of ketamine to the sewers due to veterinary use and local police activity. Although direct disposal doesn't occur frequently, it cannot be excluded and may affect the ketamine/norketamine ratio, as already observed for other drugs in some WBE studies (Mastroianni et al., 2017).

It is important to note that the average ketamine/norketamine ratio in wastewater was much higher than that in urine and many WBE studies did not estimate ketamine consumption (Table 1). One of the reasons could be that there was much uncertainty in the EFs as stated by Baker et al. (2014) (see their Supplement Information). The difference in ketamine/norketamine ratio between pharmacokinetic data and WBE data may also contribute to the uncertainty. At the moment, there is no explanation for this difference in ratios. From the literature, we know that the stability of ketamine and norketamine in sewer and wastewater is similar (McCall et al., 2016; Gao et al., 2017), and

Table 3
Current urine excretion factors of ketamine and metabolites from pharmacokinetic studies.

Reference	Infusion routes	EFs (%)	Note	Used in WBE studies	
				Ketamine as BM[b]	Norketamine as BM
Wieber et al. (1975)	Intravenous	Ketamine, norketamine and dehydronorketamine: 20 Glucuronide conjugates types: 80	–	–	–
Baselt (2008)	–	Ketamine: 2.3 Norketamine: 1.6 Dehydronorketamine: 16.2 Glucuronide conjugates types: 80	Generalized based on the results of Anonymous (1970)[a] and Wieber et al. (1975).	Andrés-Costa et al. (2014)	Lai et al. (2013)van Dyken et al. (2014, 2016)
Nutt and Williams (2004)	–	Ketamine: 2	Generalized based on previous studies	–	–
Yargeau et al. (2014)	–	Ketamine: 30	Sourced from a website, but cannot be traced (www.drugs.com).	Yargeau et al. (2014) Du et al. (2015)	–

[a] This anonymous reference cannot be traced.
[b] Biomarker.

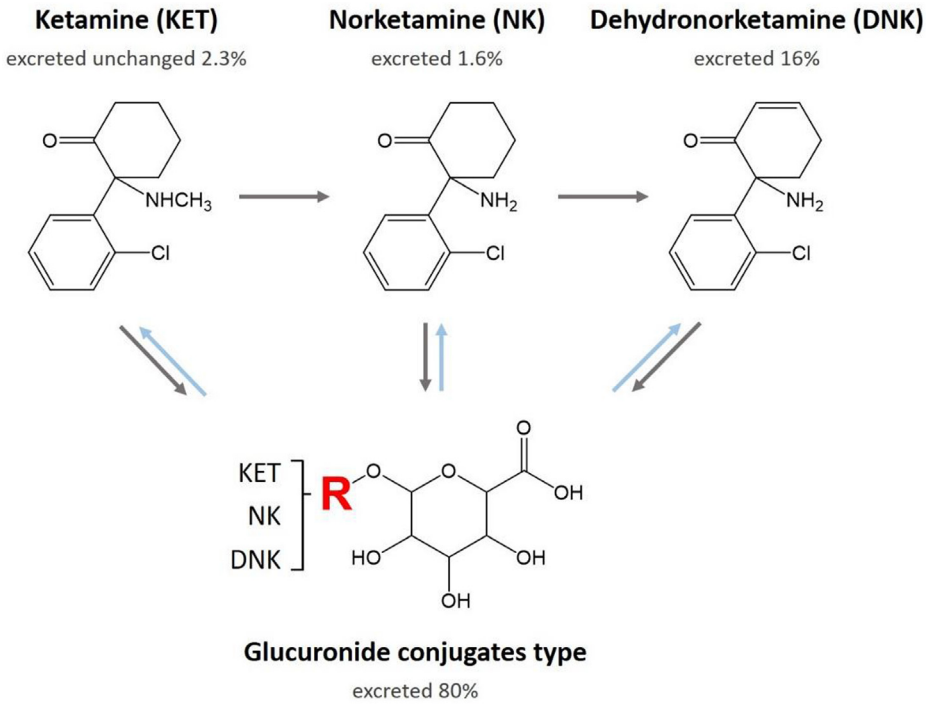


Fig. 1. Ketamine metabolism and excretion by humans (Baselt, 2008).

degradation of the same rate would not change the ratio.

A potential explanation is the hydrolysis of conjugated ketamine during sewer transport. In human body, ketamine is metabolized to dehydronorketamine (16%) and glucuronide conjugates of ketamine, norketamine and dehydronorketamine (80%), and excreted with free nortakamine and unchanged ketamine in urine (Fig. 1) (Anonymous, 1970; Wieber et al., 1975). It is thus likely that during sewer transport, the conjugated form of ketamine was hydrolyzed similar to other glucuronide compounds as shown by Gao et al. (2017) and changed the ketamine/norketamine ratio from low (like in urine samples) to higher (like in wastewater samples).

3.4. New excretion factors for ketamine and norketamine

As discussed above, the pharmacokinetic data for deriving appropriate EFs for ketamine and norketamine are limited, therefore in this study we utilized the data from WBE studies, including the ketamine/norketamine ratio in wastewater samples for refining the EFs used in WBE as proposed by Thai et al. (2016, 2019).

Of the two biomarkers, norketamine could be a more suitable biomarker for monitoring and back-estimating ketamine use by WBE because it is a product of human metabolism, which reduces the uncertainty in estimation due to occasional direct disposal of ketamine. However, norketamine has a much lower concentration and detection frequency in most WBE studies so it is more practical to use ketamine as a biomarker. In wastewater, the ratio of ketamine/norketamine is consistently higher than 1 (with the average value of 5.36). Therefore, the EF of ketamine in wastewater must be higher than that of norketamine, different to the values derived from pharmacokinetic data.

Data from two large-scale WBE monitoring campaigns of Du et al. (2015) and Li et al. (2019), in which ketamine and methamphetamine use was prevalent, were used to derive the new ketamine EFs with the seizure data. In these studies, the average influent mass load of ketamine and WBE estimated methamphetamine consumption across China were 16.4 mg/1000 inh/d and 157.7 mg/1000 inh/d in 2014 (Du et al., 2015), and the values were 5.7 mg/1000 inh/d and 97.7 mg/1000 inh/d in 2016 (Li et al., 2019). The official seizure methamphetamine/ketamine ratios were 2.31 in 2014 (OCNNCC, 2015) and 2.79 in 2016 (OCNNCC, 2017), respectively. Based on the Eqs. (1), (2) and (3), the calculated values of new ketamine EF for 2014 and 2016 data are 24% and 16%, respectively. For reducing the variation from different data sources, we propose 20% (average value) as the new EF of ketamine used in WBE estimation, which fits with the data as shown in Fig. 2. Assuming the average wastewater ratio of 5.36 between ketamine and norketamine, it means that the corresponding new EF for norketamine in WBE is around 4%.

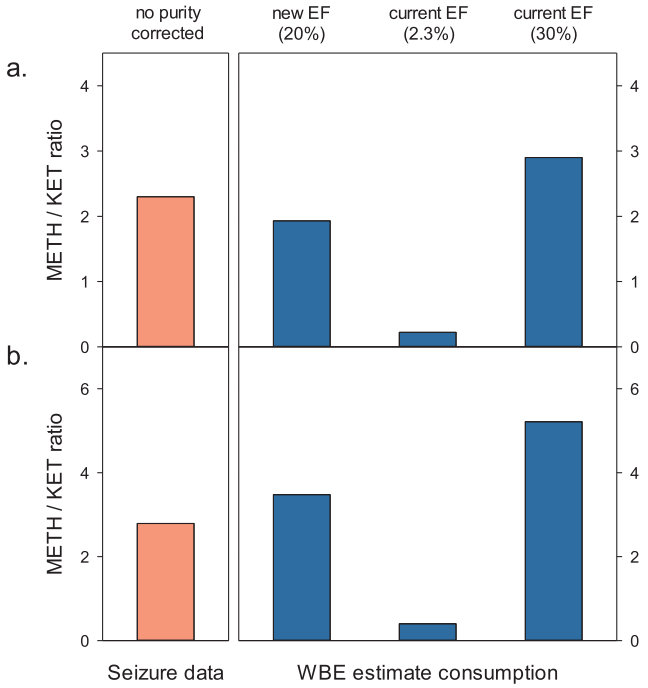


Fig. 2. Comparison and deduction of the new EF for ketamine using WBE data in 2014 (a) and 2016 (b) across China.

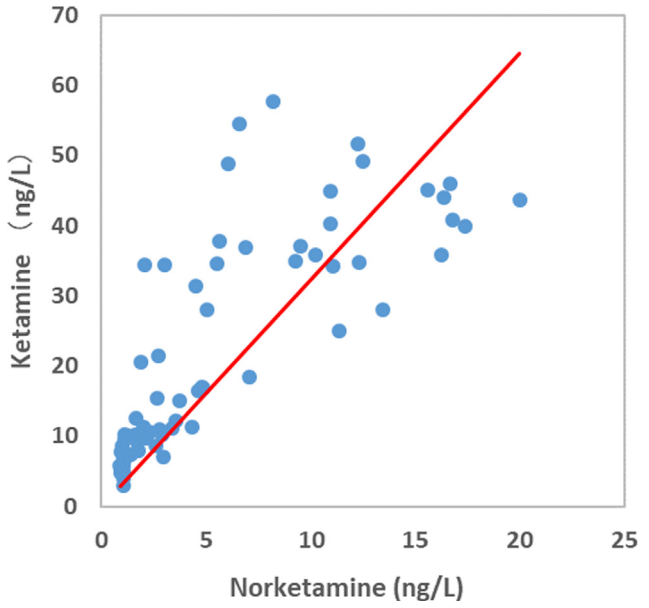


Fig. 3. Ratio of ketamine and norketamine in wastewater.

3.5. Validation using real data

For the wastewater samples analyzed in this study, the frequency of ketamine, norketamine and methamphetamine detection was 91%, 42% and 100%, suggesting that ketamine and methamphetamine are commonly used drugs. Fig. 3 shows the concentrations of ketamine were consistently higher than those of norketamine and the ratio of ketamine/norketamine found in this study (5.12), similar to what was observed in other WBE studies (Table 1).

Subsequently, back-estimation of ketamine consumption over this period was performed using both the revised and existing EFs for ketamine. Norketamine data were not used because of low detection frequency. We calculated the ratios of estimated methamphetamine/ketamine (METH/KET) consumption as well as the ratio of the officially recorded seizure data from the China National Narcotic Control Committee. The METH/KET consumption ratio using the new EF value matched well with the METH/KET ratio of official seizure data (Fig. 4). It suggested that the new EF for ketamine is appropriate for this purpose.

Due to low detection frequency and low concentration of norketamine in wastewater samples, the validation for new norketamine EF based on the seizure data is not feasible.

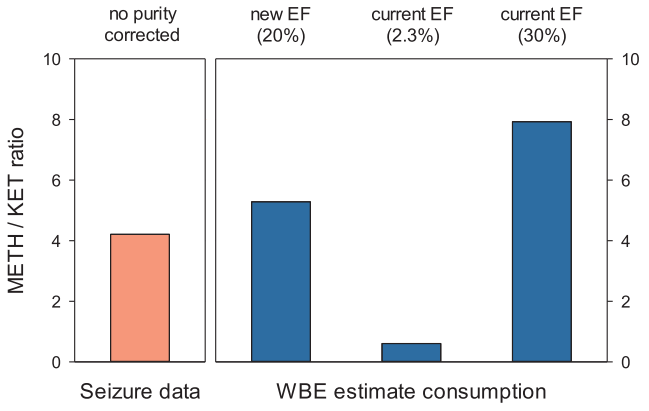


Fig. 4. Seizure methamphetamine/ketamine ratio compared with WBE estimated consumption ratio using the WBE data across China in 2017.

3.6. Advantages and limitations

As demonstrated by previous studies, the back-estimations in WBE using excretion factors derived from pharmacokinetic data only will require an additional correction factor for the degradation of chemicals and deconjugation of glucuronide-bound chemicals during the transport from the toilet to the inlet of the treatment plant (D'Ascenzo et al., 2003; Gao et al., 2017; O'Brien et al., 2017). The new EFs derived by utilising wastewater and seizure data are more suitable for WBE estimation because they include potential transformation processes in-sewer (i.e., deconjugation of glucuronide-bound chemicals and their transformation). The EF derived by this study supports more realistic estimates of ketamine consumption than the EF derived only from pharmacokinetic data.

We also acknowledge that our approach has a number of limitations. Firstly, a lack of accurate consumption data for ketamine is an important limitation of this study. Fortunately, we were able to rely on the unique set of seizure data for ketamine and methamphetamine over the years provided by the China National Narcotic Control Committee to derive the EF although the assumption that drug consumptions are proportional to their seizures was not fully proven. The use of seizure data, despite its general correlation with consumption, could contain bias due to the nature of enforcement and drug trafficking. The newly derived EFs, while acceptable when pharmacokinetic data are limited, should be refined when new data are available. Secondly, the EF of ketamine derived in this study is an average estimate from a large population. While it is the most representative value we could derive from available data, we acknowledge that EF is always associated with considerable uncertainty and can vary as showed by the data in 2014 and 2016. We haven't evaluated the uncertainty of the new EF even though there were variation in ketamine/norketamine ratio in both urine and wastewater samples. That would require further investigation to assess its impact.

4. Conclusions

In this study, new EFs of ketamine and norketamine for use in WBE studies were derived, combining wastewater and seizure data, in order to improve the accuracy of ketamine use estimates. Based on our literature review, it is more practical to use ketamine as WBE biomarker than norketamine as it is present at a higher concentration and is more frequently detected in wastewater. With the best data available, we found that the appropriate EF for ketamine is 20%. Systematic review to utilise WBE studies is a complementary approach to refine EFs for chemicals which lack adequate pharmacokinetic data.

CRediT authorship contribution statement

Peng Du: Data curation, Formal analysis, Resources, Writing - original draft. **Qiuda Zheng:** Data curation, Formal analysis, Investigation, Writing - original draft. **Kevin V. Thomas:** Writing - review & editing. **Xiqing Li:** Resources, Writing - review & editing. **Phong K. Thai:** Conceptualization, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work is supported by the National Natural Science Foundation of China (Grant No. 41701543 and 41877508) and the Fundamental

Research Funds for the Central Universities (Grant No. 2018NTST20 and 2017XTCX02). The authors extremely grateful to all the personnel at the 47 wastewater treatment plants for their assistance in wastewater sampling. Qiuda Zheng receives a PhD scholarship from the University of Queensland.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105645.

References

Adamowicz, P., Kala, M., 2005. Urinary excretion rates of ketamine and norketamine following therapeutic ketamine administration: Method and detection window considerations. J. Anal. Toxicol. 29, 376–382.

Anonymous, 1970. Ketamine. Parke-Davis and Company, Morris Plains, New Jersey.

Andrés-Costa, M.J., Rubio-Lopez, N., Morales Suarez-Varela, M., Pico, Y., 2014. Occurrence and removal of drugs of abuse in wastewater treatment plants of Valencia (Spain). Environ. Pollut. 194, 152–162.

ACIC (Australian Criminal Intelligence Commission). National Wastewater Drug Monitoring Program—Report 6, December 2018. Australia, 2018. (https://www.acic.gov.au/publications/intelligence- products/national-wastewater-drug-monitoring-program-report).

Baldwin, N., Gray, R., Goel, A., Wood, E., Buxton, J.A., Rieb, L.M., 2018. Fentanyl and heroin contained in seized illicit drugs and overdose-related deaths in British Columbia, Canada: An observational analysis. Drug Alcohol Depend 185, 322–327.

Baselt, R., 2008. Disposition of Toxic Drugs and Chemicals in Man. Biomedical Publications, Foster City, CA.

Blonk, M.I., Koder, B.G., van den Bemt, P.M., Huygen, F.J., 2010. Use of oral ketamine in chronic pain management: a review. Eur. J. Pain 14, 466–472.

Baker, D.R., Ocenaskova, V., Kvicalova, M., Kasprzyk-Hordern, B., 2012. Drugs of abuse in wastewater and suspended particulate matter–further developments in sewage epidemiology. Environ. Int. 48, 28–38.

Baker, D.R., Kasprzyk-Hordern, B., 2011b. Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1218, 7901–7913.

Baker, D.R., Kasprzyk-Hordern, B., 2011a. Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive electrospray ionisation tandem mass spectrometry. J. Chromatogr. A 1218, 1620–1631.

Baker, D.R., Kasprzyk-Hordern, B., 2013. Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: new developments. Sci. Total Environ. 454–455, 442–456.

Baker, D.R., Barron, L., Kasprzyk-Hordern, B., 2014. Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A: chemical analysis and drug use estimates. Sci. Total Environ. 487, 629–641.

Bokor, G., Anderson, P.D., 2014. Ketamine: An update on its abuse. J. Pharm. Pract. 27, 582–586.

Bruno, R., Hall, W., Kirkbride, K.P., Lai, F.Y., O'Brien, J.W., Prichard, J., Thai, P.K., Mueller, J.F., 2014. Commentary on Ort et al. (2014): What next to deliver on the promise of large scale sewage-based drug epidemiology? Addiction 109, 1353–1354.

Borova, V.L., Maragou, N.C., Gago-Ferrero, P., Pistos, C., Thomaidis, N.S., 2014. Highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 406, 4273–4285.

Cohen, M.L., Trevor, A.J., 1974. On the cerebral accumulation of ketamine and the relationship between metabolism and its pharmacological effects. J. Pharmacol. Exp. Ther. 189, 351–358.

Chou, S.L., Yang, M.H., Ling, Y.C., Giang, Y.S., 2004. Gas chromatography-isotope dilution mass spectrometry preceded by liquid-liquid extraction and chemical derivatization for the determination of ketamine and norketamine in urine. J. Chromatogr. B 799, 37–50.

Chen, C.Y., Lee, M.R., Cheng, F.C., Wu, G.J., 2007. Determination of ketamine and metabolites in urine by liquid chromatography–mass spectrometry. Talanta 72, 1217–1222.

Castiglioni, S., Bagnati, R., Melis, M., Panawennage, D., Chiarelli, P., Fanelli, R., et al., 2011. Identification of cocaine and its metabolites in urban wastewater and comparison with the human excretion profile in urine. Water Res. 45, 5141–5150.

Castiglioni, S., Thomas, K.V., Kasprzyk-Hordern, B., Vandamd, L., Griffiths, P., 2014. Testing wastewater to detect illicit drugs: State of the art, potential and research needs. Sci. Total Environ. 487, 613–620.

Castiglioni, S., Borsotti, A., Senta, I., Zuccato, E., 2015. Wastewater analysis to monitor spatial and temporal patterns of use of two synthetic recreational drugs, ketamine and mephedrone, in Italy. Environ. Sci. Technol. 49, 5563–5570.

Castiglioni, S., Gracia-Lor, E., 2016. Target drugs residues in wastewater, In Assessing Illicit Drugs in Wastewater. European Monitoring Centre for Drugs and Drug Addiction, Chapter 2.

Cheng, P.S., Lee, C.H., Liu, C., Chien, C.S., 2008. Simultaneous determination of ketamine, tramadol, methadone, and their metabolites in urine by gas chromatography-mass spectrometry. J. Anal. Toxicol. 32, 253–259.

Chiaia, A.C., Banta-Green, C., Field, J., 2008. Eliminating Solid Phase Extraction with Large-Volume Injection LC/MS/MS: Analysis of Illicit and Legal Drugs and Human Urine Indicators in US Wastewaters. Environ. Sci. Technol. 42, 8841–8848.

Corazza, O., Assi, S., Schifano, F., 2013. From "Special K" to"Special M": The evolution of the recreational use of ketamine and methoxetamine. CNS Neurosci. Ther. 19, 454–460.

D'Ascenzo, G., Di Corcia, A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., Samperi, R., 2003. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci. Total Environ. 302, 199–209.

Delimbeuf, N., Petit, A., Karila, L., Lejoyeux, M., 2014. Ketamine: Psychiatric indications and misuses. Rev. Med. Liege. 69, 434–440.

de Bairros, A.V., Lanaro, R., de Almeida, R.M., Yonamine, M., 2014. Determination of ketamine, norketamine and dehydronorketamine in urine by hollow-fiber liquid-phase microextraction using an essential oil as supported liquid membrane. Forensic Sci. Int. 243, 47–54.

Du, P., Li, K., Li, J., Xu, Z., Fu, X., Yang, J., et al., 2015. Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology. Water Res. 84, 76–84.

Du, P., Thai, P.K., Bai, Y., Zhou, Z., Xu, Z., Zhang, X., Wang, J., Zhang, C., Hao, F., Li, X., 2019. Monitoring consumption of methadone and heroin in major Chinese cities by wastewater-based epidemiology. Drug Alcohol Depend 205 107532.

Du, P., Liu, X., Zhong, G., Zhou, Z., Thomes, M.W., Lee, C.W., Bong, C.W., Zhang, X., Hao, F., Li, X., Zhang, G., Thai, P.K., 2020. Monitoring consumption of common illicit drugs in Kuala Lumpur, Malaysia, by wastewater-cased epidemiology. Int. J. Environ. Res. Public Health 17 (3), 889. https://doi.org/10.3390/ijerph17030889.

EMCDDA (European Monitoring Centre for Drugs and Drug Addiction). Assessing illicit drugs in wastewater: Advances in wastewater-based drug epidemiology, European, 2016.

Fassauer, G.M., Hofstetter, R., Hasan, M., Oswald, S., Modess, C., Siegmund, W., Link, A., 2017. Ketamine metabolites with antidepressant effects: Fast, economical, and eco-friendly enantioselective separation based on supercritical-fluid chromatography (SFC) and single quadrupole MS detection. J. Pharmaceut. Biomed. 146, 410–419.

Gracia-Lor, E., Zuccato, E., Castiglioni, S., 2016. Refining correction factors for back-calculation of illicit drug use. Sci. Total Environ. 573, 1648–1659.

Gao, J., Banks, A., Li, J., Jiang, G., Lai, F.Y., Mueller, J.F., Thai, P.K., 2017. Evaluation of in-sewer transformation of selected illicit drugs and pharmaceutical biomarkers. Sci. Total Environ. 609, 1172–1181.

Goulding, N., Hickman, M., Reid, M., Amundsen, E.J., Baz-Lomba, J.A., O'Brien, J.W., Tscharke, B.J., de Voogt, P., Emke, E., Kuijpers, W., Hall, W., Jones, H.E., 2020. A comparison of trends in wastewater-based data and traditional epidemiological indicators of stimulant consumption in three locations. in press. Addiction. https://doi.org/10.1111/add.14852.

Huang, M.K., Liu, C., Li, J.H., Huang, S.D., 2005. Quantitative detection of ketamine, norketamine, and dehydronorketamine in urine using chemical derivatization followed by gas chromatography-mass spectrometry. J. Chromatogr. B. 820, 165–173.

Huang, M.H., Wu, M.Y., Wu, C.H., Tsai, J.L., Lee, H.H., Liu, R.H., 2007. Performance characteristics of ELISAs for monitoring ketamine exposure. Clin. Chim. Acta 379, 59–65.

Harun, N., Anderson, R.A., Miller, E.I., 2009. Validation of an enzyme-linked immunosorbent assay screening method and a liquid chromatography-tandem mass spectrometry confirmation method for the identification and quantification of ketamine and norketamine in urine samples from Malaysia. J. Anal. Toxicol. 33, 310–321.

Khan, U., Nicell, J.A., 2011. Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. Environ. Int. 37, 1236–1252.

Khan, U., Nicell, J.A., 2012. Sewer epidemiology mass balances for assessing the illicit use of methamphetamine, amphetamine and tetrahydrocannabinol. Sci. Total Environ. 421–422, 144–162.

Khan, U., van Nuijs, A.L., Li, J., Maho, W., Du, P., Li, K., Hou, L., Zhang, J., Meng, X., Li, X., Covaci, A., 2014. Application of a sewage-based approach to assess the use of ten illicit drugs in four Chinese megacities. Sci. Total Environ. 487, 710–721.

Kim, E.M., Lee, J.S., Choi, S.K., Lim, M.A., Chung, H.S., 2008. Analysis of ketamine and norketamine in urine by automatic solid-phase extraction (SPE) and positive ion chemical ionization-gas chromatography-mass spectrometry (PCI-GC-MS). Forensic Sci. Int. 174 (2-3), 197–202.

Kim, K.Y., Lai, F.Y., Kim, H.Y., Thai, P.K., Mueller, J.F., Oh, J.E., 2015. The first application of wastewater-based drug epidemiology in five South Korean cities. Sci. Total Environ. 524–525, 440–446.

Lai, F.Y., Bruno, R., Leung, H.W., Thai, P.K., Ort, C., Carter, S., et al., 2013. Estimating daily and diurnal variations of illicit drug use in Hong Kong: a pilot study of using wastewater analysis in an Asian metropolitan city. Forensic Sci. Int. 233, 126–132.

Lin, H.R., Lua, A.C., 2004. Detection of acid-labile conjugates of ketamine and its metabolites in urine samples collected from pub participants. J. Anal. Toxicol. 28, 181–186.

Lin, A.Y., Lee, W.N., Wang, X.H., 2014. Ketamine and the metabolite norketamine: persistence and phototransformation toxicity in hospital wastewater and surface water. Water Res. 53, 351–360.

Li, X., Du, P., Zhang, W., 2019. Application of Wastewater-Based Epidemiology in China—From Wastewater Monitoring to Drug Control Efforts. Wastewater-Based Epidemiology: Estimation of Community Consumption of Drugs and Diets, Chapter 6. American Chemical Society, Washington, DC.

Lua, A.C., Lin, H.R., 2004. A rapid and sensitive ESI-MS screening procedure for ketamine and norketamine in urine samples. J. Anal. Toxicol. 28, 680–684.

Moore, K.A., Sklerov, J., Levine, B., Jacobs, A.J., 2001. Urine concentrations of ketamine and norketamine following illegal consumption. J. Anal. Toxicol. 25, 583–588.

Moaddel, R., Venkata, S.L.V., Tanga, M.J., Bupp, J.E., Green, C.E., Iyer, L., Furimsky, A., Goldberg, M.E., Torjman, M.C., Wainer, I.W., 2010. A parallel chiral-achiral liquid

chromatographic method for the determination of the stereoisomers of ketamine and ketamine metabolites in the plasma and urine of patients with complex regional pain syndrome. Talanta 82, 1892–1904.
Mackul'ak, T., Mosny, M., Skubak, J., Grabic, R., Birosova, L., 2015. Fate of psychoactive compounds in wastewater treatment plant and the possibility of their degradation using aquatic plants. Environ. Toxicol. Pharmacol. 39, 969–973.
Mackul'ak, T., Birosova, L., Gal, M., Bodik, I., Grabic, R., Ryba, J., Skubak, J., 2016. Wastewater analysis: the mean of the monitoring of frequently prescribed pharmaceuticals in Slovakia. Environ. Monit. Assess. 188, 18.
McCall, A.K., Scheidegger, A., Madry, M.M., Steuer, A.E., Weissbrodt, D.G., Vanrolleghem, P.A., Kraemer, T., Morgenroth, E., Ort, C., 2016. Influence of different sewer biofilms on transformation rates of drugs. Environ. Sci. Technol. 50, 13351–13360.
Mastroianni, N., López-García, E., Postigo, C., Barceló, D., de Alda, M.L., 2017. Five-yearmonitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends. Sci. Total Environ. 609, 916–926.
Nutt, D.J., Williams, T.M., 2004. Ketamine: An update 2000–2004. Psychopharmacology Unit University of Bristol.
Östman, M., Fick, J., Nasstrom, E., Lindberg, R.H., 2014. A snapshot of illicit drug use in Sweden acquired through sewage water analysis. Sci. Total Environ. 472, 862–871.
OCNNCC (Office of China National Narcotic Control Committee), 2014. 2014 Report of Drug Situation of China, Beijing.
OCNNCC (Office of China National Narcotic Control Committee), 2015. 2015 Report of Narcotics Control of China, Beijing.
OCNNCC (Office of China National Narcotic Control Committee), 2016. 2016 Report of Drug Situation of China, Beijing.
OCNNCC (Office of China National Narcotic Control Committee), 2017. 2017 Report of Narcotics Control of China, Beijing.
OCNNCC (Office of China National Narcotic Control Committee), 2018. 2018 Report of Drug Situation of China, Beijing.
O'Brien, J.W., Banks, A.P.W., Novic, A.J., Mueller, J.F., Jiang, G.M., Ort, C., Eaglesham, G., Yuan, Z.G., Thai, P.K., 2017. Impact of in-sewer degradation of pharmaceutical and personal care products (PPCPs) population markers on a population model. Environ. Sci. Technol. 51, 3816–3823.
Parkin, M.C., Turfus, S.C., Smith, N.W., Halket, J.M., Braithwaite, R.A., Elliott, S.P., Osselton, M.D., Cowan, D.A., Kicman, A.T., 2008. Detection of ketamine and its metabolites in urine by ultra high pressure liquid chromatography-tandem mass spectrometry. J. Chromatogr. B. 876, 137–142.
Stenberg, P., Idvall, J., 1981a. Does ketamine metabolite ii exist in vivo? Br. J. Anaesth. 7, 7.
Schifano, F., Corkery, J., 2007. Cocaine/crack cocaine consumption, treatment demand, seizures, related offences, prices, average purity levels and deaths in the UK (1990–2004). J. Psychopharmacol. 22, 71–79.
Schifano, F., Corkery, J., Deluca, P., Oyefeso, A., Ghodse, A.H., 2005. Ecstasy (MDMA, MDA, MDEA, MBDB) consumption, seizures, related offences, prices, dosage levels and deaths in the UK (1994–2003). J. Psychopharmacol. 20, 456–463.
Senta, I., Krizman, I., Ahel, M., Terzic, S., 2015. Multiresidual analysis of emerging amphetamine-like psychoactive substances in wastewater and river water. J. Chromatogr. A 1425, 204–212.
Soto, E., Stewart, D.R., Mannes, A.J., Ruppert, S.L., Baker, K., Zlott, D., Handel, D., Berger, A.M., 2012. Oral ketamine in the palliative care setting: a review of the literature and case report of a patient with neurofibromatosis type 1 and glomus tumor-associated complex regional pain syndrome. Am. J. Hosp. Palliat. Care 29, 308–317.
Stenberg, P., Idvall, J., 1981b. Does ketamine metabolite II exist in vivo? Brit. J. Anaesth. 53, 778.
Trujillo, K.A., Smith, M.L., Sullivan, B., Heller, C.Y., Garcia, C., Bates, M., 2011. The neurobehavioral pharmacology of ketamine: implications for drug abuse, addiction, and psychiatric disorders. ILAR J. 52, 366–378.
Thai, P.K., Lai, F.Y., Bruno, R., van Dyken, E., Hall, W., O'Brien, J., et al., 2016. Refining the excretion factors of methadone and codeine for wastewater analysis - Combining data from pharmacokinetic and wastewater studies. Environ. Int. 94, 307–314.
Thai, P.K., O'Brien, J.W., Tscharke, B., Mueller, J.F., 2019. Analyzing wastewater samples collected during Census to determine the correction factors of drugs for wastewater-based epidemiology: the case of codeine and methadone. Enviro. Sci. Tech. Let. 6, 265–269. https://doi.org/10.1021/acs.estlett.8b00673.
van Dyken, E., Thai, P., Lai, F.Y., Ort, C., Prichard, J., Bruno, R., Hall, W., Kirkbride, K.P., Mueller, J.F., 2014. Monitoring substance use in prisons: Assessing the potential value of wastewater analysis. Sci. Justice 54, 338–345.
van Dyken, E., Lai, F.Y., Thai, P.K., Ort, C., Bruno, R., Hall, W., Kirkbride, K.P., Mueller, J.F., Prichard, J., 2016. Challenges and opportunities in using wastewater analysis to measure drug use in a small prison facility. Drug Alcohol Rev. 35, 138–147.
van Nuijs, A.L., Gheorghe, A., Jorens, P.G., Maudens, K., Neels, H., Covaci, A., 2014. Optimization, validation, and the application of liquid chromatography-tandem mass spectrometry for the analysis of new drugs of abuse in wastewater. Drug Test Anal. 6, 861–867.
Wieber, J., Gugler, R.J., Hengstmann, H., Dengler, H.J., 1975. Pharmacokinetics of ketamine in man. Anaesthesist 24, 260–263.
Wang, K.C., Shih, T.S., Cheng, S.G., 2005. Use of SPE and LC/TIS/MS/MS for rapid detection and quantitation of ketamine and its metabolite, norketamine, in urine. Forensic Sci. Int. 147, 81–88.
Wills, S., 2005. Drugs of abuse, second ed. Pharmaceutical Press, London, UK.
Wu, G.J., Chan, H., Lee, M.R., Chen, C.Y., Yang, D.Y., Cheng, F.C., 2007. Simultaneous measurement of urinary ketamine, norketamine, and dehydronorketamine by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J. Chin. Chem. Soc-Taip. 54, 351–355.
WHO (World Health Organization). "Ketamine (INN): questionnaire report", presented to the WHO Expert Committee on Drug Dependence at its thirty-seventh meeting, held in Geneva from 16 to 20 November 2015.
Xu, J., Lei, H., 2014. Ketamine-an update on its clinical uses and abuses. CNS Neurosci. Ther. 20, 1015–1020.
Yargeau, V., Taylor, B., Li, H., Rodayan, A., Metcalfe, C.D., 2014. Analysis of drugs of abuse in wastewater from two Canadian cities. Sci. Total Environ. 487, 722–730.
Zhang, H., Yang, J., Du, P., Wang, C., Li, X., 2016. Concentrations of ketamine and norketamine in the water environment in Beijing. Environ. Sci. 37, 2522–2529 (In Chinese).

Environment International 136 (2020) 105492

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Using wastewater-based epidemiology to estimate consumption of alcohol and nicotine in major cities of China in 2014 and 2016

Jianfa Gao[a,b,1], Qiuda Zheng[b,1], Foon Yin Lai[b,c], Coral Gartner[b,d], Peng Du[e,f,*], Yuan Ren[g,h,*], Xiqing Li[f], Degao Wang[j], Jochen F. Mueller[b], Phong K. Thai[b]

[a] College of Chemistry and Environmental Engineering, Shenzhen University, Shenzhen 518060, China
[b] Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, 20 Cornwall Street, Woolloongabba, QLD 4102, Australia
[c] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), P.O. Box 7050, SE-75007 Uppsala, Sweden
[d] School of Public Health, The University of Queensland, Herston, QLD 4006, Australia
[e] Beijing Key Laboratory of Urban Hydrological Cycle and Sponge City Technology, College of Water Sciences, Beijing Normal University, Beijing 100875, China
[f] Laboratory of Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China
[g] The Key Laboratory of Environmental Protection and Eco-Remediation of Guangdong Regular Higher Education Institutions, Guangzhou 510006, China
[h] The Key Laboratory of Pollution Control and Ecosystem Restoration in Industry Clusters, Ministry of Education, Guangzhou 510006, China
[j] College of Environmental Science and Engineering, Dalian Maritime University, 1 Linghai Road, Dalian, Liaoning 116023, China

ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Biomarker
Cotinine
Ethyl-sulphate
LC–MS/MS
Wastewater-based epidemiology

ABSTRACT

Monitoring the use of alcohol and tobacco in the population is important for public health planning and evaluating the efficacy of intervention strategies. The aim of this study was to use wastewater-based epidemiology (WBE) to estimate alcohol and tobacco consumption in a number of major cities across China and compare WBE estimates with other data sources. Daily composite influent wastewater samples were collected from wastewater treatment plants (WWTPs) across China in 2014 (n = 53) and 2016 (n = 45). The population-normalized daily consumption estimated by WBE were compared with other data sources where available. The average consumption of alcohol was 8.1 ± 7.0 mL ethanol/person aged 15+/day (EPD) in the investigated cities of 2016 while those involved in 2014 had an average consumption of 4.7 ± 3.0 EPD. The average tobacco consumption was estimated to be 3.7 ± 2.2 cigarettes/person aged 15+/day (CPD) in 2016 and 3.1 ± 1.9 CPD in 2014. The changes in the average consumption in those cities from 2014 to 2016 were supported by the results from a limited number of WWTPs where samples were collected in both years. Consumption of alcohol and tobacco in urban China is at a medium level compared with other countries on a per capita basis. WBE estimates of tobacco consumption were relatively comparable with results of traditional surveys and sales statistics. WBE estimates of alcohol consumption were lower than WHO survey results, probably due to EtS degradation and uncertainty in the EtS excretion factor.

1. Introduction

Alcohol and tobacco are the most widely used recreational substances worldwide (World Health Organisation, 2015; World Health Organization, 2017). Consumption of these two substances contributes considerably to the global burden of diseases and injuries (Gakidou, 2017). China is the world's largest consumer of alcohol and tobacco due to its large population with levels of per capita consumption slightly above the world average. China incurs a substantial health burden as a consequence: nearly one third of the world's smokers live in China, and approximately one million deaths in China every year are associated with tobacco smoking (Zhang et al., 2011). Recently, smoking prevalence was reported to be gradually decreasing in China (World Health Organization, 2018b), whereas alcohol consumption and alcohol related diseases were increasing (Griswold et al., 2018; Jiang et al., 2015). Therefore, continuing to monitor the consumption of alcohol and tobacco in China is important for tailoring public health policies in order to mitigate the burdens of related diseases.

* Corresponding authors at: Beijing Key Laboratory of Urban Hydrological Cycle and Sponge City Technology, College of Water Sciences, Beijing Normal University, Beijing 100875, China (P. Du). The Key Laboratory of Environmental Protection and Eco-Remediation of Guangdong Regular Higher Education Institutions, Guangzhou 510006, China (Y. Ren).
E-mail addresses: dup@bnu.edu.cn (P. Du), ceyren@scut.edu.cn (Y. Ren).
[1] These authors contributed equally to this work

https://doi.org/10.1016/j.envint.2020.105492
Received 1 November 2019; Received in revised form 11 January 2020; Accepted 13 January 2020

Traditional methods for estimating alcohol and tobacco consumption in the population include sales statistics (Black et al., 2011) and population surveys (Bush et al., 1998; World Health Organization, 2000), which provide additional demographic and consumer behaviour information. However, survey methods may not accurately reflect actual consumption levels due to the potential sampling bias of incorrect answers (intentional or unintentional) and non-representativeness of the samples. Wastewater-based epidemiology (WBE) is a complementary tool for monitoring consumption of substances including alcohol and tobacco. The method is based on the systematic collection of wastewater samples at the inlet of wastewater treatment plants (WWTPs) and subsequent determination of the concentration of parent and/or metabolites of substances that can then be used to back-calculate consumption rates of target compounds. WBE has been used to monitor alcohol and tobacco consumption in a number of countries, with both temporal and geographical consumption profiles be inginvestigated (Andres-Costa et al., 2016; Castiglioni et al., 2015; Chen et al., 2019; Mackie et al., 2019; Mastroianni et al., 2014; Tscharke et al., 2016; van Wel et al., 2016). In China, alcohol consumption has not been evaluated using WBE, while tobacco consumption estimation has been conducted only in Northeastern China with good agreement with survey results (Zheng et al., 2017).

This study thus aims to estimate the consumption of alcohol and tobacco in a number of large cities across China in 2014 and 2016 using WBE. Small towns and rural areas were not included due to the lack of WWTPs and sampling accessibility. The WBE estimates were then compared with sales statistics and survey reports to obtain a better understanding of the consumption of the two substances in China.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards of ethyl-sulphate (EtS), nicotine (NIC), cotinine (COT), trans-3′-hydroxycotinine (OH-COT), EtS-d5, NIC-d4, COT-d3, OH-COT-d3 were purchased from Sigma Aldrich (Castle Hill, Australia). Liquid chromatography–mass spectrometry grade methanol and acetonitrile were purchased from Merck (Germany). Formic acid was purchased from Fisher Scientific (US). Regenerated cellulose syringe filter (0.45 μm) was purchased form Agilent (Mulgrave, Australia). Water was produced by a MilliQ system (Millipore, 0.22 μm filter, 18.2 MΩ cm^{-1}).

2.2. Wastewater sample collection

Influent wastewater samples were collected from WWTPs across China in 2014 and 2016. Daily composite samples were collected using auto-samplers. Details of the sampling campaigns can be found in Gao et al. (2016) and Du et al. (2019). Specifically for this study, in 2014, 53 samples were collected from 29 WWTPs in 17 cities, and in 2016, 45 samples were collected from 19 WWTPs in 16 cities (Tables S1 and S2). All the WWTPs served the urban area of the city and most cities involved (15 of total 17 in 2014, 13 of 16 in 2016) are the provincial capitals or are under direct administration by the central government. The remaining cities have similar population and economic development status as their provincial capitals. It should be noted that due to logistic reasons, the set of cities participating in the 2014 campaign is different from those participating in 2016, with 6 catchments being sampled in both campaigns. The cities included in this study located across all seven geographical regions of China, which allows for the evaluation of spatial variation of consumptions. We used the Qinling-Huaihe Line as the boundary of Northern and Southern China for comparison purpose (Almond et al., 2009).

2.3. Sample preparation and instrumental analysis

Wastewater samples were acidified to pH 2 on site using 2 M HCl and kept frozen before pre-treatment. Filtration of samples were conducted using regenerated cellulose syringe filters (Agilent, 0.45 μm) and a 1 mL filtered sample was pipetted into a 2 mL amber vial. The alcohol consumption biomarker, EtS, was analysed using a previously reported method (Lai et al., 2018). Briefly, 10 μL of 1 mg/L mass-labelled EtS was added to each 1 mL filtered and acidified wastewater sample. These samples were analysed by liquid chromatography (Shimadzu Nexera HPLC) coupled to tandem mass spectrometer (Sciex, 5500-QTrap®) (LC-MS/MS) in direct injection mode. The mass spectrometer parameters are shown in Table S3. The tobacco consumption biomarkers, COT and OH-COT as well as NIC, were determined by the same LC-MS/MS system in direct injection mode using a method validated by Gao et al. (2018). Mass spectrometer parameters for COT, OH-COT and NIC are shown in Table S4. Concentration of analytes were determined by internal calibration method, e.g. comparing transition intensity ratios between the sample and the calibration curve produced with several calibration standards in the same batch. The performance of the analytical method is provided in Table S5.

2.4. Data analysis

The per capita consumption of alcohol (Vol_{Alcohol}) was calculated by Equation (1):

$$Vol_{\mathrm{Alcohol}}\,(\text{ethanol (ml)/person aged 15+/day; EPD}) = \frac{C_{\mathrm{EtS}} \times F \times f_{\mathrm{Alcohol}}}{R_{15+} \times P \times \rho_{\mathrm{Alcohol}}} \quad (1)$$

where C_{EtS} is the concentration of EtS in wastewater (μg/L), F is the daily influent flow (L/d), f_{Alcohol} is the correction factor of EtS after alcohol consumption, ρ_{Alcohol} is the density of alcohol (g/mL). P is the WWTP catchment population (million inhabitants). R_{15+} is the ratio of population aged ≥15 years (15+) in the catchment (National Bureau of Statistics, 2015). Detail information is provided in Table S6.

Since COT demonstrated higher in-sewer stability (Banks et al., 2018; Gao et al., 2019), the per capita consumption of nicotine (m_{NIC}) is calculated by Equation (2).

$$m_{\mathrm{NIC}}\,(\text{mg/person/day}) = \frac{C_{\mathrm{COT}} \times F \times f_{\mathrm{NIC}} \times 1000}{P} \quad (2)$$

where C_{COT} is the concentration in wastewater (μg/L) and f_{NIC} is the correction factor of COT after tobacco smoking. We also back calculated NIC consumption using OH-COT and both COT and OH-COT, respectively (Table S7). In China, tobacco smoking is the predominant source of nicotine consumption since the contribution from other nicotine containing products (such as electronic cigarettes and nicotine replacement therapy products) are negligible due to their high cost (Gravely et al., 2014; Lam et al., 2005). The daily tobacco consumption (n_D) is calculated from nicotine consumption by using Equation (3).

$$n_{\mathrm{D}}\,(\text{cigarettes/person aged 15+/day; CPD}) = \frac{m_{\mathrm{NIC}}}{D \times Y \times R_{15+}} \quad (3)$$

where D is the content of NIC in an average cigarette (mg/cigarette), Y the average yield of NIC uptake during smoking (%) (Charles et al., 2009; Wang et al., 2016).

For accessing uncertainty and sensitivity of the estimation, Monte Carlo simulation (Oracle Crystal Ball software, Version 7.3.1) was used (Wang et al., 2016). The Monte Carlo simulation was based on repeated random sampling of the probability distributions defined for the principal factors of variation of each input parameter (Table S8). Graphpad Prism 7 and Origin 2018 were used for statistical analysis and graphing.

Table 1
Biomarker concentration and estimated alcohol and tobacco consumption in China.

WWTPs-City	COT (μg/L)	EtS (μg/L)	COT/OH-COT	NIC consumption (mg/person/day)	Tobacco consumption (CPD)	Alcohol consumption (EPD)
29 WWTPs across China in 2014	1.6 ± 0.7	3.1 ± 1.4	0.8 ± 0.1	1.6 ± 0.9	3.1 ± 1.9	4.7 ± 3.0
19 WWTPs across China in 2016	2.3 ± 1.3	5.7 ± 4.0	0.5 ± 0.1	1.9 ± 1.1	3.7 ± 2.2	8.1 ± 7.0

COT/OH-COT is the concentration ratio of COT and OH-COT in the same sample; CPD = cigarettes/person aged 15+/day; EPD = ethanol (mL)/person aged 15+/day; data presented in average ± standard deviation.

3. Results

3.1. Occurrences of consumption biomarkers in wastewater

Biomarkers of alcohol and tobacco consumption were detected in all samples. Concentration of alcohol metabolite EtS ranged from 0.5 μg/L (WH-1) to 12.1 μg/L (CD-1) with a median of 3.5 μg/L. Concentration of COT ranged from 0.4 (WH-1) to 4.7 μg/L (LZ-2). The concentration of OH-COT was higher than COT in most samples, ranging from 0.5 (WH-1) to 10.8 μg/L (LZ-2) (Table S7).(see Table 1)

3.2. Spatial variation

Overall, the consumption of both alcohol and tobacco in Northern China was higher than in Southern China (Fig. 1). In 2014, the lowest alcohol consumption was observed in WH-1 with 1.3 EPD and the highest level was observed in JN-1 with 14.4 EPD. The alcohol consumption in cities located in Northern China (6.1 ± 3.3 EPD) was significantly higher than that in Southern China (3.0 ± 1.3 EPD) in 2014 (t test, p = 0.001). However, this geographical difference was much smaller in 2016, as 8.1 ± 3.4 EPD in Northern China and 7.0 ± 6.9 EPD in Southern China (t test, p = 0.23).

In 2014, Northern China had significantly higher tobacco consumption than Southern China (t test, p = 0.002) with the CPD values of 3.9 ± 2.1 and 2.0 ± 0.8, respectively. This geographical difference is similar in 2016 with significantly higher (t test, p = 0.009) tobacco consumption in Northern China (4.5 ± 1.7 CPD) than in Southern China (3.2 ± 2.3 CPD). The lowest tobacco consumption was found at GY-1 (Guiyang) in the South with 1.0 CPD, while YC-1 (Yinchuan) in the North had the highest tobacco consumption of 8.4 CPD in 2014.

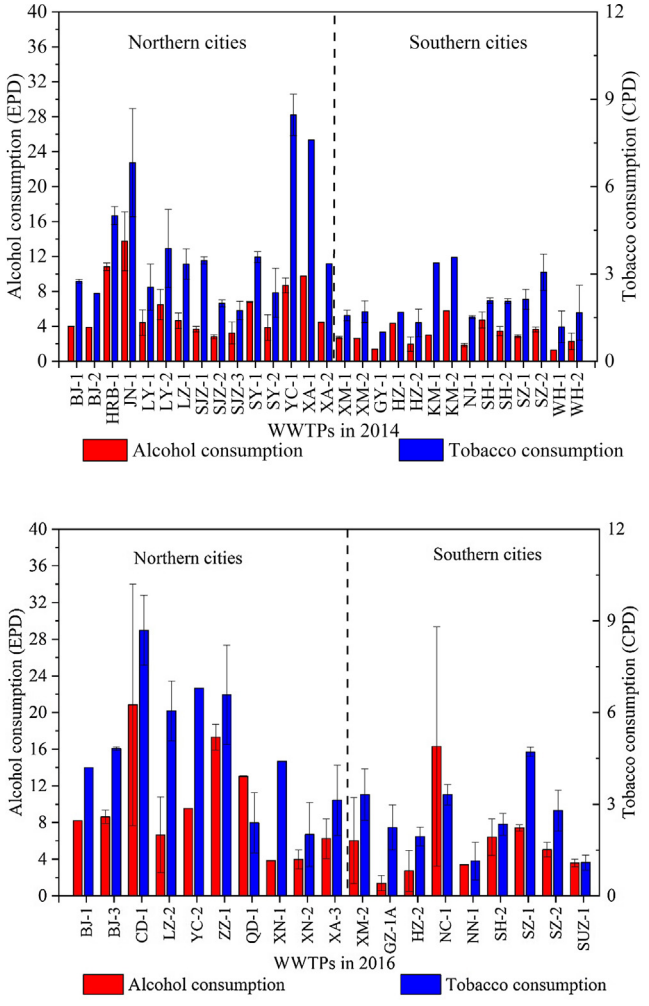


Fig. 1. Consumption of alcohol and tobacco in different catchments in China in 2014 and 2016. CPD = cigarettes/person aged 15+/day; EPD = ethanol (mL)/person aged 15+/day.

3.3. Variations between 2014 and 2016

The average alcohol consumption in the urban catchments included in this study was significantly higher in 2016 (8.1 ± 7.0 EPD) than in 2014 (4.7 ± 3.0 EPD) (t test, p = 0.002). The level of increase observed by WBE is much higher than has been reported by traditional statistics (Statista, 2018; World Health Organisation, 2014). Unlike the observed increase of alcohol consumption, tobacco consumption across urban areas in China in 2016 (3.7 ± 2.2 CPD) was slightly higher than 2014 (3.1 ± 1.9 CPD) (t test, p > 0.05).

In the six WWTPs where samples were obtained in both 2014 and 2016, we observed some level of increases of alcohol and tobacco consumption. However, the tobacco consumption between 2014 and 2016 is statistically non-significant (p > 0.05) (Fig. 2).

4. Discussion

4.1. Consumption comparison between Northern and Southern China

Our results indicated that consumption of both alcohol and tobacco in Northern China was higher than in Southern China. The geographical pattern of alcohol consumption in this study is in good agreement with traditional survey results, which also reported higher drinking rates in Northern China (Cochrane et al., 2003). Normally, colder weather and fewer sunlight hours will increase alcohol consumption (Ventura-Cots et al., 2019). Thus, the difference in climate between Northern and Southern China is one of the reasons for the geographical pattern of alcohol consumption. In addition, higher alcohol consumption is also reported in rural areas compared to urban areas in Northern China (Zhou et al., 2011).

A recent WHO report on tobacco consumption in 14 Chinese cities in 2013–2014 showed that tobacco consumption in Northern China (3.25 CPD) was higher than in Southern China (2.39 CPD) (World Health Organization, 2015). These WHO estimates were similar to our WBE-based results in 2014. The higher tobacco consumption in the North of China could be attributed to cultural and habitual factors as well as economic factor. A previous study has suggested that smoking rates generally declines with better economic status (Chaloupka and Warner, 2000). As a guide to economic prosperity between Northern and Southern China, Northern China contributed 42% of total Chinese GDP while 58% was contributed by Southern China in 2014.

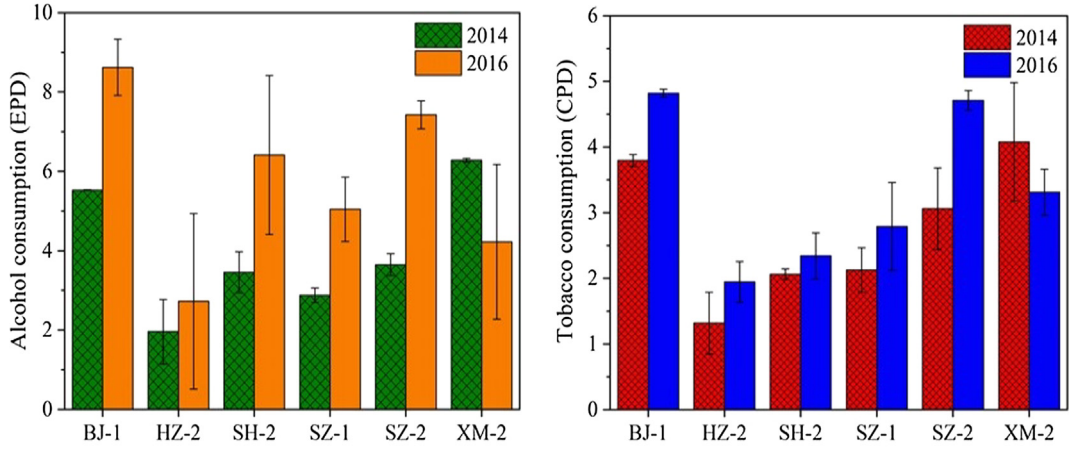


Fig. 2. Variation of alcohol and tobacco consumption in catchments with samples collected in both 2014 and 2016 (In 2014, $n = 2$ for all WWTPs except SZ-2 which $n = 3$. In 2016, $n = 2$ for SH-2, SZ-2, XM-2 and BJ-1, $n = 3$ for HZ-2 and SZ-2) CPD = cigarettes/person aged 15 + /day; EPD = ethanol (mL)/person aged 15 + /day.

4.2. Comparison between WBE estimation and other data sources

The average alcohol consumption in large cities across China measured in this study (4.7 and 8.1 EPD in 2014 and 2016, respectively) was much lower compared with the WHO survey report for 2015–2017, which reported an estimate of approximately 19.7 EPD (World Health Organization, 2018a). Lower alcohol consumption estimated by WBE studies compared to traditional surveys was also reported in other WBE studies. Twelve out of 14 WBE studies reported lower alcohol consumption levels while 2 studies showed similar consumption levels (Table 2). A consistent underestimation of alcohol consumption by WBE compared to sales data was also observed in a 6-year monitoring study (Zheng et al., 2020). The relatively lower WBE estimates could be attributed to the following factors: (i) the WHO report covered both urban and rural areas while most WBE studies only cover more urbanized areas, and rural areas were reported to have higher alcohol consumption than urban areas (Fang et al., 2018; Millwood et al., 2013); (ii). The degradation of EtS during sewer transport and uncertainty around the EtS excretion factor may have caused an underestimate of alcohol consumption ; and (iii) Uncertainty related to standardisation of alcohol products could also lead to the discrepancy in consumption rates between WBE studies and self-reported questionnaire surveys (Zheng et al., 2019).

The average tobacco consumption in China estimated by WBE in this study (3.1 and 3.7 CPD in 2014 and 2016) was similar to the Chinese adult tobacco survey in 2015 (3.6 CPD) (CCDC, 2015). However, our estimation is lower than the value of 4.7 CPD reported by the 2015 Global Adult Tobacco Survey (GATS, 2015) and the value of 5.6 CPD estimated from sales statistics extracted from the 2016 national tobacco report (CNTC, 2017).

Tobacco consumption in some of the cities involved in our study were also evaluated by a WHO survey conducted in 2014, which provides the unique opportunity to compare WBE results with survey data (World Health Organization, 2015). Five out of seven cities showed good agreement with < 20% difference (Fig. 3). In HRB, a much higher tobacco consumption was reported by WHO survey whereas in HZ, the WBE estimate was higher than the WHO value. For HRB, the lower WBE estimates may be due to the investigated WWTP catchment only covering 0.55 million of the urban population, while there were 4.9 million people in both suburban and rural areas (of the 9.5 million total population in HRB) covered by the WHO report. For HZ, the WBE sampling covered more than half of the population, so the lower WHO estimates may be attributed to the sampling bias in the WHO survey, a factor which warrants further evaluation.

4.3. Comparison of WBE results with other countries

Alcohol consumption in major cities across China estimated by WBE in this study was lower than those in other countries including Australia (7–24 EPD), Spain (18 EPD), Belgium (15.3 EPD), the United States (6–93 EPD), as well as the values from 20 cities in 11 countries (6.4–44.3 EPD, median 20.9 EPD) (Boogaerts et al., 2016; Chen et al., 2019; Lai et al., 2018; Mastroianni et al., 2014; Ryu et al., 2016) (Table 2). The lower alcohol consumption observed in China is also supported by the WHO survey report (World Health Organisation, 2014). The lower alcohol consumption in Chinese cities compared to those in other countries such as Australia and Belgium could be partially attributed to the lower percentage of regular consumers of alcohol (17.3% in China, 52.4% in Australia, and 41.7% in Belgium).

As the nicotine content in cigarettes vary, using the per capita nicotine consumption for comparison is more useful than using the number of cigarettes smoked, especially for comparison between different cultures and demographics (Wayne et al., 2006). Since we noted that different excretion factors for nicotine biomarkers were used in different WBE studies, a standard conversion of nicotine consumption was used for comparison (Table 3). The nicotine consumption in China (1.5 ± 0.9 mg/person/day in 2014 and 1.8 ± 1.1 mg/person/day in 2016) was similar to Australia (1.7 ± 0.6 mg/person/day) and Spain (1.7 mg/person/day), but much lower than Italy (3.4 mg/person/day), the United States (2.7 mg/person/day) and Portugal (2.6 mg/person/day) (Castiglioni et al., 2015; Chen et al., 2019; Lai et al., 2018; Lopes et al., 2014; Rodriguez-Alvarez et al., 2014b).

4.4. Uncertainties and limitations

In this study, we have used the two stability benchmarking chemicals, acesulfame – a stable biomarker and paracetamol – an unstable one, to evaluate the potential degradation of the consumption biomarkers of alcohol and tobacco during transport and preservation in this study (Gao et al., 2017; Gao et al., 2019; O'Brien et al., 2017). Our data showed that degradation did not have a significant effect on our samples (Table S9).

The general uncertainties for WBE applications and specifically for alcohol and tobacco biomarkers apply for this study, such as the instrumental measurement and real-time population estimation (Castiglioni et al., 2013; Zheng et al., 2019). For alcohol consumption, excretion factor of EtS, ranging from 0.010 to 0.016%, is probably the largest contributor to the overall uncertainty. For tobacco consumption, the uncertainty of D, large variance of nicotine amongst each cigarette (0.4–1.2 mg) in different brands was observed and attributed to smokers' habits (Zheng et al., 2017). Meanwhile excretion of NIC to COT

Table 2
Comparison of alcohol consumption in different countries.

City, Country	Year	WWTPs(n)	Population	Alcohol consumption range (average) (EPD)	Excretion factor used (%)	Reference	Alcohol consumption in 2015–2017 reported by WHO (national average, EPD)
Barcelona, Spain	2013–2015	1	998,846–992,301 (R15 +)	7–31 (17.6)	0.01	(Mastroianni et al., 2017)	27.4
Lesvos, Greece	2015	3	1,250–26,000 (All)	1.7–11.2 (5.4)	0.012	(Gatidou et al., 2016)	28.5
Galicia, Spain	2012	1	100,000 (All)	9.3–23.5 (16.3)	0.010–0.016	(Rodriguez-Alvarez et al., 2014a)	28.4*
Oslo, Norway	2009	1	500,000 (All)	12.4–19.8	0.010–0.016	(Reid et al., 2011)	24.6*
Santiago de Compostela, Spain	2012–2014	1	136,500 (All)	3.8–22.6 (13.6)	0.012	(Rodriguez-Alvarez et al., 2015)	27.4
Milan, Italy	2012–2014	1	1,150,000 (All)	3.2–10.5 (5.1)	0.012	(Ryu et al., 2016)	20.8
20 Cities in 11 countries	2014–2015	23	34,495–1,500,000 (All)	6.4–44.3	0.012	(Mastroianni et al., 2014)	27.4
Barcelona, Spain	–	1	1,157,000 (R15 +)	12–27 (18)	0.011	(Andres-Costa et al., 2016)	27.4
Valencia, Spain	2014	3	Sum of 1,500,000 (R15 +)	1.07–56.1	0.010–0.016	(Baz-Lomba et al., 2016)	
8 European cities	2015	8	Sum of 5,000,000 (All)	2.1–9.6 (5.1)	–	(Lai et al., 2018)	29.0
Australia	2014–2015	18	Sum of 10,800,000 (All)	(7–24)	0.012	(Nguyen et al., 2018)	No data
Ho Chi Minh City, Vietnam	2015	2	Sum of 54,5830 (All)	(2.7–3.9)	0.012	(Boogaerts et al., 2016)	32.2
Belgium	2013–2015	8	78,441–953,987 (R15 +)	5.29–33.3 (15.3)	0.012	(Chen et al., 2019)	26.8
3 cities in the U.S.	2015–2016	3	53,000–125,000 (R15 +)	5.7–92.9 (29.4)	0.012	(Zheng et al., 2020)	29.0
1 city in Australia	2012–2017	1	87,124–109,294 (All)	15.7–25.3 (17.6)	0.012	This study	19.7
17 cities in China	2014	29	200,000–3,450,000 (R15 +)	1.34–14.4 (4.8)	0.012	This study	19.7
21 cities in China	2016	19	107,600–3,338,500 (R15 +)	1.38–20.8 (7.5)	0.012		

* Data reported during 2009–2011.

Fig. 3. Comparison of tobacco consumption between WBE estimates and WHO results in 2014; (n = 5 for SZ, 4 for LY and SY, 3 for BJ and HZ, 2 for HRB and LZ for WBE).

could be different due to influences of enzymes, age, gender, diet and medications, which can contribute to uncertainty in the excretion factor (Hukkanen et al., 2005).

Using Monte Carlo simulation, the average alcohol consumption across China in 2014 and 2016 was estimated to be 4.3 EPD (95% CI: 3.4–5.3 EPD) and 8.2 EPD (95% CI: 6.0–11.9), respectively. The average tobacco consumption estimates were 3.4 CPD (95%CI: 2.0–5.8 CPD) in 2014 and 4.8 CPD (95%CI: 3.0–8.0 CPD) in 2016 (**Fig. S1**). The Monte Carlo simulation results were in good agreement with the estimation based on the mean value of input parameters used above.

Although our study provided geographical profiles of alcohol and tobacco consumption across China, we acknowledge some limitations. First, the population size of the catchments included in this study were from the best estimates provided by the WWTP managers and the Census data. Such population counts have unknown uncertainty due to catchment development and population migration. Second, the catchments investigated in 2016 were not exactly the same as those in 2014, so the national average comparison between 2014 and 2016 may be affected by geographical differences. Third, due to the lack of detailed understanding of the catchment sewer profile (HRT distribution, A/V distribution), we could not compensate the in-sewer degradation of biomarkers in our back-calculation. Fourth, due to logistical reasons, a limited number of samples (1–3) were provided by the WWTPs each year. This adds to the uncertainty in the yearly consumption estimation. Future campaigns should carefully evaluate the number of samples required for annual estimation of alcohol and tobacco consumption, similar to evaluations made in relation to other substances (e.g. Ort et al., 2014; Humphries et al., 2016). Furthermore, like many other WBE studies, the consumption estimates using WBE do not include the demographic characteristics of the population such as age distribution, consumption frequency and prevalence of heavy users. Therefore, strict interpretation of our results should consider the uncertainties and limitations outlined above.

5. Conclusion

According to the results of this study, the average alcohol consumption in urban China in 2016 was higher than that in 2014. Tobacco consumption showed no statistical difference between the two years. Northern China had higher level of alcohol and tobacco consumption compared to Southern China. Overall, the WBE estimates for alcohol consumption in China were lower than those reported in the WHO surveys, but the geographical difference between Northern and Southern China was well reflected in both approaches. Temporal and geographical profiles of tobacco consumption derived from WBE showed relatively good agreement with survey reports and sales

Table 3
Comparison of nicotine and tobacco consumption in different countries.

City, Country	Year	WWTPs (n)	Population	Nicotine consumption (average) (mg/day/person)	Tobacco consumption (average) (CPD)	Excrete factor used (%)	Reference	Standard conversion using excretion factor (COT:32.3%, OHCOT: 43.4%)
Jilin, China	2016	10	61,300–1,270,000 (All)	1.0–6.1	1.82–11.7	COT: 32.3	(Zheng et al., 2017)	1.0–6.1
Dalian, China	2015	11	65,000–350,000 (All)	1.6–2.4 (2.0)	(3)	COT: 32.3 OH-COT: 43.4	(Wang et al., 2016)	1.6–2.4 (2.0)
Lier, Belgium	2014	1	30,600(All)	5.6	(4.5)	–	(van Wel et al., 2016)	
8 Italian cities	2012	–	79,926–1,201,490 (R15 +)	2.2–4.5 (3.5)	1.8–3.4	COT: 30 OH-COT: 44	(Castiglioni et al., 2015)	2.2–4.4 (3.4)
Lisbon, Portugal	2011	3	–	4.9–12.9 (5.9)	–	COT:14.1	(Lopes et al., 2014)	2.1–5.6 (2.6)
Australia	2014–2015	18	Sum of 10,800,000 (All)	0.75–3.02 (1.8)	0.85–3.4	COT: 28.05 OH-COT: 45.23	(Lai et al., 2018)	0.7–2.9 (1.7)
Galicia, Spain	2012–2014	1	130,000 (All)	0.9–2.6 (1.8)	–	COT: 27 OH-COT: 44.5	(Rodriguez-Alvarez et al., 2014a)	0.9–2.5 (1.7)
Ho Chi Minh City, Vietnam	2015	2	Sum of 545,830 (All)	1.1–1.3	–	COT and OHCOT:13	(Nguyen et al., 2018)	0.4–0.5
Czech Republic	2014	2	120,000–1,300,000 (All)	3.6–4.3	4.5–5.4	COT:14.1	(Mackuľak et al., 2015)	1.6–1.9
Slovak Republic	2014	5	35,000–450,000 (All)	2.2–8.0	2.8–10.1	COT:14.1		1.0–3.5
3 cities in the U.S.	2015–2016	3	53,000–125,000 (All)	0.8–4.1 (2.7)	–	COT + OH-COT:74	(Chen et al., 2019)	1.7–8.7
1 city in Australia	2010–2017	1	87,000–10,9000 (All)	1.9–2.3	2.5–3.5	COT:32	(Mackie et al., 2019)	2.5–3.5
17 cities in China	2014	29	200,000–3,450,000 (R15 +)	0.5–3.9	1.0–8.4	COT:32.3	This study	0.5–3.9
21 cities in China	2016	19	107,600–3,338,500 (R15 +)	0.6–4.5	1.1–8.7	COT:32.3	This study	0.6–4.5

statistics but could be obtained in a shorter timeframe. More efforts are required to reduce the uncertainties related to WBE estimation to improve the accuracy of the consumption estimates.

CRediT authorship contribution statement

Jianfa Gao: Data curation, Formal analysis, Methodology, Software, Writing - original draft. **Qiuda Zheng:** Formal analysis, Investigation, Visualization, Writing - original draft. **Foon Yin Lai:** Methodology, Validation, Writing - review & editing. **Coral Gartner:** Investigation, Writing - review & editing. **Peng Du:** Project administration, Resources, Writing - review & editing. **Yuan Ren:** Resources, Writing - review & editing. **Xiqing Li:** Funding acquisition, Writing - review & editing. **Degao Wang:** Writing - review & editing. **Jochen F. Mueller:** Funding acquisition, Writing - review & editing. **Phong K. Thai:** Conceptualization, Supervision, Validation, Writing - review & editing.

Acknowledgement

The Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland gratefully acknowledges the financial support of the Queensland Department of Health. Jianfa Gao receives ARC and UQI scholarship. Jochen Mueller is funded through the UQ Fellowship. Qiuda Zheng receives a PhD scholarship from an Australian Research Council Linkage project (LP150100364). Foon Yin Lai was funded through Marie Skłodowska-Curie Individual Fellowship (749845 (APOLLO), European Commission, Horizon 2020). The QUT Vice Chancellor Research Fellowship funded Phong K. Thai during part of this study. Peng Du acknowledged the supported by the National Natural Science Foundation of China (Grant No. 41701543) and the Fundamental Research Funds for the Central Universities (Grant No. 2018NTST20). Yuan Ren is supported by the National Natural Science Foundation of China (Grant No. 41877466), Research Fund of SIT of Guangzhou (Grant No. 201707010158). Thanks also to Chris Paxman and Zhe Wang for their assistance.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105492.

References

Almond, D., Chen, Y., Greenstone, M., Li, H., 2009. Winter heating or clean air? Unintended impacts of China's Huai river policy. J. Am. Econ. Rev. 99, 184–190.
Andres-Costa, M.J., Escriva, U., Andreu, V., Pico, Y., 2016. Estimation of alcohol consumption during “Fallas” festivity in the wastewater of Valencia city (Spain) using ethyl sulfate as a biomarker. Sci. Total Environ. 541, 616–622.
Banks, A.P.W., Lai, F.Y., Mueller, J.F., Jiang, G., Carter, S., Thai, P.K., 2018. Potential impact of the sewer system on the applicability of alcohol and tobacco biomarkers in wastewater-based epidemiology. Drug Test. Anal. 10, 530–538.
Baz-Lomba, J.A., Salvatore, S., Gracia-Lor, E., Bade, R., Castiglioni, S., Castrignano, E., et al., 2016. Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. BMC Public Health 16, 1035.
Black, H., Gill, J., Chick, J., 2011. The price of a drink: levels of consumption and price paid per unit of alcohol by Edinburgh's ill drinkers with a comparison to wider alcohol sales in Scotland. Addiction 106, 729–736.
Boogaerts, T., Covaci, A., Kinyua, J., Neels, H., van Nuijs, A.L., 2016. Spatial and temporal trends in alcohol consumption in Belgian cities: a wastewater-based approach. Drug Alcohol Dependence 160, 170–176.
Bush, K., Kivlahan, D.R., McDonell, M.B., Fihn, S.D., Bradley, K.A., for the Ambulatory Care Quality Improvement, P., 1998. The audit alcohol consumption questions (audit-c): an effective brief screening test for problem drinking. Arch. Intern. Med. 158, 1789–1795.
Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernandez, F., Reid, M., et al., 2013. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environ. Sci. Technol. 47, 1452–1460.
Castiglioni, S., Senta, I., Borsotti, A., Davoli, E., Zuccato, E., 2015. A novel approach for monitoring tobacco use in local communities by wastewater analysis. Tob. Control

24, 38–42.
CCDC, 2015. Chinese adult tobacco survey.
Chaloupka, F.J., Warner, K.E., 2000. The Economics of Smoking. Handbook of Health Economics. Elsevier (Chapter 29).
Charles, F.K.S., Kabbani, A.A., Borgerding, M.F., 2009. Estimating tar and nicotine exposure: human smoking versus machine generated smoke yields. Regul. Toxicol. Pharm. 56, 100–110.
Chen, J., Venkatesan, A.K., Halden, R.U., 2019. Alcohol and nicotine consumption trends in three US communities determined by wastewater-based epidemiology. Sci. Total Environ. 656, 174–183.
CNTC, 2017. C.N.T.C. Report of National Cigarette Market in 2016.
Cochrane, J., Chen, H., Conigrave, K.M., Hao, W., 2003. Alcohol use in China. J. Alcohol Alcoholism 38, 537–542.
Du, P., Thai, P.K., Bai, Y., Zhou, Z., Xu, Z., Zhang, X., et al., 2019. Monitoring consumption of methadone and heroin in major Chinese cities by wastewater-based epidemiology. Drug Alcohol Depend. 205, 107532.
Fang, Y., He, Y., Bai, G., Zhao, W., 2018. Prevalence of alcohol drinking in males aged 20–79 years in China, 2012. Chin. J. Epidemiol. 39, 280–285.
Gakidou, E., 2017. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 390, 1345–1422.
Gao, J., Banks, A., Li, J., Jiang, G., Lai, F.Y., Mueller, J.F., et al., 2017. Evaluation of in-sewer transformation of selected illicit drugs and pharmaceutical biomarkers. Sci. Total Environ. 609, 1172–1181.
Gao, J., Li, J., Jiang, G., Shypanski, A., Nieradzik, L.M., Yuan, Z., et al., 2019. Systematic evaluation of biomarker stability in pilot scale sewer pipes. Water Res. 151, 447–455.
Gao, J., Li, J., Jiang, G., Yuan, Z., Eaglesham, G., Covaci, A., et al., 2018. Stability of alcohol and tobacco consumption biomarkers in a real rising main sewer. Water Res. 138, 19–26.
Gao, J., O'Brien, J., Du, P., Li, X., Ort, C., Mueller, J.F., et al., 2016. Measuring selected PPCPs in wastewater to estimate the population in different cities in China. Sci. Total Environ. 568, 164–170.
Gatidou, G., Kinyua, J., van Nuijs, A.L., Gracia-Lor, E., Castiglioni, S., Covaci, A., et al., 2016. Drugs of abuse and alcohol consumption among different groups of population on the Greek Island of Lesvos through sewage-based epidemiology. Sci. Total Environ. 563–564, 633–640.
GATS, 2015. Global Adult Tobacco Survey. The GATS Atlas 2015 CDC Foundation, Atlanta, GA.
Gravely, S., Fong, G.T., Cummings, K.M., Yan, M., Quah, A.C., Borland, R., et al., 2014. Awareness, trial, and current use of electronic cigarettes in 10 countries: findings from the ITC project. Int. J. Environ. Res. Public Health 11, 11691–11704.
Griswold, M.G., Fullman, N., Hawley, C., Arian, N., Zimsen, S.R.M., Tymeson, H.D., et al., 2018. Alcohol use and burden for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 392, 1015–1035.
Hukkanen, J., Jacob, P., Benowitz, N.L., 2005. Metabolism and disposition kinetics of nicotine. Pharmacol. Rev. 57, 79–115.
Humphries, M.A., Bruno, R., Lai, F.Y., Thai, P.K., Holland, B.R., O'Brien, J.W., et al., 2016. Evaluation of monitoring schemes for wastewater-based epidemiology to identify drug use trends using cocaine, methamphetamine, MDMA and methadone. Environ. Sci. Technol. 50, 4760–4768. https://doi.org/10.1021/acs.est.5b06126. In press.
Jiang, H., Room, R., Hao, W., 2015. Alcohol and related health issues in China: action needed. Lancet Global Health 3, e190–e191.
Lai, F.Y., Gartner, C., Hall, W., Carter, S., O'Brien, J., Tscharke, B.J., et al., 2018. Measuring spatial and temporal trends of nicotine and alcohol consumption in Australia using wastewater-based epidemiology. Addiction 113, 1127–1136.
Lam, T.H., Abdullah, A.S.M., Chan, S.S.C., Hedley, A.J., 2005. Adherence to nicotine replacement therapy versus quitting smoking among Chinese smokers: a preliminary investigation. Psychopharmacology 177, 400–408.
Lopes, A., Silva, N., Bronze, M.R., Ferreira, J., Morais, J., 2014. Analysis of cocaine and nicotine metabolites in wastewater by liquid chromatography-tandem mass spectrometry. Cross abuse index patterns on a major community. Sci. Total Environ. 487, 673–680.
Mackie, R.S., Tscharke, B.J., O'Brien, J.W., Choi, P.M., Gartner, C.E., Thomas, K.V., et al., 2019. Trends in nicotine consumption between 2010 and 2017 in an Australian city using the wastewater-based epidemiology approach. Environ. Int. 125, 184–190.
Mackuľak, T., Birošová, L., Grabic, R., Škubák, J., Bodík, I., 2015. National monitoring of nicotine use in Czech and Slovak Republic based on wastewater analysis. Environ. Sci. Pollut. Res. 22, 14000–14006.
Mastroianni, N., Lopez-Garcia, E., Postigo, C., Barcelo, D., Lopez de Alda, M., 2017. Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends. Sci. Total Environ. 609, 916–926.
Mastroianni, N., Lopez de Alda, M., Barcelo, D., 2014. Analysis of ethyl sulfate in raw wastewater for estimation of alcohol consumption and its correlation with drugs of abuse in the city of Barcelona. J. Chromatogr. A 1360, 93–99.
Millwood, I.Y., Li, L., Smith, M., Guo, Y., Yang, L., Bian, Z., et al., 2013. Alcohol consumption in 0.5 million people from 10 diverse regions of China: prevalence, patterns and socio-demographic and health-related correlates. Int. J. Epidemiol. 42, 816–827.
National Bureau of Statistics, 2015. Statistical Bulletin of National Economic and Social Development in 2015.
Nguyen, H.T., Thai, P.K., Kaserzon, S.L., O'Brien, J.W., Eaglesham, G., Mueller, J.F., 2018. Assessment of drugs and personal care products biomarkers in the influent and effluent of two wastewater treatment plants in Ho Chi Minh City, Vietnam. Sci. Total Environ. 631, 469–475.
O'Brien, J.W., Banks, A.P.W., Novic, A.J., Mueller, J.F., Jiang, G., Ort, C., et al., 2017. Impact of in-sewer degradation of pharmaceutical and personal care products (PPCPs) population markers on a population model. Environ. Sci. Technol. 51, 3816–3823.
Ort, C., Eppler, J.M., Scheidegger, A., Rieckermann, J., Kinzig, M., Sorgel, F., 2014. Challenges of surveying wastewater drug loads of small populations and generalizable aspects on optimizing monitoring design. Addiction 109 (3), 472–481. https://doi.org/10.1111/add.12405. In press.
Reid, M.J., Langford, K.H., Morland, J., Thomas, K.V., 2011. Analysis and interpretation of specific ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the quantitative measurement of regional alcohol consumption. Alcohol. Clin. Exp. Res. 35, 1593–1599.
Rodriguez-Alvarez, T., Racamonde, I., Gonzalez-Marino, I., Borsotti, A., Rodil, R., Rodriguez, I., et al., 2015. Alcohol and cocaine co-consumption in two European cities assessed by wastewater analysis. Sci. Total Environ. 536, 91–98.
Rodriguez-Alvarez, T., Rodil, R., Cela, R., Quintana, J.B., 2014a. Ion-pair reversed-phase liquid chromatography-quadrupole-time-of-flight and triple-quadrupole-mass spectrometry determination of ethyl sulfate in wastewater for alcohol consumption tracing. J. Chromatogr. A 1328, 35–42.
Rodriguez-Alvarez, T., Rodil, R., Rico, M., Cela, R., Quintana, J.B., 2014b. Assessment of local tobacco consumption by liquid chromatography-tandem mass spectrometry sewage analysis of nicotine and its metabolites, cotinine and trans-3'-hydroxycotinine, after enzymatic deconjugation. Anal. Chem. 86, 10274–10281.
Ryu, Y., Barcelo, D., Barron, L.P., Bijlsma, L., Castiglioni, S., de Voogt, P., et al., 2016. Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: an international study in 20 cities. Sci. Total Environ. 565, 977–983.
Statista, 2018. China: Consumption of alcoholic beverages 2016-2020.
Tscharke, B.J., White, J.M., Gerber, J.P., 2016. Estimates of tobacco use by wastewater analysis of anabasine and anatabine. Drug Test. Anal. 8, 702–707.
van Wel, J.H., Gracia-Lor, E., van Nuijs, A.L., Kinyua, J., Salvatore, S., Castiglioni, S., et al., 2016. Investigation of agreement between wastewater-based epidemiology and survey data on alcohol and nicotine use in a community. Drug Alcohol Depend. 162, 170–175.
Ventura-Cots, M., Watts, A.E., Cruz-Lemini, M., Shah, N.D., Ndugga, N., McCann, P., et al., 2019. Colder weather and fewer sunlight hours increase alcohol consumption and alcoholic cirrhosis worldwide. Hepatology 69, 1916–1930.
Wang, D.G., Dong, Q.Q., Du, J., Yang, S., Zhang, Y.J., Na, G.S., et al., 2016. Using Monte Carlo simulation to assess variability and uncertainty of tobacco consumption in a city by sewage epidemiology. Bmj Open 6, e010583.
Wayne, F.G., Connolly, G.N., Henningfield, J.E., 2006. Brand differences of free-base nicotine delivery in cigarette smoke: the view of the tobacco industry documents. Tob. Control 15, 189–198.
World Health Organisation, 2014. Global status report on alcohol and health 2014. World Health Organisation.
World Health Organisation, 2015. WHO global report on trends in prevalence of tobacco smoking 2015. World Health Organization.
World Health Organization, 2000. International guide for monitoring alcohol consumption and related harm. World Health Organization, Geneva.
World Health Organization, 2015. Report of China City Adult Tobacco Survey 2013-14 A 14-city experience. World Health Organization.
World Health Organization, 2017. World Health Statistics 2017: Monitoring Health for the SDGs Sustainable Development Goals. World Health Organization.
World Health Organization, 2018a. Global status report on alcohol and health 2018. World Health Organization.
World Health Organization, 2018b. Smoking prevalence, total, ages 15+, China. 2010-2016: World Health Organization.
Zhang, J., Ou, J.X., Bai, C.X., 2011. Tobacco smoking in China: prevalence, disease burden, challenges and future strategies. Respirology 16, 1165–1172.
Zheng, Q., Lin, J., Pei, W., Guo, M., Wang, Z., Wang, D., 2017. Estimating nicotine consumption in eight cities using sewage epidemiology based on ammonia nitrogen equivalent population. Sci. Total Environ. 590–591, 226–232.
Zheng, Q., Tscharke, B., O'Brien, J., Gerber, C., Mackie, R., Gao, J., et al., 2019. Uncertainties in estimating alcohol and tobacco consumption by wastewater-based epidemiology. Curr. Opin. Environ. Sci. Health.
Zheng, Q., Tscharke, B.J., Krapp, C., O'Brien, J.W., Mackie, R.S., Connor, J., et al., 2020. New approach for the measurement of long-term alcohol consumption trends: Application of wastewater-based epidemiology in an Australian regional city. Drug Alcohol Depend. 207, 107795.
Zhou, L., Conner, K.R., Caine, E.D., Xiao, S., Xu, L., Gong, Y., et al., 2011. Epidemiology of alcohol use in rural men in two provinces of China. J. Stud. Alcohol Drugs 72, 333–340.

Science of the Total Environment 643 (2018) 1602–1609

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Estimating population exposure to phthalate esters in major Chinese cities through wastewater-based epidemiology

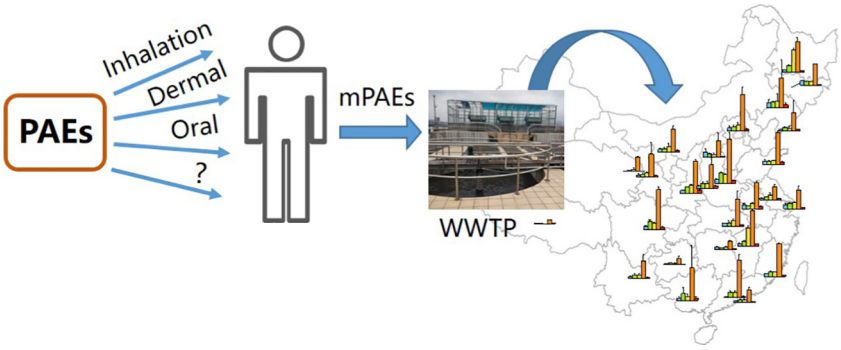
Peng Du, Zilei Zhou, Hongmei Huang, Sheng Han, Zeqiong Xu, Ya Bai, Xiqing Li *

Laboratory of Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, 100871 Beijing, PR China

HIGHLIGHTS

- Phthalate esters metabolites in wastewater across China were measured.
- Nationwide phthalate esters exposure was estimated for the first time.
- Health risk of phthalate esters for children cannot be overlooked.
- Wastewater-based epidemiology is a good tool to assess health risks of pollutants.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 April 2018
Received in revised form 26 June 2018
Accepted 26 June 2018
Available online xxxx

Editor: Adrian Covaci

Keywords:
Phthalate esters
Wastewater
Wastewater-based epidemiology
Health risk
China

ABSTRACT

Phthalate esters (PAEs) are widely used in consumer and industrial products and may thus pose significant health risks. Urine analysis, which has usually been applied to assess the health risks of PAEs, has the drawback of small sample sizes and insufficient representativeness. Wastewater-based epidemiology (WBE) collects wastewater samples containing urine from the entire community and thus is more representative than urine samples. In this work, exposure levels and health risks of PAEs were estimated on a national scale for the first time through the WBE approach. Wastewater samples were collected from 54 wastewater treatment plants in 27 major cities that cover all of the geographic regions of China. The estimated ∑5PAEs exposure levels ranged from 290 μg/inh/d (Lhasa) to 3642 ± 467 μg/inh/d (Zhengzhou) with a mean level of 2184 ± 1173 μg/inh/d. Di-n-butyl phthalate accounted for the highest proportion (65%) in the total exposure level. The ∑5PAEs exposure levels in Southwest China were significantly lower than those in other regions due to the low production and consumption of plastics in the region. The health risks of PAEs were assessed by comparing the estimated daily exposure levels to the acceptable daily exposure levels. For adults, the hazard index that represents cumulative risk of PAEs was above or below 1 depending on a particular reference dose (total daily intake values or the reference dose for anti-androgenicity) that was used for risk calculation. In contrast, the hazard index was above 1 for a significant number of cities for children, regardless of which reference dose was used. The results indicate that health risks of PAE exposure in China cannot be overlooked. Children in China are under considerably greater risks than adults, which warrants further research or proper regulation of PAE use in China.

1. Introduction

Phthalate esters (PAEs) are widely applied in consumer and industrial products. In general, PAEs are additives that improve the plasticity and toughness of such products as textiles, pesticides, detergents,

* Corresponding author.
E-mail address: xli@urban.pku.edu.cn (X. Li).

https://doi.org/10.1016/j.scitotenv.2018.06.325
0048-9697/

medical devices, food packages, vinyl toys, and personal care products (Heudorf et al., 2007). The global production of PAEs increased from 1.8 million tons in 1975 to >8 million tons in 2011 (Net et al., 2015). In China, the consumption of PAEs has also grown rapidly in recent years, reaching 3.3 million tons in 2015 (CPPIA, 2016). PAEs are not chemically bound to the products and can be released into the environment through various routes (Gong et al., 2016). Therefore, PAEs have been ubiquitously detected in soil, sea water, fresh water, sediments, atmosphere (including indoor air), particulates, and even food products (Gao and Wen, 2016; Tsai et al., 2018). Thus, long-term individual exposure to PAEs can occur by dermal, inhalation, and oral pathways (Itoh et al., 2007; Gong et al., 2016).

PAEs are a class of endocrine-disrupting compounds, and it has been demonstrated that long-term exposure to them could cause adverse health risks. Dimethyl phthalate (DMP), diethyl phthalate (DEP) and di-n-butyl phthalate (DnBP) are recognized to play important roles in delaying and speeding the onset of puberty in children (Katsikantami et al., 2016), such as delayed pubic hair and testis development for boys and earlier menarche onset and accelerated breast development for girls (Zhang et al., 2015). In addition, childhood obesity, allergic symptoms, asthma, hypertension, poor attentional performance, and DNA damage in children can also be induced by PAEs exposure (Arbuckle et al., 2016; Huen et al., 2016). Due to the immature metabolism and excretory systems for fetuses and newborns, the adverse effects are more serious for babies (Katsikantami et al., 2016). For adults, the exposure can lead to reduction of semen quality, sex hormone level decline (Bloom et al., 2015), endometriosis, leiomyoma (Upson et al., 2013), breast cancer (Lopez-Carrillo et al., 2010), and type-2 diabetes (Sun et al., 2014). Thus, exact PAEs exposure levels, as well as their geographic distribution, are highly important for the health risk assessment and regulation of their use.

In the human body, PAEs are hydrolyzed to their corresponding monoesters, and certain monoesters can be further oxidized (Koch et al., 2004, 2005). In general, metabolites are excreted in urine as glucuronide conjugates, similar to glucuronated estrogens that deconjugate rapidly in wastewater via the β-glucuronidase enzymes produced by fecal bacteria (D'Ascenzo et al., 2003). At present, the daily PAEs exposure levels are estimated mainly by analyzing concentrations of the respective metabolites in urine (Axelsson et al., 2015). This analysis provides highly specific information about single individuals, which can eventually be used to link exposure to health end-points. However, due to the highly specificity, this approach is unsuitable for identifying geographical and temporal trends of PAEs exposure on a regional or national scale.

Over the past decade, a novel approach called wastewater-based epidemiology (WBE) was developed to estimate community drug consumption. The consumption is estimated mainly based on the concentrations of target residues in influents, the flow rate of wastewater treatment plants (WWTPs), the population served by the WWTPs, and correction factors that account for excretion rates and stability in wastewater. Since Zuccato et al. (2008) estimated drug consumption by WBE, dozens of research groups worldwide have applied and refined this methodology. Recently, national scale reconnaissance of temporal variation of drug consumption has been performed in many countries (Zuccato et al., 2016; Lai et al., 2016; Du et al., 2015, 2017; Mastroianni et al., 2017). Following successful application of WBE for drug consumption estimation, the approach has also been used to estimate the exposure to environmental pollutants, such as pesticides (Rousis et al., 2017a, 2017b) and organophosphorus flame retardants (Been et al., 2017). As a priority pollutant, the PAEs exposure levels can be estimated through WBE as well (Gracia-Lor et al., 2017). Recently, Gonzalez-Marino et al. (2017) performed the first WBE study in the coastal northwest region of Spain (six WWTPs were sampled) and demonstrated that the WBE was effective in estimating PAEs exposure. Compared to urine analysis, the WBE approach cannot provide highly specific information of individual exposure, but it shows geographical and temporal trends of PAEs exposure. The two approaches complement each other for assessing health risk. However, WBE survey of PAEs exposure has not been performed in other countries or on a larger national scale.

The objective of this study was to estimate six PAEs (DMP, DEP, DnBP, di-isobutyl phthalate (DiBP), benzyl butyl phthalate (BzBP), and di(2-ethylhexyl) phthalate (DEHP)) exposure levels in major cities across China through WBE for the first time. Wastewater samples were collected from 54 WWTPs in 27 provincial capital or equivalent cities that cover all the geographic regions of the country. Composition and geographic distribution of nationwide individual exposure to PAEs were examined based on estimated PAEs mass loads in wastewater. Furthermore, health risks of PAEs exposure were also assessed by comparing estimated exposure levels with reference acceptable values.

2. Materials and methods

2.1. Reagents and materials

Standard solutions of monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-i-butyl phthalate (MiBP), mono-n-butyl phthalate (MnBP) and monobenzyl phthalate (MBzP) were purchased from AccuStandard (New Haven, CT, USA). In addition, mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) was obtained from Toronto Research Chemicals (Toronto, ON, Canada). The deuterated analogs MMP-d4 and MEHHP-d4 (utilized as internal standards) were also purchased from Toronto Research Chemicals, and MnBP-d4 was obtained from CDN Isotopes (Pointe-Claire, Quebec, Canada). Methanol (MeOH) was purchased from Fisher Scientific, USA (HPLC grade). HPLC grade acetic acid was obtained from CNW Technologies GmbH (Düsseldorf, Germany). Hydrochloric acid (AR) was purchased from Beijing Chemical Works (Beijing, China). Oasis HLB SPE cartridges (60 mg, 3 mL) were obtained from Waters Corporation (Milford, MA, USA). Ultrapure water was prepared using a Milli-Q ultrapure system (Millipore, MA, USA).

2.2. Sample collection

Wastewater samples were collected from 27 major Chinese cities distributed in all seven geographic regions of China (Fig. 1): Yinchuan (YC), Lanzhou (LZ), Xining (XN) and Xi'an (XA) of Northwest China; Chengdu (CD), Kunming (KM), Guiyang (GY) and Lhasa (LS) of Southwest China; Harbin (HRB), Changchun (CC), Shenyang (SY) and Dalian (DL) of Northeast China; Beijing (BJ) and Taiyuan (TY) of North China; Shanghai (SH), Nanjing (NJ), Hefei (HF), Nanchang (NC), Qingdao (QD), and Xiamen (XM) of East China; Zhengzhou (ZZ), Luoyang (LY) Changsha (CS) and Wuhan (WH) of Central China; Guangzhou (GZ) Shenzhen (SZ) and Nanning (NN) of South China. Twenty-two of the above cities are provincial capitals or municipalities directly under the central government (BJ and SH). The other five cities (DL, LY, QD, XM and SZ) are equivalent to provincial capitals in terms of economic development and population sizes.

In total, 54 WWTPs were selected for sampling in the above 27 cities, and two or more WWTPs were selected in most sampled cities. The WWTPs were named, for example, LZ-4 for the fourth WWTP of LZ and HRB-1 for the first WWTP of HRB. The sum of the population served by these WWTPs is 41 million (approximately 3% of the entire population of the country). The sampling campaign was conducted from July to December of 2016. Each WWTP was sampled for three or four days, except CC-1, DL-1, DL-2, LS-1 (sampled for one day) and BJ-4, BJ-5 (sampled for two days) (Table S1). Auto-samplers, such as FC-9624 (GRASP Science & Technology Co., LTD, Beijing), ISCO 3700, 6712 (Teledyne Technologies Inc., Lincoln, NE, USA), and Sigma-SD900 (HACH Inc., USA) were used to collect time-proportional composite samples. Each auto-sampler was programmed to draw 100 mL influent per hour, and a composite sample was obtained by mixing the samples collected

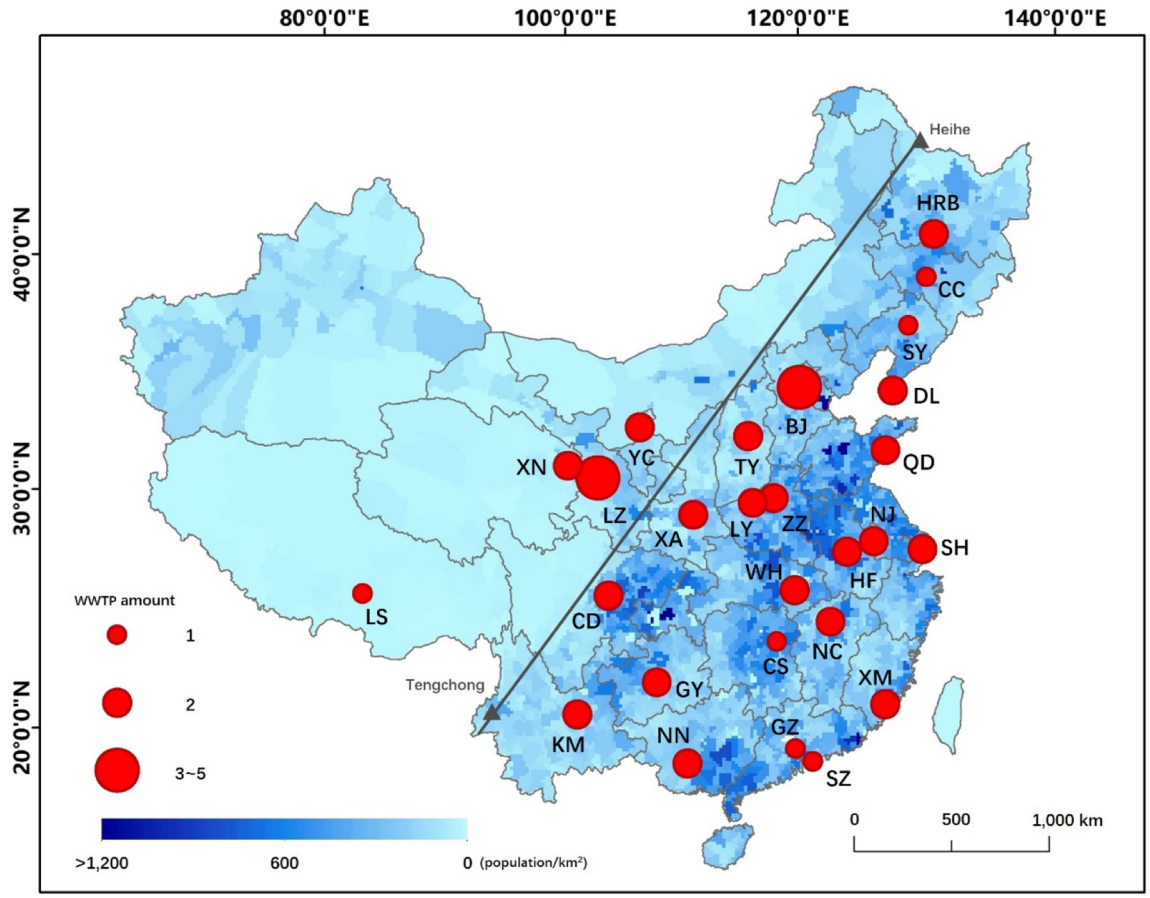


Fig. 1. Locations of cities in mainland China where wastewater samples were collected. The sizes of the circles represent the amount of sampled WWTPs in cities. The gray "Hu Huanyong Line", proposed in 1935 by the famous Chinese geographer, Hu Huanyong, separate the country into two regions of different levels of human activities. The date on the distribution of the Chinese population is from the census information in 2016 (http://data.cnki.net/).

over a 24-h period. Effluent samples were collected following the same procedure at selected WWTPs. Following collection, the composite samples were acidified to pH = 2 by hydrochloric and frozen at −20 °C at each WWTP immediately. All samples were carried back to the laboratory in a frozen state and stored at −20 °C for less than one month until analysis. Details of sample collections and information of each WWTP (flow rates and served inhabitants) are provided in Supporting Information (Table S1).

2.3. Analysis

Sample pretreatment and analysis followed the procedure described in previous publications (Mu et al., 2015; Gonzalez-Marino et al., 2017) with minor modifications. First, the wastewater was passed through a glass filter to remove solid particles, and deuterated internal standards (50 μL, 500 μg/L) was added to 50 mL filtered wastewater samples for quantification. An Oasis HLB cartridge was conditioned in sequence with 3 mL MeOH and 3 mL ultrapure water (pH = 2) at a rate of 1 to 2 mL/min. Next, the spiked wastewater was loaded to the conditioned Oasis HLB cartridge under vacuum at the same rate. The following step was to dry the cartridge with vacuum, and elution was conducted with 5 mL of MeOH. The eluate was evaporated to below 0.5 mL by a N_2 stream and diluted to a constant volume of 0.5 mL with MeOH. A further filtration step was performed by a 0.2 μm centrifugal filter (modified nylon, VWR International, Radnor, PA, USA).

Target compounds were separated using an ultra-fast liquid chromatography (UFLC) system (20 AD-XR, Shimadzu, Japan) with a Phenomenex Gemini C18 column (100 mm × 2 mm, 3 μm) with an injection volume of 5 μL. The mobile phase was composed of 0.1% acetic acid in ultrapure water (A) and MeOH (B). The elution gradient was as follows: 0–0.1 min: 50% B; 0.1–13.0 min: 60% B; 13.0–14.0 min: 90% B; 14.0–16.0 min: 90% B; 16.0–16.3 min: 50% B; 16.3–17.0 50% B, with the flow rate 0.3 mL/min. Concentrations were determined using an API-4000 triple quadrupole mass spectrometer (AB SCIEX, USA) with an electrospray interface operating in negative ionization mode. The quantification of the MS system was operated in multiple reaction monitoring (MRM) mode. Details of MS parameters (quantifier and qualifier ions), retention time and internal standard are described in Table S2. The analytical methods were subjected to strict quality assurance measures. The method detection limit (MDL) (S/*N* = 3) and method quantification limit (MQL) (S/*N* = 10) of the whole method were determined by examining extracts of wastewater with low concentrations of target PAEs. Recoveries were determined by spiking ultrapure water (pH = 2) with target compounds (200 and 500 ng/L) following the same pretreatment procedures (Table S3). The differences in detected concentrations of the spiked and un-spiked samples were divided by the actual spiked concentrations to yield recovery. The differences in responses between wastewater extracts and MeOH spiked with target compounds (200 and 500 ng/L) were used to assess the matrix effects. The recoveries and matrix effects of target compounds ranged from 82.9% ± 8.6% to 111.0% ± 3.5% and from −12.5% ± 3.5% to 13.0% ± 1.3%, respectively (Table S3). Intra- and inter-day repeatability of instrument and method ranged from 1.7% to 3.5% and from 3.2% to 8.9%, respectively (Table S3). Procedure blanks using ultrapure water (pH = 2) spiked with internal standards to check the potential interference and contamination were included in every 11 samples. MMP, MEP, MBzP and MEHHP were below detection limits in all procedure blanks, but a relatively stable levels was found for MiBP and MnBP ranging from

65 to 87 ng/L. Finally, concentrations of MiBP and MnBP were corrected by subtracting the levels found in blanks.

2.4. Daily exposure levels estimation through WBE

The daily exposure level of each PAE per inhabitant was estimated according to the influent concentration of each target metabolite at a specific WWTP by the following equation:

$$Exposure_i(\mu g/inh/d) = \frac{C_i(\mu g/L) \times F_{in}(m^3/d) \times 1000}{PS} \times CF \quad (1)$$

$$CF = \frac{MW_{pi}}{MW_{mi}} \times \frac{1}{EF_i} \quad (2)$$

where C_i is the influent concentration of the target phthalate ester metabolites (mPAEs), PS is the number of inhabitants served by the WWTP (based on the census data of the WWTP service areas), F_{in} is the mean influent flow of the WWTP (obtained from the staff in each respective WWTP), and CF is the corresponding correction factor. The CF for each mPAE is obtained by Eq. (2), where MW_{pi} is the molecular weight of the parent PAE, MW_{mi} is the molecular weight of the mPAE, and EF_i is excretion fraction of a given dose of PAE excreted as metabolites through urine. Excretion fractions for MMP and MEP are not available, thus MnBP excretion was used by an analogy structure (Saravanabhavan et al., 2014; Gonzalez-Marino et al., 2017). A correction factor of 1.8 for DnBP, 1.76 for DiBP, 1.55 for DMP, 1.65 for DEP, 1.68 for BzBP, and 8.4 for DEHP was reported by Gonzalez-Marino et al. (2017) and was used in this study.

2.5. Health risk assessment

Based on the ratio of estimated exposure level ($Exposure_i$) and acceptable exposure level ($Acceptable\ Exposure_i$), a hazard quotient (HQ) was used to assess the risk from individual PAEs exposure:

$$HQ_i = \frac{\frac{Exposure_i(\mu g/inh/d)}{BW(kg)}}{AcceptalbeExposure_i(\mu g/BW/d)} \quad (3)$$

where BW is the body weight, the average body weights for adults and children in this study are 70 kg and 15 kg, respectively (U.S. EPA, 2013). The WBE approach assumes identical exposure levels for adults and children. Tolerable daily intake (TDI) values proposed by the European Food Safety Authorities (EFSA) (EFSA, 2005a, 2005b, 2005c), the reference dose for anti-androgenicity (RfD-AA) proposed by Kortenkamp and Faust (2010), and the reference dose (RfD) proposed by the U.S. Environmental Protection Agency (EPA) (U.S. EPA, 1990, 1993a, 1993b, 1993c) were selected as reference acceptable values (Acceptable $Exposure_i$) (Table S4). The EFSA-TDI values for DnBP, benzyl butyl phthalate (BzBP) and di(2-ethylhexyl) phthalate (DEHP) were based on anti-androgenic outcomes in animal experiments (damage to germ cells and spermatozoa concentrations). The RfD-AA for di-isobutyl phthalate (DiBP), DnBP, BzBP and DEHP were based on specific anti-androgenic endpoints. The RfD values for DEP, MnBP, BzBP and DEHP were primarily based on organ weight or increased mortality in animal testing, and not all were developed on the basis of anti-androgenic effects. Hazard index (HI), cumulative risk for each participant, is the sum of each HQ_i and was assessed by Eq. (4). In this study, HI was calculated according to EFSA-TDI and RfD-AA, which were based on similar toxicological endpoints for each HQ_i (Søeborg et al., 2012).

$$HI = \sum HQ_i \quad (4)$$

There is a potential adverse health risk from multiple PAEs exposure when the HI value is >1.

2.6. Statistical analysis

Normality was examined through the Kolmogorov-Smirnov test before statistical analysis. Pearson correlation analysis used to assess the correlation between exposure levels of target PAEs. The geographical pattern of exposure was assessed via one-way ANOVA. All statistical analysis was performed using SPSS 20 (IBM Co., USA), with a p-value <0.05 meaning the difference is statistically significant.

3. Results and discussion

3.1. Phthalate metabolite concentrations in influents and effluents

MMP, MEP and MnBP were detected in all the influent samples analyzed, with concentrations ranging from 23 ng/L to 2670 ng/L (mean ± STD, 269 ± 258 ng/L), 6 ng/L to 1581 ng/L (mean ± STD, 401 ± 282 ng/L) and 93 ng/L to 6921 ng/L (mean ± STD, 2303 ± 1282 ng/L), respectively (Table 1, Table S5). MiBP concentrations were below MDL in only one influent sample and above MQL in the others. The maximum MiBP concentration was 2599 ng/L (mean ± STD, 572 ± 473 ng/L). MEHHP concentrations ranged from <MDL to 102 ng/L (mean ± STD, 34 ± 22 ng/L), with a detection frequency of 96%. High detection frequencies of these mPAEs indicate that exposure to PAEs are ubiquitous in major Chinese cities. MBzP was detected in only 16 influent samples, with the concentrations ranging from <MDL to 31 ng/L (mean ± STD, 1 ± 3 ng/L). Compared with previous publications, the mean concentrations of mPAEs (MMP, MEP, MnBP, MBzP and MEHHP) in influents of HRB in this study are 1 to 3 orders of magnitude lower than corresponding PAEs (Gao et al., 2014). A similar result was also reported in Spanish (northwest region) urban influents in 2017 (Gonzalez-Marino et al., 2017). The excretion rate for a given dose of parent PAE as a corresponding mPAE ranged from 0.16 to 0.73 (Gonzalez-Marino et al., 2017). This means that PAEs in wastewater were not entirely derived from metabolism. Compared to surface water and sea water, the levels of MMP, MEP, MiBP and MnBP in influents in the present study are 1 or 2 orders of magnitude higher (Suzuki et al., 2001; Blair et al., 2009).

MMP, MEP, MnBP and MiBP were detected in most effluent samples, with detection frequencies of 90%, 81%, 57% and 71%, respectively. MEHHP had a low detection rate (5%), whereas MBzP concentrations were below MDL in all effluent samples. Among the six analyzed metabolites in effluents, MnBP (52 ± 66 ng/L) had the highest mean concentration followed by MiBP (31 ± 50 ng/L) and MMP (16 ± 13 ng/L). The range, median, arithmetic mean concentrations and standard deviations (STD) are presented in Table 1 and Table S6. MEHHP was almost completely removed by wastewater treatment processes (Table 2). The apparent removal rate of MEHHP was 100% at all sampled WWTPs except GZ-1 (92.3 ± 13.3%). MiBP and MnBP removals were also high with mean apparent removal rates above 90%. Removal of MMP and MEP were significant, although lower than those of the

Table 1
Statistics of mPAEs concentrations in wastewater across China.

Chemical residue		n	Detection frequency (%)	Mean ± STD	Median	Min	Max
				ng/L			
Influent	MMP	188	100	269 ± 258	214	23	2670
	MEP	188	100	401 ± 282	386	6	1581
	MiBP	188	99.5	572 ± 473	455	<LOD	2599
	MnBP	188	100	2303 ± 1282	2273	93	6921
	MBzP	188	9	1 ± 3	<LOD	<LOD	31
	MEHHP	188	96	34 ± 22	29	<LOD	102
Effluent	MMP	21	90	16 ± 13	16	<LOD	47
	MEP	21	81	8 ± 9	7	<LOD	38
	MiBP	21	57	31 ± 50	7	<LOD	176
	MnBP	21	71	52 ± 66	28	<LOD	261
	MBzP	21	0	–	–	–	–
	MEHHP	21	5	0.2 ± 1	<LOD	<LOD	4

Table 2
Apparent removal rates of MMP, MEP, MiBP, MnBP and MEHHP.

WWTP	Treatment process	Apparent removal rate (%)				
		MMP	MEP	MiBP	MnBP	MEHHP
HRB-1	A/O[a]	86.9 ± 0.3	99.0 ± 0	90.6 ± 0.8	100	100
XM-1	BAF[b]	91.6 ± 0	89.9 ± 0.2	100	90.0 ± 1.1	100
LY-2	A/A/O[c]	94.1 ± 2.0	98.7 ± 1.1	96.4 ± 4.4	98.5 ± 1.2	100
GZ-1	A/A/O	97.3 ± 3.2	99.1 ± 0.8	96.7 ± 4.2	99.1 ± 0.3	92.3 ± 13.3
CD-1	A/A/O	94.7 ± 2.9	98.6 ± 0.2	94.2 ± 4.4	97.1 ± 1.3	100
GY-2	A/A/O	89.9 ± 4.0	81.8 ± 21.9	100	100	100
LS-1	A/A/O	89.0	83.2	–	93.2	100

[a] Anoxic-Oxic.
[b] Biological Aerated Filter.
[c] Anaerobic-Anoxic-Oxic.

above 3 mPAEs (with mean apparent removal rates above 80%). Negative removal rates of MMP and MEHHP were reported previously (Gonzalez-Marino et al., 2017) but were not observed in this study. The main treatment process of XM-1 is BAF, HRB-1 is A/O, and the other five WWTPs are A/A/O. Table 2 shows that there was no significant difference in apparent removal rates among different treatment processes for the 5 mPAEs. LY-2 (Central China), GZ-1 (South China), CD-1 and GY-2 (Southwest China) are located in different geographic regions with various climate types, and the four WWTPs employ A/A/O as their main treatment process. However, similar apparent removal rates were found in these WWTPs, which indicate geographic location had no effect on removal of the five PAEs during wastewater treatment process.

3.2. Estimation of PAEs exposure

Phthalate monoesters are major human metabolites of the corresponding parent PAEs. Degradation and potential formation of phthalate monoesters in wastewater are negligible (Gonzalez-Marino et al., 2017). MMP, MEP, MiBP, MnBP, MBzP and MEHHP are considered to be the most suitable biomarkers to estimate the exposure to their corresponding parent PAEs (DMP, DEP, DiBP, DnBP, BzBP and DEHP) (Barr et al., 2003). However, both DnBP and BzBP can both be transformed into MnBP during human metabolism with average excretion rates of 69% and 6%, respectively, of the parent PAEs doses (Anderson et al., 2001). Low detection frequencies and concentration levels of MBzP (a major metabolite of BzBP) indicates that BzBP exposure was very low (Table 1). As a result, MnBP (a minor metabolite) from BzBP was much lower than that from DnBP metabolism in influent. Thus, MnBP in influents was assumed to come predominantly from DnBP metabolism (Gonzalez-Marino et al., 2017). Hence, exposure levels of the six PAEs were estimated using Eq. (1) and Eq. (2) based on concentrations of corresponding mPAEs. For each city, population-weighted averages were used to evaluate the average exposure levels of PAEs.

Exposure levels of DMP, DEP, DiBP, DnBP and DEHP in major Chinese cities ranged from 21 ± 5 μg/inh/d to 284 ± 170 μg/inh/d, 40 μg/inh/d to 567 ± 5 μg/inh/d, <1 μg/inh/d to 1132 ± 105 μg/inh/d, 204 μg/inh/d to 2263 ± 325 μg/inh/d, and 18 μg/inh/d to 178 ± 40 μg/inh/d, respectively (Fig. 2, Table S7). The highest DEP and DnBP exposure levels were both found in ZZ followed by CD (435 ± 88 μg/inh/d for DEP and 2044 ± 118 μg/inh/d for DnBP). DEP exposure levels in the others cities were below 350 μg/inh/d. DnBP exposure was between 1000 μg/inh/d to 2000 μg/inh/d at 15 cities (HRB, CC, SY, BJ, XA, LZ, YC, LY, WH, QD, XM, HF, NC, GZ and NN) and below 1000 μg/inh/d at the other cities. DMP exposure was >200 μg/inh/d at only five cities, TY (284 ± 170 μg/inh/d), SY (255 ± 153 μg/inh/d), CC (223 ± 0 μg/inh/d), SH (211 ± 65 μg/inh/d) and ZZ (202 ± 68 μg/inh/d). HRB (1132 ± 105 μg/inh/d) and NC (944 ± 104 μg/inh/d) had the highest DiBP exposure levels, two times higher than that in ZZ (447 ± 67 μg/inh/d) and XA (412 ± 38 μg/inh/d), and nearly one order of magnitude higher than that at other cities. The highest DEHP exposure level (178 ± 40 μg/inh/d) was observed in XA followed by ZZ (163 ± 2 μg/inh/d) and SY (158 ± 98 μg/inh/d). LS had the lowest exposure levels to DEP (40 μg/inh/d), DiBP (<1 μg/inh/d), DnBP (204 μg/inh/d) and DEHP (18 μg/inh/d), whereas XN had the lowest DMP exposure (21 ± 5 μg/inh/d).

Statistically significant positive correlations were found between exposure levels of DMP, DEP, DiBP, DnBP and DEHP (Table S8) in most Chinese cities except HRB and NC. These results reveal the fact that there was simultaneous exposure to PAEs mixtures across the country. Similar simultaneous exposure was also observed by Wang et al. (2015) for school children in three regions of the Yangtze River Delta in China. In addition, the observed correlations indicate that potential common sources of exposure may exist at different cities in China. The high exposure levels corroborate with the high DiBP concentrations observed in surface water and soil in HRB and NC (Niu et al., 2014; Zhang et al., 2012).

The average estimated exposure level of ∑5PAEs was 2184 ± 1173 μg/inh/d (population-weighted average ± STD) in China (Fig. 2). High STD indicates that a high geographical variation of ∑5PAEs exposure levels appeared across the seven regions. The highest level was found in ZZ (3642 ± 467 μg/inh/d) followed by NC (3327 ± 229 μg/inh/d), HRB (3255 ± 400 μg/inh/d), and CD (3189 ± 282 μg/inh/d). In total, there were four cities (CC, GY, LS and XN) with levels below 1000 μg/inh/d, and the lowest was observed in LS (290 μg/inh/d). The exposure levels of ∑5PAEs in all seven regions followed normal distributions (Kolmogorov-Smirnov test, $P > 0.05$) in this study. One-way ANOVA reveals that ∑5PAEs exposure levels display a clear geographical trend ($P < 0.05$). The exposure levels of ∑5PAEs in Southwest China were significantly lower than in the other regions. No significant difference in exposure was found in Northeast China, North China, Northwest China, East China, Central China and South China. The low ∑5PAEs exposure levels in Southwest China may be attributed to the low production and consumption of plastics in the region (CPPIA, 2016).

The average contributions of individual PAEs for total average exposure levels were further calculated (Fig. S1). Low detection rates and low concentrations of MBzP in influent wastewater samples indicate that the contribution from BzBP exposure was negligible. DnBP accounted for the highest proportion of ∑5PAEs exposure in this study with a national average contribution of 65% and the maximum contribution of 78% in XN. DiBP and DEP exposure ranked second and third, which contributed 14% and 11% to ∑5PAEs, respectively. DMP (6%) and DEHP (4%) exposure contributed the remaining 10%. The average estimated PAEs exposures were converted into mPAEs concentrations in urine. The composition of total mPAEs concentrations estimated by WBE agreed reasonably well with the results (urine analysis) in previous publications in China (Mu et al., 2015; Duan et al., 2017) (Fig. S2). This finding demonstrated that WBE is a useful tool for PAEs exposure estimation.

The average exposure levels of DMP, DEP, DiBP, DnBP and DEHP across all sampled cities of China were 141 ± 87 μg/inh/d, 230 ± 147 μg/inh/d, 306 ± 236 μg/inh/d, 1413 ± 649 μg/inh/d, and 94 ± 54 μg/inh/d, respectively (Table S7). In the literature, there was only one

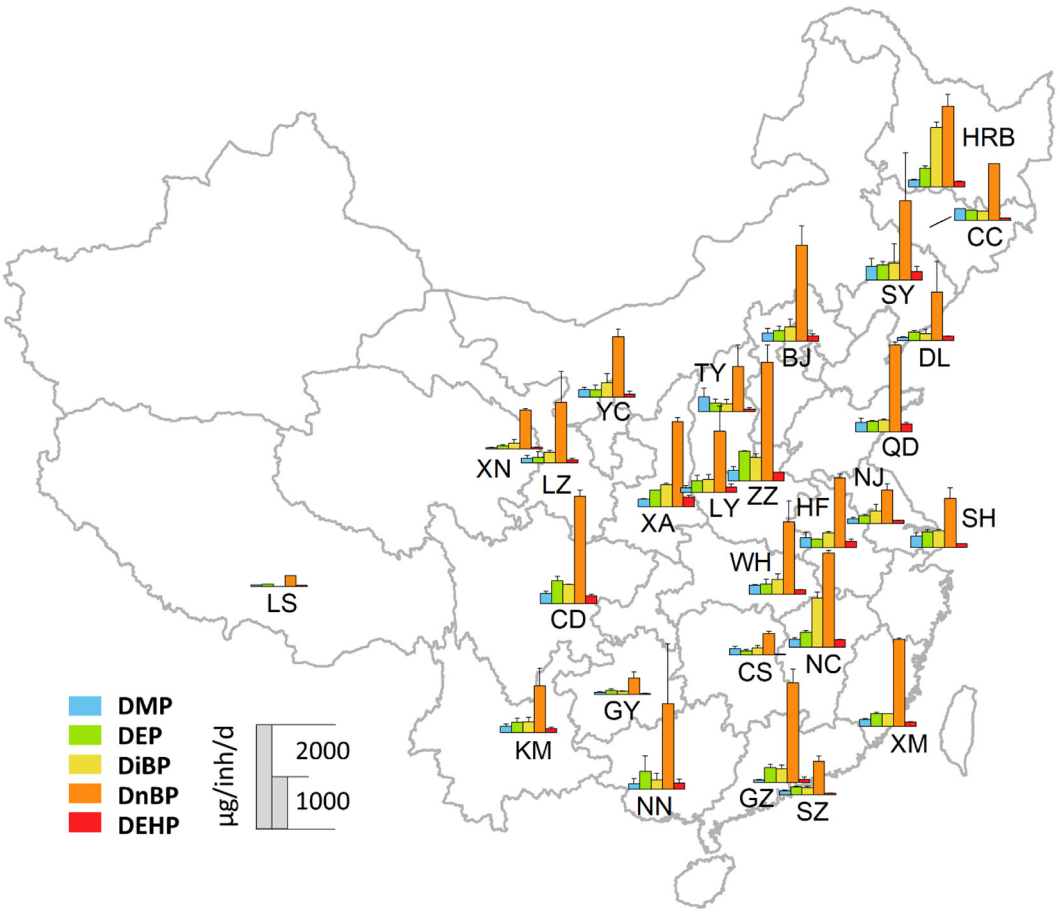


Fig. 2. Average exposure levels of DMP, DEP, DiBP, DnBP and DEHP in major cities of mainland China.

publication that estimated the exposure levels (population-weight average) and composition of PAEs (in Spain) through WBE (Gonzalez-Marino et al., 2017). DMP (214 μg/inh/d), DEP (714 μg/inh/d), and DEHP (145 μg/inh/d) exposure levels in Spain were higher than those in China by a factor of 1.5, 3.1 and 1.5, respectively. In contrast, DiBP was nearly two times higher, and DnBP was one order of magnitude higher than the levels in Spain (137 μg/inh/d and 138 μg/inh/d). In general, the ∑5PAEs exposure levels in China were also higher than that in Spain (1348 μg/inh/d). There was a clear difference in the composition of PAE exposure between China and Spain. DEP was the predominant contributor (53% of ∑5PAEs) in Spain, whereas the DnBP accounted for the highest proportion in China. Contributions of DMP, DiBP and DEHP to ∑5PAEs were not significantly different between Spain and China. The differences agreed reasonably well with the results estimated through conventional human biomonitoring in Spain and China (Herrero et al., 2015; Mu et al., 2015; Cutanda et al., 2015; Duan et al., 2017).

3.3. Health risks of PAEs in major Chinese cities

The health risks associated with PAEs exposure were assessed with the estimated daily exposure levels (Table 3). The exposure levels of DnBP for adults were below EFSA-TDI (10 μg/kg BW/d) at only five cities (Table S9). For children, the exposure levels of DnBP were higher than EFSA-TDI in all selected cities. These results show that both adults and children were at high DnBP exposure risk (HQ > 1) across China. For BzBP and DEHP, the HQ was below 1 based on EFSA-TDI (50 μg/kg BW/d and 500 μg/kg BW/d) for adults and children. Hence, the exposure levels of BzBP and DEHP were acceptable in China. $HI_{EFSA\text{-}TDI}$ was obtained by combining HQ_{DnBP}, HQ_{BzBP} and HQ_{DEHP}. HQ_{DnBP} contributed 98% of $HI_{EFSA\text{-}TDI}$, and the remaining 2% were attributed to HQ_{BzBP} and HQ_{DEHP}. $HI_{EFSA\text{-}TDI}$ were all above 1 in all selected cities for children (Table S9). For adults, $HI_{EFSA\text{-}TDI}$ were above 1 at all selected cities except NJ, SZ, CS, GY and LS ($HI_{EFSA\text{-}TDI}$ < 1). Recently, more and more studies suggested that the anti-androgenic effects of PAEs exposure in humans could arise at all life stages (Joensen et al., 2012). Thus, the high $HI_{EFSA\text{-}TDI}$ levels (>1) means a relatively high cumulative risk of anti-androgenic effects for both adults and children in most selected cities in China.

Using RfD-AA as a reference dose, the HQ (for DiBP, DnBP, BzBP and DEHHP) and $HI_{RfD\text{-}AA}$ was below 1 for all adults at all the cities (Table S10). The low risks for adults were observed primarily because RfD-AA is 10 times higher than EFSA-TDI for DnBP (which means that the EFSA-TDI approach is more conservative). However, HQ_{DnBP} and $HI_{RfD\text{-}AA}$ were above 1 for children at half of the cities examined (CC, TY, DL, XN, LZ, CS, NJ, SH, SZ, GY, KM, LS).

When EPA-RfD was used as the acceptable exposure level, the daily exposure levels of DEP, DnBP, BzBP and DEHP were also within acceptable levels for adults at all sampled cities (HQ < 1) (Table S11). However, exposure levels of DnBP exceeded the EPA-RfD for children (HQ > 1) at 11 out of the 27 cities (HRB, SY, BJ, XA, ZZ, QD, XM, NC, GZ, NN and CD) (Table S11). These results indicate that the children from these 11 cities were at a high non-cancer risk of DnBP exposure. Apparently, the risk for children, regardless of which approach is adopted, is higher than that for adults and is above accepted levels at many Chinese cities. Gonzalez-Marino et al. (2017) also reported higher risks for children than for adults in Spain. Furthermore, previous studies showed that the exposure opportunities and levels of PAEs are considerably higher for children than adults (CDC, 2009; Kasper-Sonnenberg et al., 2012). However, the WBE approach does not differentiate the PAEs in

Table 3
Hazard quotients (HQ) and hazard Indexes (HI) based on EFSA-TDI, RfD-AA and EPA-RfD for adults and children in sampled cities of China.

PAEs	Adults					Children				
	MIN	Median	95P	MAX	$n > 1$	MIN	Median	95P	MAX	n > 1
$HQ_{EFSA-TDI}$										
DnBP	0.29	1.67	2.86	3.23	22	1.36	7.79	13.33	15.09	27
BzBP	1.43E-05	1.43E-05	3.86E-05	2.00E-04	0	6.67E-05	6.67E-05	1.80E-04	9.33E-04	0
DEHP	4.86E-03	0.02	0.04	0.05	0	0.02	0.10	0.22	0.24	0
HI	0.30	1.70	2.89	3.28	22	1.38	7.93	13.49	15.30	27
HQ_{RfD-AA}										
DiBP	3.57E-05	0.02	0.03	0.07	0	1.67E-04	0.08	0.15	0.31	0
DnBP	0.03	0.17	0.29	0.32	0	0.14	0.78	1.34	1.51	11
BzBP	4.33E-05	4.33E-05	8.66E-05	3.03E-04	0	2.02E-04	2.02E-04	4.04E-04	1.41E-03	0
DEHP	0.01	0.04	0.08	0.08	0	0.04	0.17	0.36	0.40	0
HI	0.04	0.24	0.39	0.43	0	0.18	1.11	1.83	2.02	13
$HQ_{EPA-RfD}$										
DEP	7.14E-04	3.41E-03	0.01	0.01	0	3.33E-03	0.02	0.03	0.05	0
DnBP	0.03	0.17	0.29	0.32	0	0.14	0.78	1.34	1.51	11
BzBP	3.57E-05	3.57E-05	7.14E-05	5.00E-04	0	1.67E-04	1.67E-04	3.33E-04	2.33E-03	0
DEHP	0.01	0.06	0.12	0.13	0	0.06	0.26	0.54	0.59	0

n = Number of cities with the HQ or HI above 1.

wastewater excreted by children from those by adults. Rather, this approach assumes identical levels of PAEs exposure for children and adults, which means that it underestimates the PAE exposure level of children. In other words, the WBE method underestimates the health risks of children. Thus, the actual health risks of PAEs exposure to children in China is even higher than the values estimated in this instance and warrants further research.

Currently, health risk assessment of PAEs exposure is conducted by urine analysis. In this approach, most studies collected urine samples from several hundred subjects and later examined the health risks of PAEs exposure for sampled people (Kranich et al., 2014; Axelsson et al., 2015). However, sample sizes in these studies were usually small, ranging from dozens to several hundred (corresponding to resident populations ranging from several hundred thousand to several millions, respectively). Such small sample sizes may not yield risk assessment results that are sufficiently representative. Because of the high cost and difficulty of collecting urine samples, it is not feasible to perform regional or nationwide assessment via the urine analysis approach. In contrast, the WBE approach collects wastewater samples that contain urine from the entire community. Clearly, the wastewater samples, if properly sampled (e.g. using flow-proportional sampling mode, regular and long enough sampling periods), are more representative and easier to obtain compared to urine samples. Thus, the WBE is a useful approach for regional and nationwide health risk assessment of pollutants. However, there were also uncertainties in the WBE (Castiglioni et al., 2013), such as behavior of conjugated phthalates in different wastewaters, stability of phthalates and corresponding monoesters in wastewater and sewage (Gonzalez-Marino et al., 2017), and the excretion rates for different races. Further research is needed for a more accurate estimate. In addition, longer sampling periods should be conducted to assess temporal variations.

4. Conclusions

As the first study to report levels of mPAEs in wastewater on a national scale in China, this paper fills a gap in the literature and provides a new approach for exposure estimation and health risk assessment of PAEs. Estimated $\sum$5PAEs exposure levels in major Chinese cities ranged from 290 μg/inh/d (Lhasa) to 3642 ± 467 μg/inh/d (Zhengzhou) with a mean level of 2184 ± 1173 μg/inh/d. The $\sum$5PAEs exposure levels in Southwest China were significantly lower than in the other geographic regions due to low production and consumption of plastics in the region. PAE exposure poses considerably greater health risks to children in China than to adults, which warrants further research or proper regulation of PAE use in China. The WBE is a useful tool for regional and nationwide health risk assessment of pollutants and provides valuable information for policy makers.

Acknowledgements

This work is based on work funded by the National Science Foundation of China (Grant No. 41701543 and 41371442). The authors wish to thank all the personnel at the 54 wastewater treatment plants for their assistance in wastewater sampling.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.325.

References

Anderson, W.A., Castle, L., Scotter, M.J., Massey, R.C., Springall, C., 2001. A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Addit. Contam. 18 (12), 1068–1074.
Arbuckle, T.E., Davis, K., Boylan, K., Fisher, M., Fu, J., 2016. Bisphenol A, phthalates and lead and learning and behavioral problems in Canadian children 6–11 years of age: CHMS 2007–2009. Neurotoxicology 54, 89–98.
Axelsson, J., Rylander, L., Rignell-Hydbom, A., Jonsson, B.A., Lindh, C.H., Giwercman, A., 2015. Phthalate exposure and reproductive parameters in young men from the general Swedish population. Environ. Int. 85, 54–60.
Barr, D.B., Silva, M.J., Kato, K., Reidy, J.A., Malek, N.A., Hurtz, D., et al., 2003. Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. Environ. Health Perspect. 111 (9), 1148–1151.
Been, F., Bastiaensen, M., Lai, F.Y., van Nuijs, A.L.N., Covaci, A., 2017. Liquid chromatography-tandem mass spectrometry analysis of biomarkers of exposure to phosphorus flame retardants in wastewater to monitor community-wide exposure. Anal. Chem. 89 (18), 10045–10053.
Blair, J.D., Ikonomou, M.G., Kelly, B.C., Surrideg, B., Gobas, F.A.P.C., 2009. Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine inlet by LC/ESI-MS/MS. Environ. Sci. Technol. 43 (16), 6262–6268.
Bloom, M.S., Whitcomb, B.W., Chen, Z., Ye, A., Kannan, K., Buck Louis, G.M., 2015. Associations between urinary phthalate concentrations and semen quality parameters in a general population. Hum. Reprod. 30 (11), 2645–2657.
CDC, 2009. Fourth National Report on Human Exposure to Environmental Chemicals, U.S. Department of Health and Human Services. Centers for Disease Control and Prevention, Atlanta, GA.
CPPIA (China Plastics Processing Industry Association), 2016. China Plastics Industry Yearbook 2016. China Light Industry Press, Beijing.
Cutanda, F., Koch, H.M., Esteban, M., Sánchez, J., Angerer, J., Castaño, A., 2015. Urinary levels of eight phthalate metabolites and bisphenol A in mother-child pairs from two Spanish locations. Int. J. Hyg. Environ. Health 218, 47–57.
D'Ascenzo, G., Corcia, A.D., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., et al., 2003. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci. Total Environ. 302 (1–3), 199–209.
Du, P., Li, K., Li, J., Xu, Z., Fu, X., Yang, J., et al., 2015. Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology. Water Res. 84, 76–84.

Du, P., Zhou, Z., Bai, Y., Xu, Z., Gao, T., Fu, X., et al., 2017. Estimating heroin abuse in major Chinese cities through wastewater-based epidemiology. Sci. Total Environ. 605–606, 158–165.
Duan, Y., Wang, L., Han, L., Wang, B., Sun, H., Chen, L., et al., 2017. Exposure to phthalates in patients with diabetes and its association with oxidative stress, adiponectin, and inflammatory cytokines. Environ. Int. 109, 53–63.
EFSA (European Food Safety Authority), 2005a. Opinion of the scientific panel on food additives, flavourings, processing aids and material in contact with food (AFC) on a request from the commission related to Bis(2-ethylhexyl)phthalate (DEHP) for use in food contact materials. EFSA J. 243, 1–20.
EFSA (European Food Safety Authority), 2005b. Opinion of the scientific panel on food additives, flavourings, processing aids and material in contact with food (AFC) on a request from the commission related to Butylbenzylphthalate (BBP) for use in food contact materials. EFSA J. 241, 1–14.
EFSA (European Food Safety Authority), 2005c. Opinion of the scientific panel on food additives, flavourings, processing aids and material in contact with food (AFC) on a request from the commission related to Di-Butylphthalate (DBP) for use in food contact materials. EFSA J. 242, 1–17.
Gao, D.W., Wen, Z.D., 2016. Phthalate esters in the environment: a critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. Sci. Total Environ. 541, 986–1001.
Gao, D., Li, Z., Wen, Z., Ren, N., 2014. Occurrence and fate of phthalate esters in full-scale domestic wastewater treatment plants and their impact on receiving waters along the Songhua River in China. Chemosphere 95, 24–32.
Gong, M., Weschler, C.J., Zhang, Y., 2016. Impact of clothing on dermal exposure to phthalates: observations and insights from sampling both skin and clothing. Environ. Sci. Technol. 50 (8), 4350–4357.
Gonzalez-Marino, I., Rodil, R., Barrio, I., Cela, R., Quintana, J.B., 2017. Wastewater-based epidemiology as a new tool for estimating population exposure to phthalate plasticizers. Environ. Sci. Technol. 51 (7), 3902–3910.
Gracia-Lor, E., Castiglioni, S., Bade, R., Been, F., Castrignano, E., Covaci, A., et al., 2017. Measuring biomarkers in wastewater as a new source of epidemiological information: current state and future perspectives. Environ. Int. 99, 131–150.
Herrero, L., Calvarro, S., Fernández, M.A., Quintanilla-López, J.E., González, M.J., Gómara, B., 2015. Feasibility of ultra-high performance liquid and gas chromatography coupled to mass spectrometry for accurate determination of primary and secondary phthalate metabolites in urine samples. Anal. Chim. Acta 853, 625–636.
Heudorf, U., Mersch-Sundermann, V., Angerer, J., 2007. Phthalates: toxicology and exposure. Int. J. Hyg. Environ. Health 210 (5), 623–634.
Huen, K., Calafat, A.M., Bradman, A., Yousefi, P., Eskenazi, B., Holland, N., 2016. Maternal phthalate exposure during pregnancy is associated with DNA methylation of LINE-1 and Alu repetitive elements in Mexican-American children. Environ. Res. 148, 55–62.
Itoh, H., Yoshida, K., Masunaga, S., 2007. Quantitative identification of unknown exposure pathways of phthalates based on measuring their metabolites in human urine. Environ. Sci. Technol. 41 (13), 4542–4547.
Joensen, U.N., Frederiksen, H., Blomberg Jensen, M., Lauritsen, M.P., Olesen, I.A., Lassen, T.H., et al., 2012. Phthalate excretion pattern and testicular function: a study of 881 healthy Danish men. Environ. Health Perspect. 120 (10), 1397–1403.
Kasper-Sonnenberg, M., Koch, H.M., Wittsiepe, J., Wilhelm, M., 2012. Levels of phthalate metabolites in urine among mother-child-pairs — results from the Duisburg birth cohort study, Germany. Int. J. Hyg. Environ. Health 215 (3), 373–382.
Katsikantami, I., Sifakis, S., Tzatzarakis, M.N., Vakonaki, E., Kalantzi, O.I., Tsatsakis, A.M., et al., 2016. A global assessment of phthalates burden and related links to health effects. Environ. Int. 97, 212–236.
Koch, H.M., Drexler, H., Angerer, J., 2004. Internal exposure of nursery-school children and their parents and teachers to di(2-ethylhexyl)phthalate (DEHP). Int. J. Hyg. Environ. Health 207 (1), 15–22.
Koch, H.M., Bolt, H.M., Preuss, R., Angerer, J., 2005. New metabolites of di(2-ethylhexyl) phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch. Toxicol. 79 (7), 367–376.
Kranich, S.K., Frederiksen, H., Andersson, A.M., Jorgensen, N., 2014. Estimated daily intake and hazard quotients and indices of phthalate dieters for young Danish men. Environ. Sci. Technol. 48 (1), 706–712.
Lai, F.Y., O'Brien, J., Bruno, R., Hall, W., Prichard, J., Kirkbride, P., et al., 2016. Spatial variations in the consumption of illicit stimulant drugs across Australia: a nationwide application of wastewater-based epidemiology. Sci. Total Environ. 568, 810–818.
Lopez-Carrillo, L., Hernandez-Ramirez, R.U., Calafat, A.M., Torres-Sanchez, L., Galvan-Portillo, M., Needham, L.L., et al., 2010. Exposure to phthalates and breast cancer risk in northern Mexico. Environ. Health Perspect. 118 (4), 539–544.
Mastroianni, N., Lopez-Garcia, E., Postigo, C., Barcelo, D., Lopez de Alda, M., 2017. Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends. Sci. Total Environ. 609, 916–926.
Mu, D., Gao, F., Fan, Z., Shen, H., Peng, H., Hu, J., 2015. Levels of phthalate metabolites in urine of pregnant women and risk of clinical pregnancy loss. Environ. Sci. Technol. 49 (17), 10651–10657.
Net, S., Sempere, R., Delmont, A., Paluselli, A., Ouddane, B., 2015. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environ. Sci. Technol. 49 (7), 4019–4035.
Niu, L., Xu, Y., Xu, C., Yun, L., Liu, W., 2014. Status of phthalate esters contamination in agricultural soils across China and associated health risks. Environ. Pollut. 195, 16–23.
Rousis, N.I., Gracia-Lor, E., Zuccato, E., Bade, R., Baz-Lomba, J.A., Castrignanò, E., et al., 2017a. Wastewater-based epidemiology to assess pan-European pesticide exposure. Water Res. 121, 270–279.
Rousis, N.I., Zuccato, E., Castiglioni, S., 2017b. Wastewater-based epidemiology to assess human exposure to pyrethroid pesticides. Environ. Int. 99, 213–220.
Saravanabhavan, G., Walker, M., Guay, M., Aylward, L., 2014. Urinary excretion and daily intake rates of diethyl phthalate in the general Canadian population. Sci. Total Environ. 500-501, 191–198.
Søeborg, T., Frederiksen, H., Andersson, A.M., 2012. Cumulative risk assessment of phthalate exposure of Danish children and adolescents using the hazard index approach. Int. J. Androl. 35 (3), 245–252.
Sun, Q., Cornelis, M.C., Townsend, M.K., Tobias, D.K., Eliassen, A.H., Franke, A.A., et al., 2014. Association of urinary concentrations of bisphenol A and phthalate metabolites with risk of type 2 diabetes: a prospective investigation in the Nurses' health study (NHS) and NHSII cohorts. Environ. Health Perspect. 122 (6), 616–623.
Suzuki, T., Yaguchi, K., Suzuki, S., 2001. Monitoring of phthalic acid monoesters in river water by solid-phase extraction and GC-MS determination. Environ. Sci. Technol. 35 (18), 3757–3763.
Tsai, Y.A., Tsai, M.S., Hou, J.W., Lin, C.L., Chen, C.Y., Chang, C.H., et al., 2018. Evidence of high di(2-ethylhexyl) phthalate (DEHP) exposure due to tainted food intake in Taiwanese pregnant women and the health effects on birth outcomes. Sci. Total Environ. 618, 635–644.
U.S. EPA (United States Environmental Protection Agency), 1990. Integrated Risk Information System (IRIS) Chemical Assessment Summary, Dibutyl Phthalate; CASRN 84-74-2, Washington, DC.
U.S. EPA (United States Environmental Protection Agency), 1993a. Integrated Risk Information System (IRIS) Chemical Assessment Summary, Butyl Benzyl Phthalate; CASRN 85-68-7, Washington, DC.
U.S. EPA (United States Environmental Protection Agency), 1993b. Integrated Risk Information System (IRIS) Chemical Assessment Summary, Di(2-Ethylhexyl)Phthalate (DEHP); CASRN 117-81-7, Washington, DC.
U.S. EPA (United States Environmental Protection Agency), 1993c. Integrated Risk Information System (IRIS) Chemical Assessment Summary, Diethyl Phthalate; CASRN 84-66-2, Washington, DC.
U.S. EPA (United States Environmental Protection Agency), 2013. Mid Atlantic Risk Assessment. Regional Screening Level (RSL) Summary Table, Washington DC.
Upson, K., Sathyanarayana, S., De Roos, A.J., Thompson, M.L., Scholes, D., Dills, R., et al., 2013. Phthalates and risk of endometriosis. Environ. Res. 126, 91–97.
Wang, B., Wang, H., Zhou, W., Chen, Y., Zhou, Y., Jiang, Q., 2015. Urinary excretion of phthalate metabolites in school children of China: implication for cumulative risk assessment of phthalate exposure. Environ. Sci. Technol. 49 (2), 1120–1129.
Zhang, F., Mu, Z., Chen, Z., Qin, D., Wu, S., 2012. Monitoring phthalate esters in fishery environment of Heilongjiang River Basin. J. Environ. Health 129 (12), 1133–1134 (in Chinese).
Zhang, Y., Cao, Y., Shi, H., Jiang, X., Zhao, Y., Fang, X., et al., 2015. Could exposure to phthalates speed up or delay pubertal onset and development? A 1.5-year follow-up of a school-based population. Environ. Int. 83, 41–49.
Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., Fanelli, R., 2008. Estimating community drug abuse by wastewater analysis. Environ. Health Perspect. 116 (8), 1027–1032.
Zuccato, E., Castiglioni, S., Senta, I., Borsotti, A., Genetti, B., Andreotti, A., et al., 2016. Population surveys compared with wastewater analysis for monitoring illicit drug consumption in Italy in 2010–2014. Drug Alcohol Depend. 161, 178–188.

Science of the Total Environment 605–606 (2017) 158–165

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Estimating heroin abuse in major Chinese cities through wastewater-based epidemiology

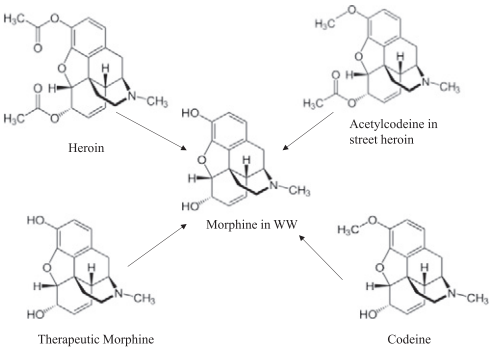
Peng Du, Zilei Zhou, Ya Bai, Zeqiong Xu, Tingting Gao, Xiaofang Fu, Xiqing Li *

Laboratory of Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, 100871 Beijing, China

HIGHLIGHTS

- First large scale wastewater-based investigation on heroin abuse in major Chinese cities
- Morphine and codeine in wastewater were predominantly from street heroin.
- Codeine abuse was evident in cities in Guangdong province.
- Heroin consumption was estimated based on morphine loads in wastewater.
- Heroin consumption in Northwest and Southwest was much higher than in other regions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 January 2017
Received in revised form 26 May 2017
Accepted 29 May 2017
Available online 27 June 2017

Editor: Kevin V. Thomas

Keywords:
Heroin
Abuse
Wastewater
Epidemiology
China

ABSTRACT

Heroin consumption in major cities across China was estimated for the first time via wastewater-based epidemiology. Influent and effluent wastewater samples were collected from 49 wastewater treatment plants (WWTPs) in 24 major cities that cover all the geographic regions of the country. Concentrations of morphine, 6-acetylmorphine, and codeine were measured. Near complete removal of morphine by wastewater treatment processes was observed, whereas removal rates of codeine were slightly lower. Morphine loads were much higher than codeine loads at most WWTPs in China, a trend opposite to that in many European countries. In addition, morphine and codeine loads were strongly correlated at most WWTPs, indicating morphine and codeine in wastewater were predominantly from the same source, street heroin. At WWTPs in Guangzhou and Shenzhen, codeine loads were considerably higher than morphine loads, consistent with previous reports of codeine abuse (e.g., as cough syrup) among middle and high school students in Guangdong province. Heroin consumption was derived based on morphine loads and taking into account therapeutic use of morphine and codeine, as well as contribution of codeine and acetylcodeine in street heroin. Highest heroin consumption was observed in northwestern and southwestern China. The average heroin consumption of the sampled cities was 64.6 ± 78.7 mg/1000 inh/d. The nation-wide average heroin consumption was much lower than that of methamphetamine, consistent with seizure data and numbers of registered heroin and methamphetamine users in China.

1. Introduction

Heroin had long been the primary drug of abuse in China since the reemergence of the drug problem in the country in early 1980s (Office of China National Narcotic Control Committee, 2016a). For

* Corresponding author.
E-mail address: xli@urban.pku.edu.cn (X. Li).

http://dx.doi.org/10.1016/j.scitotenv.2017.05.262
0048-9697/

example, registered heroin abusers (0.745 million) accounted for 82.7% of the total number of registered drug abusers (0.901 million) in China in 2000 (Office of China National Narcotic Control Committee, 2001). Although seizure of heroin was exceeded by that of methamphetamine about ten years ago (Office of China National Narcotic Control Committee, 2006), heroin abuse remains at high level (Office of China National Narcotic Control Committee, 2016b). Heroin seizure increased gradually from 5.79 t in 2007 to 9.3 t in 2014 (Office of China National Narcotic Control Committee, 2008, 2015b). However, exact heroin abuse, its spatial distribution, as well as its temporal variation were largely unknown in the country.

About a decade ago, Zuccato et al. (2005, 2008) estimated illicit drug abuse through wastewater-based epidemiology (WBE) for the first time. This methodology involves collecting wastewater samples (typically influents) from wastewater treatment plants (WWTPs) and measuring the concentrations of the drug target residues in the samples. The drug consumption of the communities served by the WWTPs is then back-calculated by taking in account flow rates of WWTPs, populations of the communities, as well as the excretion rates of the drugs (Zuccato et al., 2008). This approach uses objective measures to estimate drug use, generates results in near real time, and allows comparison of drug use between different communities and different time periods.

In the past decade, wastewater-based epidemiology has been applied in many countries, mainly in Europe (van Nuijs et al., 2009, 2011a; Subedi and Kannan, 2014; Jiang et al., 2015; Senta et al., 2015; Mackulak et al., 2015, 2016; Karolak et al., 2010; Repice et al., 2013; Andres-Costa et al., 2014; Bijlsma et al., 2014; Damien et al., 2014; de Castro et al., 2014; Guerra et al., 2014; Kankaanpaa et al., 2014; McCall et al., 2016). In particular, this approach has been used to estimate heroin use in Spain (Boleda et al., 2009; Postigo et al., 2010), Belgium (van Nuijs et al., 2011b), Italy (Zuccato et al., 2008, 2011, 2016), Switzerland (Zuccato et al., 2008; Been et al., 2015), UK (Zuccato et al., 2008), Canada (Yargeau et al., 2014), and Croatia (Terzic et al., 2010). In these studies, heroin consumption was back-calculated based on concentrations of either of its two metabolites, morphine or 6-acetylmorphine in wastewater. Morphine (in free and conjugate forms) is the major yet non-exclusive metabolite, whereas 6-acetylmorphine is the exclusive yet minor metabolite of heroin (Baselt, 2004; D'Ascenzo et al., 2003). Among the countries examined, heroin consumption ranged from a few tens to a few hundreds of milligrams per 1000 inhabitants per day.

Wastewater-based epidemiology has not been applied in mainland China until quite recently. Khan et al. (2014) applied the approach for the first time to four megacities (Beijing, Shanghai, Guangzhou, and Shenzhen). In total nine wastewater treatment plants were sampled in that study to examine the use of heroin, methamphetamine, ketamine, and other seven illicit drugs in the cities. Li et al. (2014) examined methamphetamine loads in wastewater of the urban districts of Beijing. Du et al. (2015) carried out a nation-wide reconnaissance of methamphetamine and ketamine abuse. More recently, Gao et al. (2017) used this approach to examine use of new psychoactive substances (synthetic cathinones and piperazines). However, large-scale survey of heroin use has not been performed in the country.

The objective of this work was to examine, for the first time, heroin use in major cities across China. Influent wastewater samples were collected from 49 WWTPs of 24 provincial capitals or equivalent cities that cover all the geographic regions of the country. Concentrations of morphine, 6-acetylmorphine, and codeine (a pharmaceutical that is metabolized into morphine and an impurity in street heroin) in the wastewater samples were analyzed. Heroin consumption was derived based on morphine loads in wastewater that were subtracted by contribution of therapeutic morphine and codeine as well as contribution of codeine from heroin. A clear geographic pattern of heroin use in China was then demonstrated.

2. Materials and method

2.1. Reagents and chemicals

Morphine (MOR), codeine (COD), 6-acetylmorphine (6-AM), and their deuterated analogs used as internal standards (MOR-d_3, COD-d_6, 6-AM-d_6) were purchased from Cerilliant (Round Rock, TX, USA). Formic acid and ammonium formate (HPLC grade) were purchased from CNW Technologies GmbH (Düsseldorf, Germany). HPLC grade of acetonitrile (AcN) and methanol (MeOH) was obtained from Fisher Scientific (Waltham, MA, USA). Hydrochloric acid (AR) and ammonium hydroxide (AR) were purchased from Beijing Chemical Works (Beijing, China). Ultrapure water was prepared using a Milli-Q ultrapure system (Millipore, MA, USA). Oasis MCX SPE cartridges (60 mg, 3 mL) were purchased from Waters Corporation (Milford, MA, USA).

2.2. Wastewater sampling

Wastewater samples were collected from 24 cities in China (Fig. 1, Table S1). Beijing (BJ) and Shanghai (SH) are municipalities directly administrated by the central government. Harbin (HRB), Xi'an (XA), Lanzhou (LZ), Yinchuan (YC), Taiyuan (TY), Shijiazhuang (SJZ), Wuhan (WH), Jinan (JN), Nanjing (NJ), Hangzhou (HZ), Nanning (NN), Guangzhou (GZ), Chengdu (CD), Kunming (KM) and Guiyang (GY) are provincial capitals. Dalian (DL), Luoyang (LY), Qingdao (QD), Suzhou (SuZ), Xichang (XC), Xiamen (XM), and Shenzhen (SZ) are equivalent to provincial capitals in terms of economy and population. The sampled cities are distributed in all the eight geographical regions of China: Northeast (HRB and DL); North (BJ, SJZ and TY); Northwest (LZ, XA and YC); Central China (LY and WH), East (JN, QD, NJ, SuZ and SH); Southeast (XM and HZ), Southwest (CD, XC, KM and GY), South (NN, GZ and SZ). The total population of sampled cities was 217.5 million, about 16% of the entire population of the nation.

Wastewater samples were collected from 49 WWTPs in the 24 cities. In most cities, two or more WWTPs were selected for wastewater sample collection. Only one WWTP was sampled in LZ and JN. Influent samples were collected in all 49 WWTPs, whereas effluent samples were collected in 19 selected WWTPs (Fig. 2) in 9 cities (Table S1). Most sampled WWTPs treat domestic wastewater from the urban districts of the cities. The population served by the WWTPs from which influent samples were collected totals 43.9 million, representing about 20% of the total population of the cities and 3.2% of the entire population of the country. The WWTPs are named as SZ-1 (first WWTP of Shenzhen), BJ-5 (fifth WWTP of Beijing), etc.

The sampling campaign was conducted during two stages. The wastewater in DL, TY, QD, SuZ, NN, GZ, SZ, XC, CD and HRB-2 were collected between May and September of 2015. Samples from other cities were collected from early July to early October of 2014. Sampling over a period of one year may involve seasonal changes, which may affect the spatial trend of heroin use. However, sampling across a country as vast as China was too huge an undertaking to be completed within a short sampling period. Each WWTP was sampled at least two days (one weekday and one weekend day) except LZ-1, KM-1 and HZ-2 (sampled for one day) (Table S1). Most of samples were collected by collecting 24-h time-proportional composite samples with several types of auto-samplers, such as FC-9624 (GRASP Science & Technology Co., LTD, Beijing), ISCO 3000, 3700, 4700, 6712 (Teledyne Technologies Inc., Lincoln, NE, USA), Sigma-SD900 (HACH Inc., USA). Time-proportional sampling is not an ideal approach to collect samples (Ort et al., 2010). However, flow- and volume-proportional samplers were not available at the WWTPs. In addition, time-proportional sampling was adopted in many regional and national scale WBE studies (e.g., Kankaanpaa et al., 2014; Mackulak et al., 2014; Du et al., 2015).

During collection, each auto-sampler was programmed to imbibe a specific volume (100 mL) of influent (or effluent) for each hour of sampling. A composite sample was obtained by mixing the samples

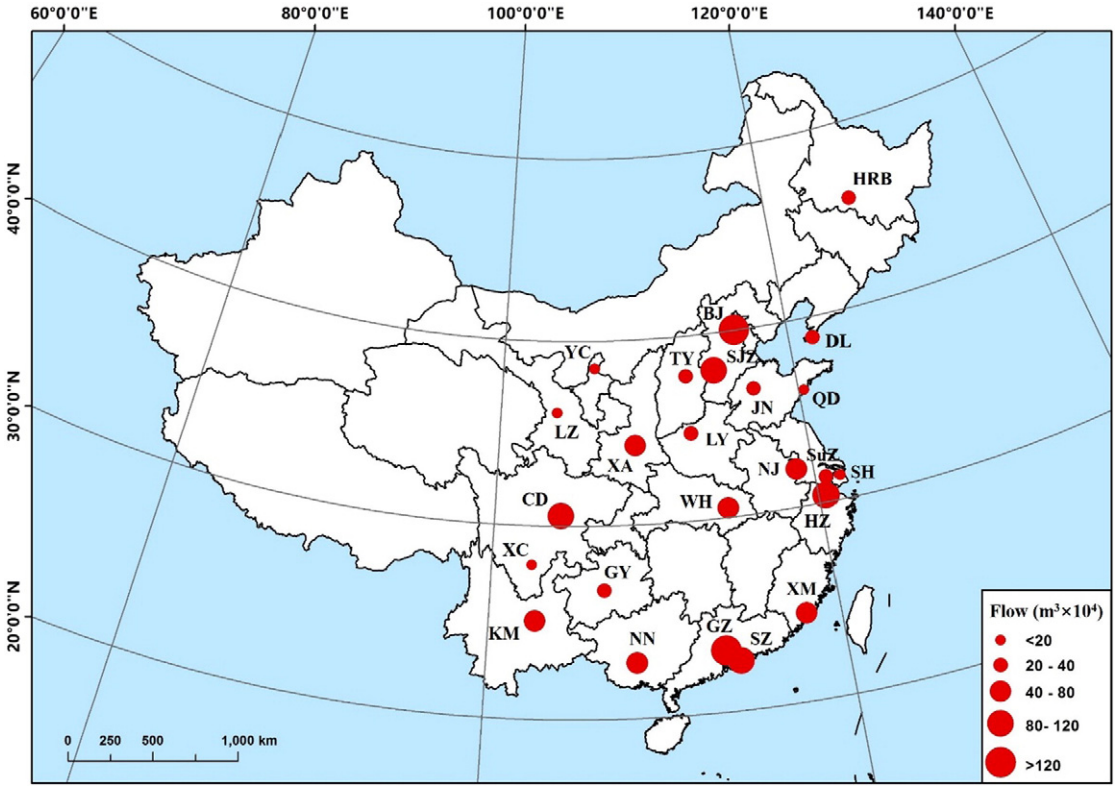


Fig. 1. Locations of cities in mainland China where wastewater samples were collected. The sizes of the circles represent the total flow rates of the WWTPs sampled in the cities.

collected in the 24-h period. LZ-1, XA-1, NN-1 and NN-2 did not install auto-samplers but disallowed entry of our own samplers. At these WWTPs, 200 mL of wastewater was grabbed manually every 4 h throughout the day and was combined to form a composite sample. Following collection, samples were mixed with hydrochloric acid to pH 2 and stored in −20 °C freezers at WWTPs. All samples were carried back to laboratory with ice and stored under −20 °C until analysis. Details of WWTPs (flow rates and served populations) and sampling information are provided in Table S1.

2.3. Analysis

Sample pretreatment procedures followed the method described in our previous publication (Du et al., 2015), with minor modifications. Briefly, the solid particles in the wastewater were removed by passing the wastewater through a glass filter. An Oasis MCX cartridge was conditioned in sequence with 6 mL MeOH, 4 mL ultrapure water and another 4 mL ultrapure water at pH = 2. Then, 50 mL filtered wastewater added with deuterated internal standards (50 μL, 200 μg/L) was loaded to the conditioned MCX cartridge at a rate of 1–2 mL/min. Following drying under vacuum, the cartridge was eluted with 4 mL of MeOH and 4 mL of 5% NH_3 in MeOH. The eluate was evaporated to dryness by N_2 stream, then redissolved using 200 μL AcN/ultrapure water (1/19, v/v), and finally filtered using a 0.22 μm centrifugal filter (VWR International, Radnor, PA, USA).

The filtered solution was injected into an UFLCXR-LC system (Shimadzu, Japan) with a Phenomenex Gemini C18 column (100 mm × 2 mm, 3 μm) to separate the target compounds. The injection volume was 5 μL. The mobile phase was composed of 30 mM ammonium formate in ultrapure water, with pH adjusted to 3.5 by formic acid (A) and MeOH (B). The flow rate of the mobile phase was controlled at 0.3 mL/min. The elution gradient was as follows: 0–0.1 min: 5% B; 0.1–3.0 min: 30% B; 3.0–5.0 min: 80% B; 5.0–9.5 min: 95% B; 9.5–14.0 min: 5% B. Concentrations were determined using an API 4000 triple quadrupole mass spectrometer (AB SCIEX, USA) equipped with an electrospray interface operating in positive ionization mode. The quantification of MS system was operated in multiple reaction monitoring (MRM) mode. MS parameters, quantifier and qualifier ions, retention time, LODs, LOQs were provided in Table S2.

The analytical methods were validated followed the procedure described in previous papers (Li et al., 2016; Gao et al., 2017), with minor modifications. Recovery was determined by spiking ultrapure water (pH = 2) with target drugs at three concentrations (40, 200 and 400 ng/L) and following the same pretreatment procedures. Matrix effects were determined by spiking the low-concentration influent wastewater with target drugs at three concentrations (40, 200 and 400 ng/L). The difference in concentrations of the spiked and unspiked samples was divided by the spiked concentrations to yield matrix effects. The recoveries and matrix effects of target drugs ranged from 87.0 ± 4.4% to 108.2 ± 1.3% and from −10.2 ± 6.7% to 19.2 ± 2.0%, respectively (Table S3). Intra- and inter-day repeatability of instrument and method ranged from 1.0 to 2.1% and from 2.7 to 8.1%, respectively (Table S3). A procedure blank using ultrapure water (pH = 2) was included in every 11 wastewater samples and followed the same pretreatment steps. All the target compounds were below detection limits in blanks (Table S3).

2.4. Removal rate calculation, mass load estimation, and statistical analysis

Removal rates of target drug residues at a specific WWTP were calculated by the following equation:

$$\text{Removal rate} = \left(\frac{C_{in} - C_{eff}}{C_{in}}\right) \times 100\% \quad (1)$$

C_{in} is the concentration of a target drug in influent, C_{eff} is the concentration of a target drug in effluent. The daily mass load of each drug target residue per 1000 inhabitants at a specific WWTP was estimated by the below equation:

$$\text{Load (mg/1000inh/d)} = \frac{RC\ (\text{ng/L}) \times F_{in}\ (\text{L/d})}{\frac{POP}{1000}} \times \frac{1}{10^6}\left(\frac{\text{mg}}{\text{ng}}\right) \quad (2)$$

where RC is the concentration of the drug target residue in influent, F_{in} is the influent flow of the WWTP, POP is the population served by the WWTP (obtained from the plant or based on the most recent census data of the service areas). Uncertainties involved in load estimation were discussed in a previous study (Li et al., 2014). The statistical analysis (K-S test, one-way ANOVA, correlation analysis, etc.) was performed using SPSS 20 (IBM Co., USA).

2.5. Heroin consumption estimation

Back-calculation of heroin is not straightforward. Heroin consumption has been usually estimated based on loads of either 6-AM or MOR. No interference from other substances exists when estimating heroin consumption based on 6-AM loads, as 6-AM is an exclusive metabolite of heroin. However, the excretion rate of 6-AM following heroin use is very low (<1.5%) (van Nuijs et al., 2011b; Smith et al., 2001; Elliott et al., 1971), which lead to high uncertainties. In addition, 6-AM is not stable in wastewater and could be not be detected even if there is significant heroin consumption. Thus 6-AM is not a suitable metabolite to back-calculate heroin consumption.

MOR (in free and conjugate forms) is the major urinary metabolite of heroin (Baselt, 2004). However, MOR may also come from metabolism of legal pharmaceuticals, mainly therapeutic MOR and COD. Thus to back-calculate heroin consumption, the contribution of therapeutic sources of MOR needs to be subtracted. The excretion rate of MOR following its therapeutic use was reported to be 77.7 ± 9.1% (Khan and Nicell, 2011; Brunk and Delle, 1974; Mikus et al., 1994). This excretion rate assumes complete cleaving of MOR conjugates (morphine-3-glucuronide and morphine-6-glucuronide). Chinese Medicine Statistics Year Book of 2014 (Ministry of Industry and Information Technology, 2015) indicates that 1.04 t morphine hydrochloride and 0.66 t morphine sulfate were produced in China and that there was not import nor export of MOR. Assuming produced MOR were all used in the same year, in average 2.55 mg/1000 inh/d MOR was consumed (based on a total population of 1.38 billion). This value is slightly higher than the MOR consumption (1.49 mg/1000 inh/d) derived from prescription data in Changshu, a city with about a 1.1 million population in Jiangsu province. Based on the national average consumption, 1.96 mg/1000 inh/d MOR from therapeutic MOR was expected to be released into wastewater.

COD is an opiate used to treat pain and coughing. Production of codeine phosphate was 1.46 t in 2014 in China (Ministry of Industry and Information Technology, 2014, 2015), translating to a national average of 2.06 mg/1000 inh/d. COD can be metabolized into COD and MOR. In the literature, excretion rate of COD as a parent compound varies dramatically. Baker et al. (2014) used an excretion rate of 63.5%, whereas van Dyken et al. (2016) use a rate of 10%. These two rates were found to underestimate and overestimate COD consumption, respectively. Recently, Thai et al. (2016) suggested an excretion rate of 30% that yielded consumption matching reasonably well with the sales statistics. Using this value, 0.62 mg/1000 inh/d COD from therapeutic COD administration was expected to be released into wastewater. Using an excretion rate of 6.51% that was commonly adopted in the literature (Khan and Nicell, 2011), a nation-wide average MOR load of 0.27 mg/1000 inh/d from therapeutic COD use is expected to enter wastewater.

In addition to therapeutic sources, there is another source of MOR that needs to be accounted for. Street heroin contains COD and acetylcodeine. Analysis of 124 heroin seizure samples across China indicated that street heroin contains 0.2 ± 0.4% COD and 14.4 ± 11.5% acetylcodeine (personal communication, National Laboratory of Narcotics of China). Contribution of COD and acetylcodeine from street heroin to MOR loads in wastewater has not been accounted for in the literature (Zuccato et al., 2008; Postigo et al., 2010; Terzic et al., 2010). Acetylcodeine is metabolized into COD and then into MOR (Staub et al., 2001). Detailed pharmacokinetic data of acetylcodeine is not available in the literature. However, urinary excretion of acetylcodeine as a parent compound was found to be negligible after 3 h following administration (Staub et al., 2001). Thus, it is reasonable to assume that administered acetylcodeine was quickly and nearly completely metabolized into COD. As such, the excretion rate of MOR following COD administration can also be used to estimate contribution of MOR loads in wastewater from acetylcodeine in street heroin (i.e., acetylcodeine was treated as COD in back-calculation).

Taking all the sources into account, pure heroin consumption (*HER Cons.*) can be estimated as following:

$$HER\ Cons. = MOR_{HER} \times \frac{1}{Excretion_{MOR-HER}} \cdot \frac{MW_{HER}}{MW_{MOR}} \quad (3)$$

where $Excretion_{Mor\text{-}HER}$ is the excretion rate of MOR following heroin abuse (77.7%); MW_{HER} and MW_{MOR} are molecular weights of heroin and MOR, respectively; MOR_{HER} is MOR load in wastewater that arose from heroin metabolism. It can be derived by subtracting contribution of therapeutic MOR ($MOR_{THER\text{-}MOR}$, 1.96 mg/1000 inh/d), therapeutic COD ($MOR_{THER\text{-}COD}$, 0.27 mg/1000 inh/d), and non-therapeutic COD and acetylcodeine ($MOR_{NTHER\text{-}COD}$) from MOR loads in wastewater (MOR_{WW}):

$$MOR_{HER} = MOR_{WW} - MOR_{THER-MOR} - MOR_{THER-COD} - MOR_{NTHER-COD} \quad (4)$$

$MOR_{NTHER\text{-}COD}$ can be derived from loads of nontherapeutic COD (from street heroin as COD and acetylcodeine) (COD_{NTEHR}):

$$MOR_{NTHER-COD} = COD_{NTHER} \times \frac{1}{Excretion_{COD-COD}} \times Excretion_{MOR-COD} \quad (5)$$

where $Excretion_{COD\text{-}COD}$ is the excretion rate of COD (30%) as a parent compound following COD administration, $Excretion_{MOR\text{-}COD}$ is the excretion rate of MOR (6.51%) following COD administration. COD_{NTEHR} divided by $Excretion_{COD\text{-}COD}$ gives the consumed amount of COD and acetylcodeine (which again is treated as COD) in street heroin. COD_{NTEHR} can be derived by subtracting the average therapeutic load of COD (0.62 mg/1000 inh/d) from total COD loads in wastewater:

$$COD_{NTHER} = COD_{WW} - COD_{THER} \quad (6)$$

Using national average of therapeutic use of MOR and COD and national average contents of COD and acetylcodeine in street heroin, as well as treating acetylcodeine as COD, undoubtedly brings in uncertainties in pure heroin consumption estimation. This is especially true for WWTPs where MOR loads were low. However, for most WWTPs with total MOR loads greater 20 mg/1000 inh/d, average MOR loads expected from therapeutic use of MOR and COD is around 10%, whereas MOR loads expected from COD and acetylcodeine in street heroin were typically <15% of the total loads in wastewater. Thus, the uncertainties involved at these WWTPs were likely low and would not change the geographic trend of heroin consumption in major cities across China. Nevertheless, future research is warranted to refine the parameters (e.g., therapeutic MOR and COD use, excretion rates of MOR and

COD following therapeutic and non-therapeutic use) used in HER consumption estimation.

3. Results and discussion

3.1. Concentrations in wastewater

The mean concentrations of MOR in the influent of all 49 sampled WWTPs ranged from <LOD to 462.3 ± 497.4 ng·L^{-1} (Table 1). The highest mean concentration was found at LZ-1. The concentrations were below LOD at DL-2, NJ-1 and GY-2. Large variations in concentrations were observed between or among WWTPs of same cities (e.g., NJ, GY). This is because in these cities, there are WWTPs that treat wastewater from suburban districts (GY-2) or from a recently developed district (NJ-2). MOR was not detected in the effluent from the 19 WWTPs except for BJ-1 (4.6 ± 7.2 ng·L^{-1}) and GZ-1 (0.2 ± 0.4 ng·L^{-1}) (Table S4). COD was detected in all influent samples with mean concentrations ranging from 2.5 ± 0.3 to 173.9 ± 108.5 ng·L^{-1} (Table 1). The highest and lowest mean COD concentrations were found at SZ-1 (173.9 ± 108.5 ng·L^{-1}) and NJ-1 (2.5 ± 0.3 ng·L^{-1}), respectively. Effluent mean COD concentrations ranged from <LOD to 22.3 ± 9.2 ng·L^{-1} in 19 WWTPs (Table S4). The mean COD concentrations were below LOD at BJ-5, KM-1, NJ-1, NJ-2, SH-1, WH-1 and WH-2, the highest was observed in BJ-1. 6-AM was detected in influent at only four WWTPs, with the highest mean concentration (10.3 ± 10.5 ng·L^{-1}) observed at TY-2 (Table 1). 6-AM concentrations were below LOD in effluent at all 19 WWTPs (Table S4).

3.2. MOR and COD removal

The removal of MOR by wastewater treatment processes was nearly complete (Fig. 2). Apparent removal rate of MOR was 100% at most WWTPs (17/19). At the other two WWTPs (BJ-1and GZ-1), the removal rate was 98.1 ± 3.0% and 99.6 ± 0.8%, respectively. Near complete removal of MOR was also observed by Baker and Kasprzyk-Hordern (2013), whereas Postigo et al. (2010) reported a slightly lower removal rate. Removal of COD was also significant, albeit lower than that of MOR. At 18 out of 19 WWTPs, the majority of COD in influent wastewater was removed, with average COD removal ranging from 75.5 ± 14.9 to 100%. The only exception was XM-1, where the apparent removal rate was 51.1 ± 9.1%. The average removal rates of COD observed here were consistent with those reported by Baker and Kasprzyk-Hordern (2013) and were higher than the result of Lin et al. (2010).

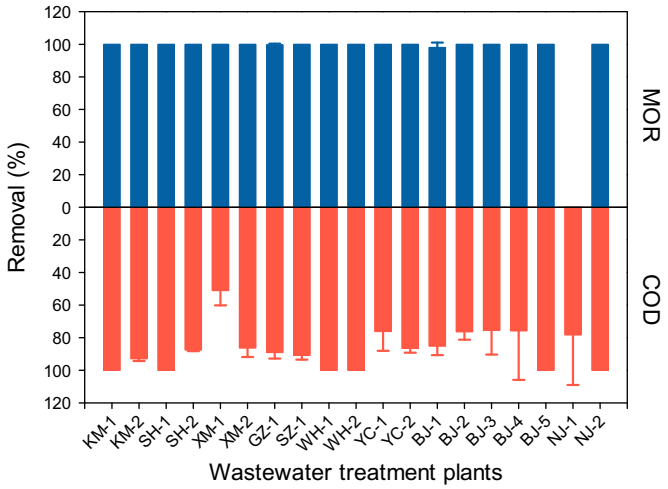


Fig. 2. Apparent removal rates of MOR and COD at 19 WWTPs.

3.3. MOR and COD loads

The loads of MOR derived from back calculation using Eq. (2) ranged from <0.1 mg/1000 inh/d (DL-2, NJ-1 and GY-2) to 123.2 ± 132.6 mg/1000 inh/d (LZ-1) (Fig. 3a, Table S5). LZ-1 is the only WWTPs with a mean load > 100 mg/1000 inh/d. Mean MOR loads were between 50 and 100 mg/1000 inh/d in XA-1, YC-2, TY-1, TY-2, CD-1, KM-1, KM-2, and <50 mg/1000 inh/d in the remaining WWTPs. COD loads ranged from 0.4 to 47.5 ± 6.2 mg/1000 inh/d (Fig. 3b, Table S5). The highest mean COD load was found at SZ-2 (47.5 ± 6.2 mg/1000 inh/d), followed by GZ-1 (36.6 ± 47.9 mg/1000 inh/d). The lowest mean COD loads was found at SuZ-2 (0.4 mg/1000 inh/d), followed by NJ-1 (0.7 ± 0.1 mg/1000 inh/d). Mean COD loads were >20 but <40 mg/1000 inh/d in LZ-1 (35.1 ± 7.6 mg/1000 inh/d), BJ-1 (31.1 ± 1.4 mg/1000 inh/d), GZ-1 (36.6 ± 47.9 mg/1000 inh/d), SZ-1 (33.4 ± 20.9 mg/1000 inh/d), CD-1 (31.7 ± 2.8 mg/1000 inh/d), XA-1 (21.1 ± 5.4 mg/1000 inh/d), YC-2 (21.1 ± 0.3 mg/1000 inh/d) and HRB-2 (20.2 ± 2.4 mg/1000 inh/d). The mean COD loads were <-20 mg/1000 inh/d but >10 mg/1000 inh/d at 10 WWTPs (HRB-1, BJ-2,5, TY-1,2, SH-2, JN-1, CD-2, XC-1, KM-1,2), and <10 mg/1000 inh/d at the remaining WWTPs.

The K-S test indicates that the MOR and COD loads at sampled WWTPs in all the eight geographic regions of China are consistent with a normal or log-normal distribution. One-way ANOVA reveals that MOR and COD loads in wastewater display a clear geographical trend ($P < 0.001$) (Fig. 3a–b). The MOR load in Northwest China (XA, LZ, YC), Southwest China (CD, KM) and North China (BJ, TY) were significantly higher than Northeast China (HRB, DL), Central China (WH, LY), East China (JN, QD, NJ, SuZ, SH), Southeast China (HZ, XM) and South China (NN, GZ, SZ). In general, COD loads in the western inland regions were also higher than those in the eastern coastal regions, a trend similar to that of MOR loads.

The mean MOR load in influent of all 49 WWTPs was 25.2 ± 27.0 mg/1000 inh/d. This value was much lower than the loads reported in most European countries, such as UK, Sweden (Ostman et al., 2014), France (Nefau et al., 2013), lower than those in Spain (Boleda et al., 2009), Croatia (Terzic et al., 2010), (Baker et al., 2014), similar to those of Finland (Vuori et al., 2014), and slightly higher than the level reported in Czech Republic (Baker et al., 2012). The mean MOR load in China was also much lower than those reported in Australia (Lai et al., 2013) and some American cities (Gerrity et al., 2011; Subedi and Kannan, 2014), similar with those reported in Hong Kong and large cities in Canada (Yargeau et al., 2014), and much higher than loads in South Korea (Kim et al., 2015). The mean COD load of China was 11.7 ± 11.4 mg/1000 inh/d. This load is much lower than those reported in most European countries: Finland (Vuori et al., 2014), UK (Baker et al., 2014; Kasprzyk-Hordern et al., 2009), Sweden (Ostman et al., 2014), Czech Republic (Baker et al., 2012), and lower than those reported in Spain (Boleda et al., 2009), and Croatia (Terzic et al., 2010). This load is also much lower than those reported in Canada (Yargeau et al., 2014), Australia (Lai et al., 2011), and higher than loads in Korea (Kim et al., 2015). The fact that loads of MOR and COD observed in this study were much lower than many countries in Europe is understandable, as therapeutic use of MOR and COD in China was much lower than those in European countries. For example, in England in 2010, 3993 kg MOR and 37,117 kg COD were distributed (Baker et al., 2014). The MOR and COD consumed per capita in England (assuming a population of 64 million) were 67 and 771 times higher than those in China in 2014, respectively.

In many European countries, COD loads were much higher than MOR loads (Baker et al., 2014; Ostman et al., 2014; Been et al., 2015; Vuori et al., 2014), likely due to much greater therapeutic use of COD in these countries. In Finland (Vuori et al., 2014) and Sweden (Ostman et al., 2014), strong correlations were observed between COD and MOR loads in wastewater. The observed correlations are expected, as MOR in wastewater in these countries mainly came from COD

Table 1
Measured influent concentrations of MOR, COD, and 6-AM at the 49 WWTPs sampled.

WWTP	MOR ng/L	COD	6-AM	WWTP	MOR ng/L	COD	6-AM
YC-1	25.0 ± 1.3	16.9 ± 3.6	<LOD	NN-1	92.0 ± 26.9	15.6 ± 6.8	<LOD
YC-2	137.7 ± 0.7	52.5 ± 0.7	<LOD	NN-2	70.6 ± 6.5	13.1 ± 1.1	<LOD
XA-1	174.2 ± 73.3	55.4 ± 14.1	<LOD	GZ-1	43.3 ± 9.6	66.0 ± 86.3	<LOD
LZ-1	462.3 ± 497.4	131.8 ± 28.6	4.4 ± 6.2	SZ-1	62.3 ± 22.4	173.9 ± 108.5	<LOD
KM-1	241.6	56.6	<LOD	SZ-2	38.7 ± 6.9	126.4 ± 16.4	<LOD
KM-2	219.7 ± 135.6	56.1 ± 36.0	<LOD	HRB-1	63.9 ± 11.2	39.9 ± 6.3	<LOD
CD-1	210.7 ± 6.7	81.3 ± 7.2	2.0 ± 0.9	HRB-2	40.0 ± 7.4	40.4 ± 4.8	<LOQ
CD-2	81.4 ± 4.5	57.7 ± 14.6	<LOD	DL-1	16.1 ± 5.3	6.2 ± 2.5	<LOD
XC-1	144.7 ± 34.4	66.6 ± 6.2	5.4 ± 0.2	DL-2	<LOD	3.7 ± 2.1	<LOD
GY-1	85.2 ± 109.2	44.0 ± 21.8	<LOD	DL-3	10.4 ± 14.7	7.6 ± 5.6	<LOD
GY-2	<LOD	7.8 ± 6.5	<LOD	QD-1	36.9 ± 15.3	10.9 ± 5.1	<LOD
TY-1	193.1 ± 21.8	64.3 ± 5.7	2.3 ± 1.1	QD-2	75.3 ± 26.5	16.3 ± 4.7	<LOD
TY-2	182.7 ± 21.5	64.3 ± 5.0	10.3 ± 10.5	JN-1	37.9 ± 1.6	14.2 ± 0.7	<LOD
BJ-1	263.5 ± 19.2	147.5 ± 6.5	<LOD	NJ-1	<LOD	2.5 ± 0.3	<LOD
BJ-2	50.0 ± 10.5	53.7 ± 7.1	<LOD	NJ-2	49.6 ± 0.3	10.8 ± 0.5	<LOD
BJ-3	19.8 ± 6.5	14.1 ± 2.9	<LOD	SuZ-1	17.7 ± 3.4	5.4 ± 0.4	<LOD
BJ-4	45.0 ± 11.9	39.7 ± 8.5	<LOD	SuZ-2	20.2 ± 13.9	5.6 ± 2.5	<LOD
BJ-5	51.2 ± 4.2	53.6 ± 6.5	<LOD	SuZ-3	33.1 ± 10.6	10.5 ± 5.7	<LOD
SJZ-1	45.4 ± 22.1	18.0 ± 4.0	<LOD	SH-1	54.4 ± 5.1	26.4 ± 6.4	<LOD
SJZ-2	27.7 ± 0.3	15.1 ± 2.1	<LOD	SH-2	111.5 ± 29.3	40.6 ± 6.5	<LOD
SJZ-3	59.6 ± 10.5	22.8 ± 1.5	<LOD	HZ-1	74.0	24.2	<LOD
LY-1	12.6 ± 10.7	14.0 ± 6.8	<LOD	HZ-2	36.4 ± 3.8	18.1 ± 4.2	<LOD
LY-2	95.2 ± 20.1	17.4 ± 2.9	<LOD	XM-1	86.4 ± 42.4	25.1 ± 11.7	<LOD
WH-1	16.7 ± 8.6	6.7 ± 4.1	<LOD	XM-2	125.9 ± 4.1	34.8 ± 1.7	<LOD
WH-2	33.6 ± 5.3	9.2 ± 0.5	<LOD				

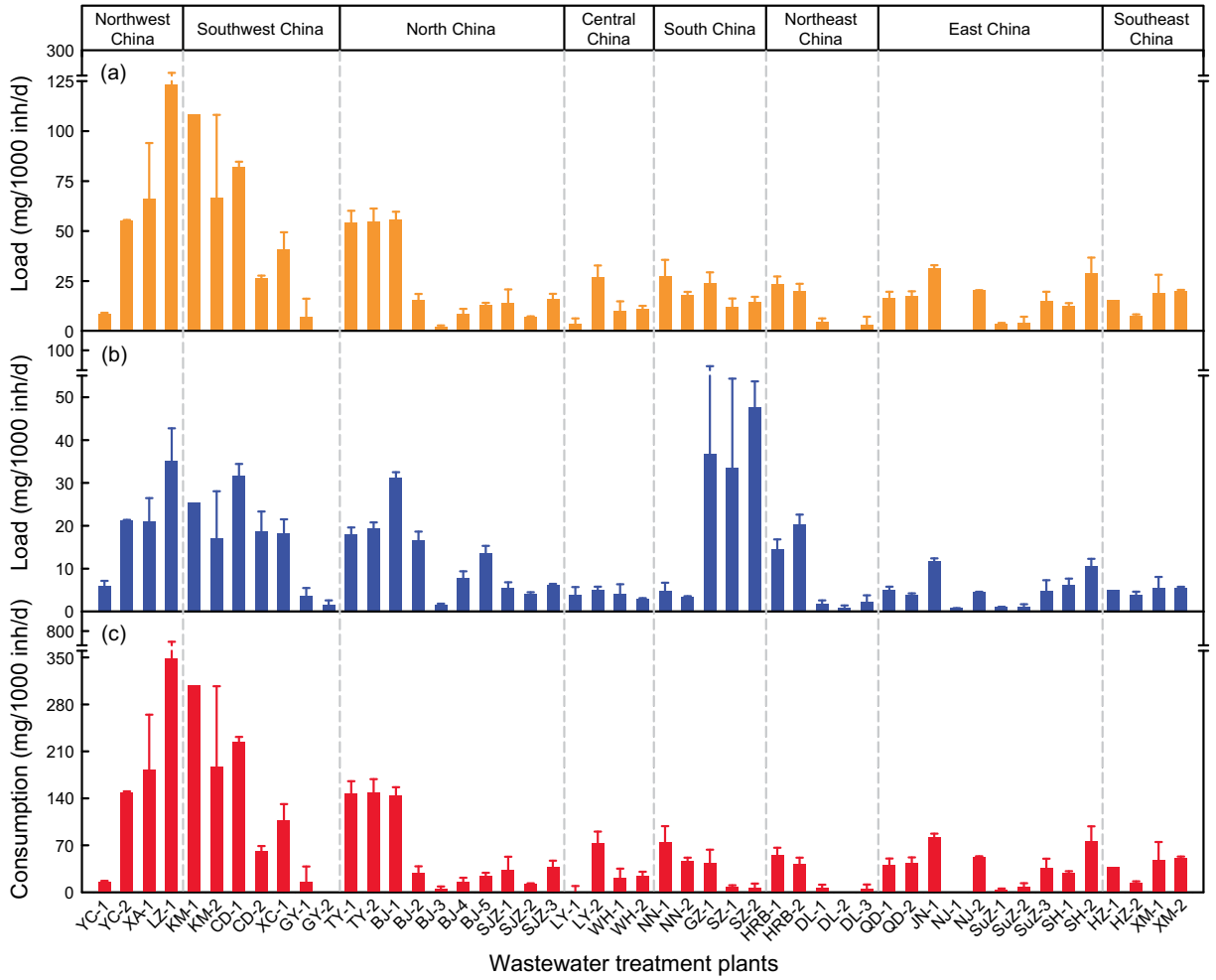


Fig. 3. Influent loads of MOR (a), COD (b), and heroin consumption (c) at the WWTPs.

metabolism. In China, at WWTPs where MOR was detected in influent (i.e., MOR loads > 0), in general MOR and COD loads were also correlated (Fig. 3a–b). However, at most WWTPs, MOR loads were greater than COD loads (Fig. 3a–b, Table S5). Lower COD loads can be explained first by the fact that therapeutic use of COD in China was less than that of MOR. Furthermore, average pure heroin contents (47.5 ± 21.0%) were much higher than those of COD (0.2 ± 0.4%) and acetylcodeine (14.4 ± 11.5%) in street heroin. In addition, excretion rate of heroin into MOR (77.7 ± 9.1%) (Khan and Nicell, 2011) was much higher those of COD and acetylcodeine into COD (~30%).

At BJ-4 and BJ-5, MOR loads were roughly equal to or slightly lower than COD loads. At GZ-1, SZ-1, and SZ-2, COD loads in wastewater were much higher than MOR loads (Fig. 3a-b, Table S5). Equivalent and greater COD loads indicate that there was COD abuse in the population served by these WWTPs. COD abuse (e.g., by taking the so-called "cough syrup") was widespread among school students in Guangdong province. A survey at 18 middle and high schools in seven cities across the province showed that 7.31 to 9.6% of the students had used COD for non-therapeutic purposes (Zhou, 2010). This explains the much higher COD loads in wastewater of Guangzhou and Shenzhen relative to other cities examined.

3.4. Heroin consumption

High uses of therapeutic MOR and COD in many European countries (Baker et al., 2014; Vuori et al., 2014) would lead to high uncertainties, if MOR loads were used to estimate heroin consumption in these countries. In China, much less therapeutic MOR and COD were used. Average MOR loads arising from therapeutic MOR and COD (1.96 and 0.27 mg/1000 inh/d, respectively) were much lower than MOR loads in wastewater at the majority of the WWTPs sampled. Thus, therapeutic MOR and COD uses in China would cause much smaller uncertainties in heroin consumption estimation.

Strong correlation between MOR and COD loads at most WWTP indicates that MOR and COD in the wastewater at these WWTPs had the same origin, street heroin. Hence, contribution of COD and acetylcodeine to MOR loads in wastewater must be corrected (see Eqs. (4)–(6)). Back-calculation at DL-2, GY-2, NJ-1 was not performed, as MOR was below LOD at these WWTPs. Heroin consumption at these WWTPs was set as 0. During back-calculation, correction of MOR loads from sources other than pure heroin metabolism (therapeutic MOR and COD uses, metabolism of COD and acetylcodeine in street heroin) yielded slightly negative heroin consumption on the first sampling day of LY-1 and second day of GY-1. When calculating the mean values at these two WWTPs, heroin consumption on these sampling days was also set as 0.

Heroin consumption in major Chinese cities ranged from 0 (DL-2, GY-2, NJ-1) to 348.8 ± 402.2 mg/1000 inh/d (LZ-1) (Fig. 3c). High heroin consumption was found at LZ-1 (348.8 ± 402.2 mg/1000 inh/d), KM-1 (308.8 mg/1000 inh/d), CD-1 (224.7 ± 6.8 mg/1000 inh/d), KM-2 (187.6 ± 119.5 mg/1000 inh/d), XA-1 (182.9 ± 81.9 mg/1000 inh/d), and YC-2 (149.4 ± 1.1 mg/1000 inh/d) (Fig. 3c). All these WWTPs were located in the southwest (KM-1, 2, CD-1) and northwest (LZ-1, XA-1, YC-2) provinces of the country, indicating overall high use of heroin in the two regions. Moderately high consumption was observed at three WWTPs in north China, namely, TY-1 (147.7 ± 17.7 mg/1000 inh/d), TY-2 (149.1 ± 19.5 mg/1000 inh/d), and BJ-1 (143.8 ± 12.8 mg/1000-inh/d). Lowest consumption was observed in Dalian and Shenzhen, with consumption below 10 mg/1000 inh/d at all WWTPs of these two cities.

The average heroin consumption of the major cities sampled was 64.6 ± 78.7 mg/1000 inh/d. This value is slightly higher than consumption of Lausanne (Switzerland) in 2013–2014 reported by Been et al. (2016), similar to those in Ebro River basin of Spain (Postigo et al., 2010) and Milan (Zuccato et al., 2008), slightly lower than consumption in Lugano of Switzerland (Zuccato et al., 2008), much lower than that in Catalonia of Spain (Boleda et al., 2009), London (Zuccato et al., 2008), Zagreb of Croatia (Terzic et al., 2010), Brussels of Belgium (van Nuijs et al., 2011b). Recent nation-wide survey of heroin consumption via wastewater-based epidemiology was rare in the literature. Zuccato et al. (2016) examined illicit drug consumption in 17 cities across Italy in 2010–2014 via both population survey and wastewater-based epidemiology. Average heroin consumption in the 17 cities in 2011–2013 was equivalent to the average consumption of the major Chinese cities. Average heroin consumption in 7 large cities in 2014 was 90 mg/1000 inh/d, slightly higher than the average consumption in Chinese cities.

The mean methamphetamine load in WWTPs of major Chinese cities sampled in 2014 was 67.8 ± 45.2 mg/1000 inh/d (Du et al., 2015). Assuming an excretion rate of methamphetamine of 42%, average methamphetamine consumption of major Chinese cities was 155.9 mg/1000 inh/d, much higher than the average consumption of heroin. This finding based on wastewater-based epidemiology was consistent with the fact that methamphetamine seizure was much higher than heroin seizure in recent years. For example, according to the 2014 Report of Narcotics Control of China (Office of China National Narcotic Control Committee, 2015b), methamphetamine seizure in 2014 was 25.9 t, whereas heroin seizure was only 9.3 t in the same year. It was also consistent with data of registered abusers in recent years. In 2014, only 19.1% of the 480,000 newly registered abusers used opioids (including heroin); whereas 70.8% of abuser used methamphetamine (Office of China National Narcotic Control Committee, 2015a).

4. Conclusions

This study is the first wastewater-based investigation of heroin abuse in major cities across China. Correlation between COD and MOR loads was evident at most WWTPs, indicating COD and MOR in wastewater at most plants had same origin. In Guangzhou and Shenzhen, COD loads in wastewater were much higher than MOR loads, indicating COD abuse in the cities. Low therapeutic use of MOR and COD allowed back-calculation of heroin consumption based on MOR loads in wastewater. Heroin consumption in western and northwestern cities of the country was much higher than that in other regions. Overall heroin consumption across the country was much lower than that of methamphetamine, consistent with seizure and registered number of abusers in recent years.

Acknowledgements

This work is based on work funded by the National Science Foundation of China (grant no. 41371442 and 41401566). The authors wish to thank all the personnel at the 49 wastewater treatment plants who assisted in wastewater sampling.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.05.262.

References

Andres-Costa, M.J., Rubio-Lopez, N., Morales Suarez-Varela, M., Pico, Y., 2014. Occurrence and removal of drugs of abuse in wastewater treatment plants of Valencia (Spain). Environ. Pollut. 194, 152–162.

Baker, D.R., Kasprzyk-Hordern, B., 2013. Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: new developments. Sci. Total Environ. 454–455, 442–456.

Baker, D.R., Ocenaskova, V., Kvicalova, M., Kasprzyk-Hordern, B., 2012. Drugs of abuse in wastewater and suspended particulate matter—further developments in sewage epidemiology. Environ. Int. 48, 28–38.

Baker, D.R., Barron, L., Kasprzyk-Hordern, B., 2014. Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A: chemical analysis and drug use estimates. Sci. Total Environ. 487, 629–641.

Baselt, R.C., 2004. Disposition of Toxic Drugs and Chemicals in Man. seventh edition. Biomedical Publications, California.

Been, F., Benaglia, L., Lucia, S., Gervasoni, J.P., Esseiva, P., Delemont, O., 2015. Data triangulation in the context of opioids monitoring via wastewater analyses. Drug Alcohol Depend. 151, 203–210.

Been, F., Bijlsma, L., Benaglia, L., Berset, J.D., Boterocoy, A.M., Castiglioni, S., et al., 2016. Assessing geographical differences in illicit drug consumption-A comparison of results from epidemiological and wastewater data in Germany and Switzerland. Drug Alcohol Depend. 163, 272.

Bijlsma, L., Beltran, E., Boix, C., Sancho, J.V., Hernandez, F., 2014. Improvements in analytical methodology for the determination of frequently consumed illicit drugs in urban wastewater. Anal. Bioanal. Chem. 406, 4261–4272.

Boleda, M.A., Galceran, M.A., Ventura, F., 2009. Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. Water Res. 43, 1126–1136.

Brunk, S.F., Delle, M., 1974. Morphine metabolism in man. Clin. Pharmacol. Ther. 16, 51–57.

de Castro, A., Lendoiro, E., Fernandez-Vega, H., Steinmeyer, S., Lopez-Rivadulla, M., Cruz, A., 2014. Liquid chromatography tandem mass spectrometry determination of selected synthetic cathinones and two piperazines in oral fluid. Cross reactivity study with an on-site immunoassay device. J. Chromatogr. A 1374, 93–101.

Damien, D.A., Thomas, N., Helene, P., Sara, K., Yves, L., 2014. First evaluation of illicit and licit drug consumption based on wastewater analysis in Fort de France urban area (Martinique, Caribbean), a transit area for drug smuggling. Sci. Total Environ. 490, 970–978.

D'Ascenzo, G., Di, C.A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., et al., 2003. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci. Total Environ. 302, 199–209.

Du, P., Li, K., Li, J., Xu, Z., Fu, X., Yang, J., et al., 2015. Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology. Water Res. 84, 76–84.

van Dyken, E., Lai, F.Y., Thai, P.K., Ort, C., Bruno, R., Hall, W., et al., 2016. Challenges and opportunities in using wastewater analysis to measure drug use in a small prison facility. Drug Alcohol Rev. 35, 138–147.

Elliott, H.W., Parke, K.D., Wright, J.A., Nomof, N., 1971. Actions and metabolism of heroin administered by continuous intravenous infusion to man. Clin. Pharmacol. Ther. 12, 806–814.

Gao, T., Du, P., Xu, Z., Li, X., 2017. Occurrence of new psychoactive substances in wastewater of major Chinese cities. Sci. Total Environ. 575, 963–969.

Gerrity, D., Trenholm, R.A., Snyder, S.A., 2011. Temporal variability of pharmaceuticals and illicit drugs in wastewater and the effects of a major sporting event. Water Res. 45, 5399–5411.

Guerra, P., Kim, M., Shah, A., Alaee, M., Smyth, S.A., 2014. Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. Sci. Total Environ. 473–474, 235–243.

Jiang, J.J., Lee, C.L., Fang, M.D., Tu, B.W., Liang, Y.J., 2015. Impacts of emerging contaminants on surrounding aquatic environment from a youth festival. Environ. Sci. Technol. 49, 792–799.

Kankaanpaa, A., Ariniemi, K., Heinonen, M., Kuoppasalmi, K., Gunnar, T., 2014. Use of illicit stimulant drugs in Finland: a wastewater study in ten major cities. Sci. Total Environ. 487, 696–702.

Karolak, S., Nefau, T., Bailly, E., Solgadi, A., Levi, Y., 2010. Estimation of illicit drugs consumption by wastewater analysis in Paris area (France). Forensic Sci. Int. 200, 153–160.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43, 363–380.

Khan, U., Nicell, J.A., 2011. Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. Environ. Int. 37, 1236–1252.

Khan, U., van Nuijs, A.L., Li, J., Maho, W., Du, P., Li, K., et al., 2014. Application of a sewage-based approach to assess the use of ten illicit drugs in four Chinese megacities. Sci. Total Environ. 487, 710–721.

Kim, K.Y., Lai, F.Y., Kim, H.Y., Thai, P.K., Mueller, J.F., Oh, J.E., 2015. The first application of wastewater-based drug epidemiology in five South Korean cities. Sci. Total Environ. 524–525, 440–446.

Lai, F.Y., Ort, C., Gartner, C., Carter, S., Prichard, J., Kirkbride, P., et al., 2011. Refining the estimation of illicit drug consumptions from wastewater analysis: co-analysis of prescription pharmaceuticals and uncertainty assessment. Water Res. 45, 4437–4448.

Lai, F.Y., Thai, P.K., O'Brien, J., Gartner, C., Bruno, R., Kele, B., et al., 2013. Using quantitative wastewater analysis to measure daily usage of conventional and emerging illicit drugs at an annual music festival. Drug Alcohol Rev. 32, 594–602.

Li, J., Hou, L., Du, P., Yang, J., Li, K., Xu, Z., et al., 2014. Estimation of amphetamine and methamphetamine uses in Beijing through sewage-based analysis. Sci. Total Environ. 490, 724–732.

Li, K., Du, P., Xu, Z., Gao, T., Li, X., 2016. Occurrence of illicit drugs in surface waters in China. Environ. Pollut. 213, 395–402.

Lin, A.Y., Wang, X.H., Lin, C.F., 2010. Impact of wastewaters and hospital effluents on the occurrence of controlled substances in surface waters. Chemosphere 81, 562–570.

Mackulak, T., Skubak, J., Grabic, R., Ryba, J., Birosova, L., Fedorova, G., Spalkova, V., Bodik, I., 2014. National study of illicit drug use in Slovakia based on wastewater analysis. Sci. Total Environ. 494–495, 158–165.

Mackulak, T., Mosny, M., Skubak, J., Grabic, R., Birosova, L., 2015. Fate of psychoactive compounds in wastewater treatment plant and the possibility of their degradation using aquatic plants. Environ. Toxicol. Pharmacol. 39, 969–973.

Mackulak, T., Birosova, L., Gal, M., Bodik, I., Grabic, R., Ryba, J., et al., 2016. Wastewater analysis: the mean of the monitoring of frequently prescribed pharmaceuticals in Slovakia. Environ. Monit. Assess. 188, 18.

McCall, A.K., Bade, R., Kinyua, J., Lai, F.Y., Thai, P.K., Covaci, A., et al., 2016. Critical review on the stability of illicit drugs in sewers and wastewater samples. Water Res. 88, 933–947.

Mikus, G., Bochner, F., Eichelbaum, M., Horak, P., Somogyi, A.A., Spector, S., 1994. Endogenous codeine and morphine in poor and extensive metabolisers of the CYP2D6 (debrisoquine/sparteine) polymorphism. J. Pharmacol. Exp. Ther. 268, 546–551.

Ministry of Industry and Information Technology, 2014. Chinese Medicine Statistics Year Book of 2013. China Press of Traditional Chinese Medicine, Beijing.

Ministry of Industry and Information Technology, 2015. Chinese Medicine Statistics Year Book of 2014. China Press of Traditional Chinese Medicine, Beijing.

Nefau, T., Karolak, S., Castillo, L., Boireau, V., Levi, Y., 2013. Presence of illicit drugs and metabolites in influents and effluents of 25 sewage water treatment plants and map of drug consumption in France. Sci. Total Environ. 461–462, 712–722.

van Nuijs, A.L., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., et al., 2009. Cocaine and metabolites in waste and surface water across Belgium. Environ. Pollut. 157, 123–129.

van Nuijs, A.L., Mougel, J.F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., et al., 2011a. A one year investigation of the occurrence of illicit drugs in wastewater from Brussels, Belgium. J. Environ. Monit. 13, 1008–1016.

van Nuijs, A.L., Mougel, J.F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., et al., 2011b. Sewage epidemiology—a real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. Environ. Int. 37, 612–621.

Office of China National Narcotic Control Committee, 2001. 2000 Report of Narcotics Control of China, Beijing.

Office of China National Narcotic Control Committee, 2006. 2005 Report of Narcotics Control of China, Beijing.

Office of China National Narcotic Control Committee, 2008. 2007 Report of Narcotics Control of China, Beijing.

Office of China National Narcotic Control Committee, 2015a. 2014 Report of Drug Situation of China, Beijing.

Office of China National Narcotic Control Committee, 2015b. 2014 Report of Narcotics Control of China, Beijing.

Office of China National Narcotic Control Committee, 2016a. 2015 Report of Drug Situation of China, Beijing.

Office of China National Narcotic Control Committee, 2016b. 2015 Report of Narcotics Control of China, Beijing.

Ort, C., Lawrence, M.G., Rieckermann, J., Joess, A., 2010. Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review. Environ. Sci. Technol. 44, 6024–6035.

Ostman, M., Fick, J., Nasstrom, E., Lindberg, R.H., 2014. A snapshot of illicit drug use in Sweden acquired through sewage water analysis. Sci. Total Environ. 472, 862–871.

Postigo, C., Lopez de Alda, M.J., Barcelo, D., 2010. Drugs of abuse and their metabolites in the Ebro River basin: occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. Environ. Int. 36, 75–84.

Repice, C., Dal Grande, M., Maggi, R., Pedrazzani, R., 2013. Licit and illicit drugs in a wastewater treatment plant in Verona, Italy. Sci. Total Environ. 463–464, 27–34.

Senta, I., Gracia-Lor, E., Borsotti, A., Zuccato, E., Castiglioni, S., 2015. Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment. Water Res. 74, 23–33.

Smith, M.L., Shimomura, E.T., Summers, J., Paul, B.D., Jenkins, A.J., Darwin, W.D., et al., 2001. Urinary excretion profiles for total morphine, free morphine, and 6-acetylmorphine following smoked and intravenous heroin. J. Anal. Toxicol. 25, 504–514.

Staub, C., Marset, M., Mino, A., Mangin, P., 2001. Detection of acetylcodeine in urine as an indicator of illicit heroin use: method validation and results of a pilot study. Clin. Chem. 47, 301–307.

Subedi, B., Kannan, K., 2014. Mass loading and removal of select illicit drugs in two wastewater treatment plants in New York State and estimation of illicit drug usage in communities through wastewater analysis. Environ. Sci. Technol. 48, 6661–6670.

Terzic, S., Senta, I., Ahel, M., 2010. Illicit drugs in wastewater of the city of Zagreb (Croatia)—estimation of drug abuse in a transition country. Environ. Pollut. 158, 2686–2693.

Thai, P.K., Lai, F.Y., Bruno, R., van Dyken, E., Hall, W., O'Brien, J., et al., 2016. Refining the excretion factors of methadone and codeine for wastewater analysis—combining data from pharmacokinetic and wastewater studies. Environ. Int. 94, 307–314.

Vuori, E., Happonen, M., Gergov, M., Nenonen, T., Jarvinen, A., Ketola, R.A., et al., 2014. Wastewater analysis reveals regional variability in exposure to abused drugs and opioids in Finland. Sci. Total Environ. 487, 688–695.

Yargeau, V., Taylor, B., Li, H., Rodayan, A., Metcalfe, C.D., 2014. Analysis of drugs of abuse in wastewater from two Canadian cities. Sci. Total Environ. 487, 722–730.

Zhou, X., 2010. Investigation on the Abuse of "Cough Syrup" with Codeine Phosphate among Middle School Students in Guangdong Province. (Master). Sun Yat-sen University, Guangzhou.

Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., et al., 2005. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. Environ. Health 4, 14.

Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., Fanelli, R., 2008. Estimating community drug abuse by wastewater analysis. Environ. Health Perspect. 116, 1027–1032.

Zuccato, E., Castiglioni, S., Tettamanti, M., Olandese, R., Bagnati, R., Melis, M., et al., 2011. Changes in illicit drug consumption patterns in 2009 detected by wastewater analysis. Drug Alcohol Depend. 118, 464–469.

Zuccato, E., Castiglioni, S., Senta, I., Borsotti, A., Genetti, B., Andreotti, A., et al., 2016. Population surveys compared with wastewater analysis for monitoring illicit drug consumption in Italy in 2010–2014. Drug Alcohol Depend. 161, 178–188.

Drug and Alcohol Dependence 205 (2019) 107532

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Full length article

Monitoring consumption of methadone and heroin in major Chinese cities by wastewater-based epidemiology

Peng Du[a,b], Phong K. Thai[c], Ya Bai[b], Zilei Zhou[b], Zeqiong Xu[b], Xuan Zhang[a], Jing Wang[d], Congbin Zhang[d], Fanghua Hao[a], Xiqing Li[b,*]

[a] Beijing Key Laboratory of Urban Hydrological Cycle and Sponge City Technology, College of Water Sciences, Beijing Normal University, Beijing 100875, China
[b] Laboratory of Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China
[c] Queensland Alliance for Environmental Health Science (QAEHS), The University of Queensland, Brisbane, Queensland 4102, Australia
[d] Yunnan Institute for Drug Abuse, Kunming 650228, China

ARTICLE INFO

Keywords:
Heroin
Codeine
Morphine
Drug consumption
Wastewater-based epidemiology
Methadone maintenance services

ABSTRACT

Background: Methadone maintenance treatment (MMT) services have been used in China for treatment of heroin dependence. But no study has been conducted to assess the appropriateness of MMT distribution and the potential abuse of methadone in China. This study aims to do that through a nationwide estimation of methadone consumption in China via wastewater-based epidemiology and subsequently compare it with MMT data and level of heroin abuse.

Methods: Wastewater samples were collected from 53 wastewater treatment plants in 27 major cities that cover all geographic regions of China. Methadone and pure heroin consumptions were estimated based on influent concentrations of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), morphine and codeine.

Results: Drug residues were detected in most samples. The ratio of EDDP/methadone was around 2 in influents and methadone and EDDP loads were strongly correlated, indicating that they originated from human consumption. Both influent methadone and EDDP loads in Southwest and Northwest China were significantly higher than those in other regions. The highest estimated consumptions of methadone and heroin in China were 22.0 ± 2.1 mg/1000 in./d and 263.9 ± 115.9 mg/1000 in./d, respectively. There was a significant positive correlation between methadone and heroin consumptions.

Conclusions: Consumption of methadone in China was primarily from MMT services. The use of methadone and heroin displayed a clear geographical pattern: it is higher in the western inland regions than in the eastern regions. This study has shown that the distribution of MMT services is reflective of the level of heroin abuse in different regions of China.

1. Introduction

Methadone is a synthetic, long acting, potent μ-opioid agonist (Monnelly et al., 2018). It is widely used for maintenance treatment of opioid dependency, particularly for heroin. In China, heroin is one of the most prevalent illicit drugs, with 0.955 million registered abusers in 2016 (OCNNCC, 2017). In order to treat people for heroin dependence in China, methadone clinics were established by the Chinese government in 2004 to provide methadone maintenance treatment services (Li et al., 2013). As a pilot work, there were only eight methadone clinics in five provinces of the country at the beginning. Up to 2006, more than one hundred methadone clinics were established, marking methadone maintenance treatment as a routine medical service in China. The consumption of methadone has increased rapidly over the past decade. Thus, understanding the relationship between the level of methadone use and heroin abuse across China as well as the geographical variation will provide more insights on the effectiveness of drug control and methadone maintenance treatment in the country.

Over the past decade, wastewater-based epidemiology (WBE) has become an alternative method to estimate community drug consumption. WBE studies have been successfully applied to estimate common illicit drug use such as cocaine, methamphetamine, amphetamine within and/or across countries and regions. Some of the results of the studies have been adopted by authorities as evidence for policy makers

* Corresponding author.
E-mail address: xli@urban.pku.edu.cn (X. Li).

https://doi.org/10.1016/j.drugalcdep.2019.06.034
Received 6 March 2019; Received in revised form 21 June 2019; Accepted 28 June 2019
Available online 22 October 2019
0376-8716/

to formulate the drug control strategies (EMCDDA, 2016; ACIC, 2018). However, other addictive drugs such as methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) have not been paid enough attention although they were sometimes included in the multi-residue analysis methods.

WBE has also been frequently applied in China. Lai et al. (2013) performed the first WBE study in Hong Kong for a range of illicit drugs. Khan et al. (2014) and Li et al. (2014) applied the approach to estimate illicit drug use in four megacities (Beijing, Shanghai, Guangzhou and Shenzhen) of mainland China. In recent years, nationwide reconnaissance through WBE has been implemented to estimate methamphetamine, ketamine, heroin and new psychoactive substances use in China (Du et al., 2015, 2017; Xu et al., 2017; Gao et al., 2017). However, methadone has not yet been included in the above WBE campaigns.

The aim of this study was to obtain, for the first time, a snapshot picture of the level of methadone and heroin in major cities across China through WBE. Concentrations of methadone, its metabolite EDDP, morphine and codeine were measured in wastewater samples to estimate the mass loads and the drug consumption in the catchments. Geographical patterns of methadone and heroin use were examined by WBE data and the estimate through methadone maintenance treatments across China.

2. Methods

2.1. Sample collection

Wastewater samples were collected from 27 major Chinese cities distributed in all seven geographic regions of China (Fig. 1). Twenty-five of the above cities are provincial capitals or equivalent to provincial capitals in terms of economic development and population sizes. Beijing and Shanghai are municipalities directly under the central government (Table S1). In total, 53 wastewater treatment plants participated in the sampling campaigns and there were two or more wastewater treatment plants participated in most sampled cities. The total population served by these wastewater treatment plants is 43 million (~3.1% of the population of China). For wastewater treatment plants from the same city, a numerical was added to the city, for example, Beijing-3 for the third wastewater treatment plant of Beijing and Qingdao-1 for the first wastewater treatment plant of Qingdao.

Due to limited resources, the sampling campaign was conducted from July to December of 2016 in most cities as described previously (Du et al., 2018) except that samples from Kunming were collected in May of 2017. Each wastewater treatment plant was sampled for three or four days, except for Changchun-1, Dalian-1, Dalian-2, Lhasa-1 where only one sample was collected (Table S1).

For influent samples, 24-h composite sample was collected using auto-sampler at the rate of 100 mL/h into a cooled sampling container. Auto-samplers (ISCO 3700/6712, Teledyne Technologies Inc., Lincoln, NE, USA; FC-9624, GRASP Science & Technology Co., LTD, Beijing; Sigma-SD900, HACH Inc., USA) were employed to collect time-proportional composite samples. After collection, the composite samples were adjusted to pH = 2 with hydrochloric and frozen immediately at −20°C at each wastewater treatment plant and were shipped back to the laboratory in frozen state. Analysis was performed as soon as the samples were received at the laboratory. Details of sample collections and information of each wastewater treatment plant (flow rates and served inhabitants) are provided in Table S1.

2.2. Analysis

Standard solutions of methadone, EDDP, morphine, codeine and corresponding deuterated analogs (methadone-d9, EDDP-d9, morphine-

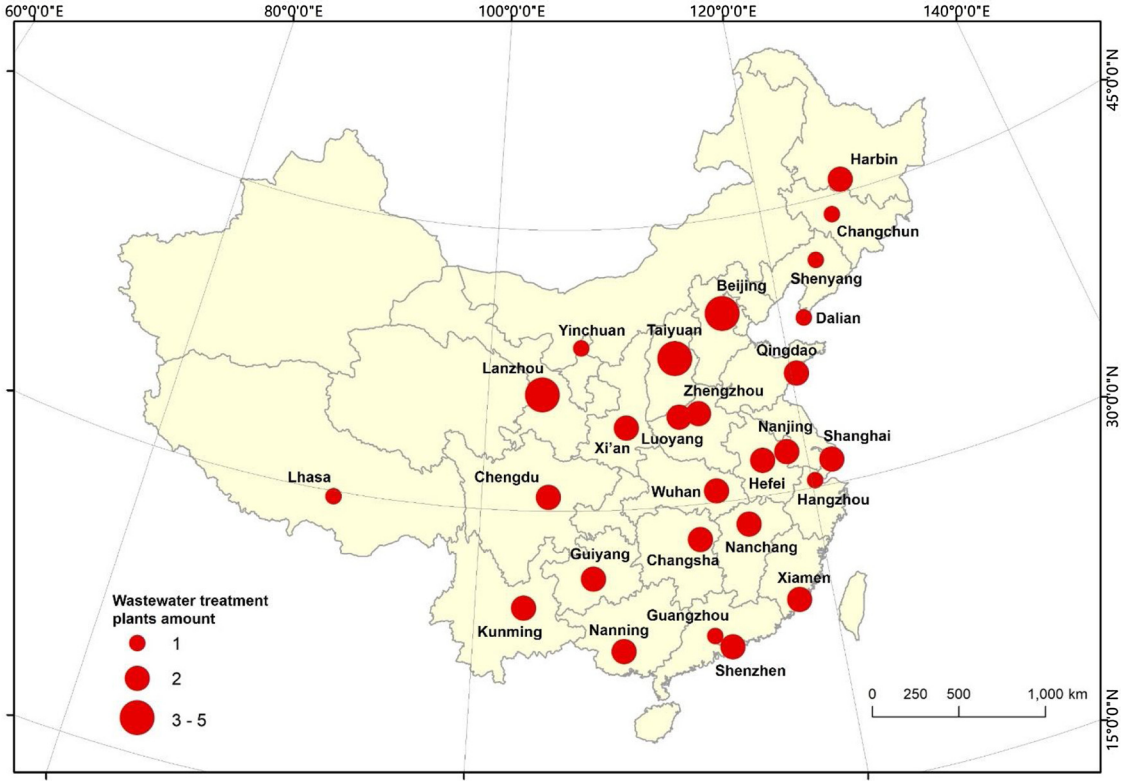


Fig. 1. Map of selected cities where wastewater samples were collected. The sizes of the circles represent the amount of sampled wastewater treatment plants. Xi'an, Lanzhou, and Yinchuan of Northwest China; Beijing and Taiyuan of North China; Shenyang, Changchun, Harbin and Dalian of Northeast China; Changsha and Wuhan, Zhengzhou and Luoyang of Central China; Shanghai, Nanjing, Hangzhou, Qingdao, Xiamen, Nanchang and Hefei of East China; Guangzhou, Shenzhen and Nanning of South China; Kunming, Chengdu, Guiyang and Lhasa of Southwest China.

Table 1
Statistics of methadone, EDDP, morphine and codeine concentrations in wastewater across China.

Chemicals	n	DF[a] (%)	Min ng/L	Median	Max	Mean ± STD
Methadone	208	82	< MDL	2.2	49.9	4.6 ± 6.8
EDDP	208	91	< MDL	4.3	108.0	10.5 ± 15.5
Morphine	208	84	< MDL	41.6	1168.0	86.4 ± 121.6
Codeine	208	99	< MDL	28.4	238.4	42.4 ± 37.9

[a] DF = Detection frequency.

d3 and codeine-d6) to be used as internal standards were all purchased from Cerilliant (Round Rock, TX, USA). Other reagents and materials can be found in Supplementary Material (Text S1).

Sample pretreatment and analysis followed the procedure described in our previous publication (Du et al., 2017) with minor modifications. The analytical method was subjected to strict quality control and quality assurance measures. The method detection limit (MDL), method quantification limit (MQL), recoveries and matrix effects were examined according to previous publication (Du et al., 2018) (Table S3). Details were described in the Supplementary Material (Text S1).

2.3. Mass load and consumption estimation

The daily mass load of each target compound per 1000 in.abitants (mg/1000 in./d) at a specific wastewater treatment plant was estimated by Eq. (1). Where C_i is the influent concentration of the target compound, F_{In} is the influent flow of the specific wastewater treatment plant (obtained from the staff in each respective plant), PS is the population served by the wastewater treatment plant (based on the census data of the wastewater treatment plant service areas).

$$Load = \frac{C_i(ng/L) \times F_{In}(L/d)}{\frac{PS}{1000}} \times \frac{1}{10^6}\left(\frac{mg}{ng}\right) \quad (1)$$

The consumption (mg/1000 in./d) of methadone was estimated by the following equation:

$$Consumption_{MTD} = Load_{MTD} \times \frac{MW_{MTD}}{MW_{EDDP}} \times \frac{1}{EF_{MTD}} \quad (2)$$

$Load_{MTD}$ is daily mass load of methadone at a specific wastewater treatment plant, MW_{MTD} is the molecular weight of methadone, MW_{EDDP} is the molecular weight of EDDP, and EF_{MTD} is excretion fraction of a given dose of methadone excreted as main metabolite (EDDP) through urine.

The consumption (mg/1000 in./d) of pure heroin was estimated based on the influent concentrations of both morphine and codeine using the procedure described in a previous paper (Du et al., 2017). Detailed description can be found in Text S2.

Uncertainties involved in the above estimation process have been discussed in previous studies (Li et al., 2014; Du et al., 2017).

2.4. Consumption in methadone maintenance treatment

Data of methadone consumption in methadone maintenance treatment were collected from the cities Kunming and Shenzhen. The data were supplied by Workgroup of Kunming Methadone Maintenance Treatment and Clean Syringe Exchange and Workgroup of Guangdong Detoxification Medicine Maintenance Treatment.

2.5. Statistical analysis

Normality was examined through the Kolmogorov-Smirnov (K-S) test before other analyses. Pearson correlation analysis was used to assess the correlation between methadone and EDDP loads, morphine and codeine loads, methadone and heroin consumptions. The significance of geographical variation of mass loads and consumptions were assessed via one-way ANOVA. Student-t test was employed for comparing the differences of consumptions in weekdays and weekends, and differences between estimated methadone consumptions and therapeutic methadone. All statistical analyses were performed using SPSS 20 (IBM Co., USA), with a p-value below 0.05 meaning the difference is statistically significant.

3. Results

3.1. Influents concentrations of biomarkers

Concentrations of methadone and EDDP in influent samples ranged from < MDL to 49.9 ng/L (mean ± STD, 4.6 ± 6.8 ng/L) and < MDL to 108.0 ng/L (mean ± STD, 10.5 ± 15.5 ng/L), with detection frequencies of 82% and 91%, respectively (Table 1). There were 7 wastewater treatment plants with the mean EDDP concentrations > 20 ng/L (Lanzhou-2, Lanzhou-3, Lanzhou-4, Xi'an-1, Kunming-1, Kunming-2 and Guiyang-1) indicating a high level of methadone consumption in the catchment (Table S4). The wastewater treatment plant with highest mean methadone and EDDP concentrations is Lanzhou-2 (32.7 ± 17.5 ng/L and 63.7 ± 38.8 ng/L) while methadone and EDDP were not detected in the influents of Harbin-1, Harbin-2, Changchun-1, Shenyang-1, Dalian-1 and Lhasa-1 (Table S4). Mean ratio of EDDP/methadone was 2.24 ± 0.86 in influent samples. The detection frequencies of morphine and codeine were 84% and 99% in influent samples, with concentrations ranging from 41.6 to 1168.0 ng/L (mean ± STD, 86.4 ± 121.6 ng/L) and 28.4 to 238.4 ng/L (mean ± STD, 42.4 ± 37.9 ng/L), respectively (Table 1). There was only one wastewater treatment plant (Lhasa-1) with both the morphine and codeine concentrations below MDL in influents (Table S4). The highest mean morphine and codeine concentrations were also found at Lanzhou-2 (528.6 ± 448.8 ng/L and 141.5 ± 77.7 ng/L). Mean ratio of morphine/codeine was 2.34 ± 1.86 in influent samples, and it was much higher than that in European countries (Östman et al., 2014; Vuori et al., 2014).

3.2. Daily loads of methadone, EDDP, morphine and codeine

The daily per capita loads of methadone and EDDP (major human metabolite of methadone) at sampled wastewater treatment plants varied from wastewater treatment plant to wastewater treatment plant, ranging from < 0.1 to 8.9 ± 4.8 mg/1000 in./d for methadone and < 0.1 to 17.4 ± 10.6 mg/1000 in./d for EDDP (Fig. 2, Table S5). The highest mean methadone load was found at Lanzhou-2 (8.9 ± 4.8 mg/1000 in./d), followed by Lanzhou-4 (5.1 ± 1.6 mg/1000 in./d). Mean methadone loads in the others wastewater treatment plants were below 5 mg/inh/d. The highest mean EDDP load was also found at Lanzhou-2 (17.4 ± 10.6 mg/1000 in./d), followed by Xi'an-1 (13.5 ± 4.1 mg/1000 in./d) and Kunming-2 (11.7 ± 3.6 mg/1000 in./d). Statistically significant positive correlation was found between influent methadone and EDDP loads ($p < 0.05$) (Fig. 2).

The average influent loads of methadone and EDDP were 1.0 ± 1.3 mg/1000 in./d and 2.5 ± 3.1 mg/1000 in./d (population-weighted average ± STD) in all 53 wastewater treatment plants. These loads were much lower (nearly one or two order of magnitude) than the loads reported in wastewater from other countries, such as Croatia (Krizman-Matasic et al., 2019), UK (Baker and Kasprzyk-Hordern, 2011), Italy (Castiglioni et al., 2006; Cosenza et al., 2018), France (Nefau et al., 2013), Switzerland (Berset et al., 2010), Spain (Martínez Bueno et al., 2011), Australia (Bade et al., 2018; Thai et al., 2016) and the USA (Foppe et al., 2018; Skees et al., 2018). The values were similar with those in Slovakia (Mackul'ak et al., 2016), Belgium (van Nuijs et al., 2011) and Sweden (Östman et al., 2014).

For morphine and codeine, the mean loads in influents at sampled wastewater treatment plants were ranging from < 0.2 mg/1000 in./d to

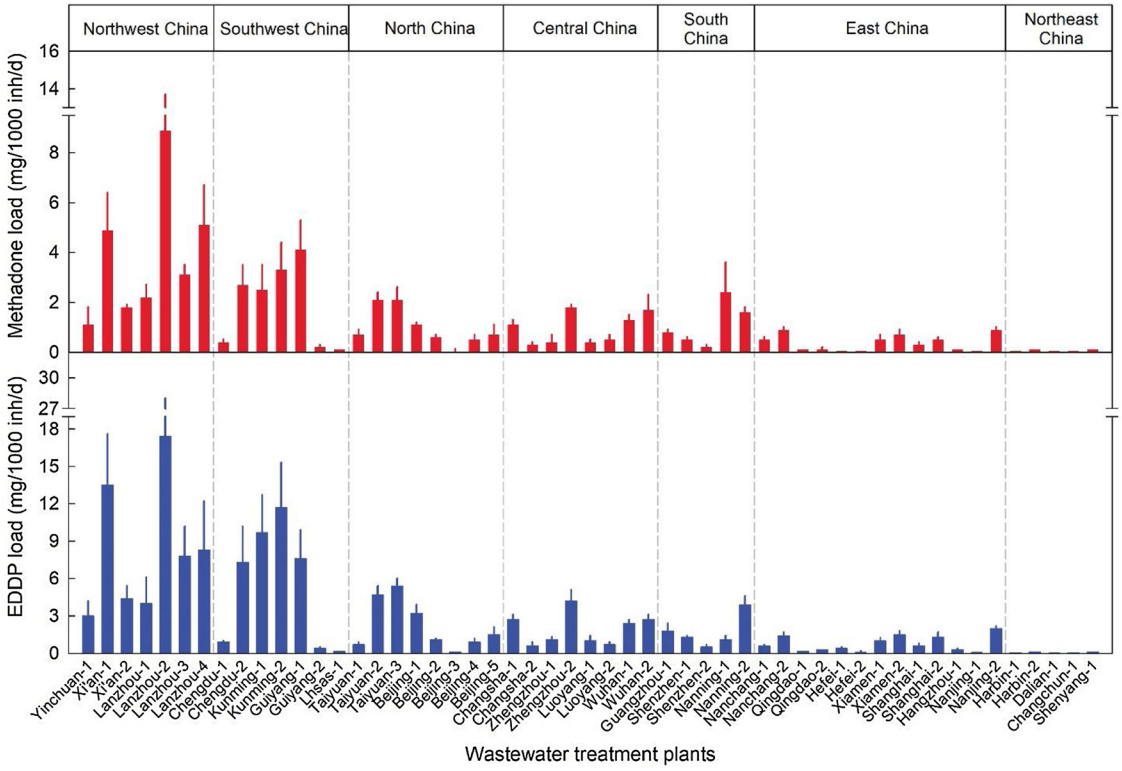


Fig. 2. Influent mean loads of methadone and EDDP at the wastewater treatment plants across China.

144.4 ± 122.6 mg/1000 in./d and < 0.2 mg/1000 in./d to 41.7 ± 4.8 mg/1000 in./d, respectively (Fig. S2, Table S5). The highest mean loads of morphine and codeine occurred in Lanzhou-2 (144.4 ± 122.6 mg/1000 in./d) and Nanchang-2 (41.7 ± 4.8 mg/1000 in./d), respectively. In most wastewater treatment plants, morphine loads were greater than that of codeine. The average influent loads of morphine and codeine were 23.5 ± 28.5 mg/inh/d and 12.7 ± 10.3 mg/inh/d (population-weighted average ± STD) across the country. These loads were lower than many countries such as UK (Baker et al., 2014), Sweden (Östman et al., 2014), Spain (Boleda et al., 2009), Australia (Lai et al., 2013), Canada (Yargeau et al., 2014), etc. There was a significantly positive correlation between morphine and codeine loads, which is consistent with our previous study (Du et al., 2017). There were no significant differences between the average morphine and codeine loads and the values in our previous study in 2014 and 2015 (Du et al., 2017) ($p <$ 0.05). Minor variation of morphine and codeine loads compared to data of 2014–2015 (Du et al., 2017) indicates a relatively stable level of heroin consumption in recent years in China. This finding was consistent with the minor variation of heroin seizures in 2014 (9.3 t) and 2016 (8.8 t) (OCNNCC, 2015, 2017).

3.3. Consumptions of methadone and heroin

We used the major human metabolite of methadone to estimate the methadone consumption. The excretion rate of EDDP (55%) was applied (Thai et al., 2016). Average methadone consumption in major Chinese cities ranged from < 0.2 mg/1000 in./d to 22.0 ± 2.1 mg/1000 in./d (population-weighted average ± STD) (Fig. 3, Table S6). There were 5 cities with the average methadone consumption above 10 mg/1000 in./d: Kunming (22.0 ± 2.1 mg/1000 in./d), Lanzhou (17.3 ± 9.6 mg/1000 in./d), Xi'an (14.1 ± 8.3 mg/1000 in./d), Guiyang (12.8 ± 5.6 mg/1000 in./d) and Chengdu (11.2 ± 5.9 mg/1000 in./d). All these cities were located in the Southwest and Northwest China, indicating overall high use of methadone in these two regions. The average consumptions in Yinchuan, Taiyuan, and Nanning were between 5 to 7 mg/1000 in./d, and the other cities with consumptions below 5 mg/ 1000 in./d.

As a major metabolite of heroin, morphine was used to estimate its consumption. However, as showed in Eq. S1 to S4 in the Supplementary Material, we had to subtract the morphine measured in wastewater by the amount of morphine originated from prescription of morphine and codeine as well as from heroin product (as impurities). As such the heroin consumption was set as zero when negative estimates were obtained. The population-weighted average consumption of pure heroin ranged from 0 (Changchun, Nanjing and Lhasa) to 263.9 ± 115.9 mg/1000 in./d (Chengdu) in major cities of China (Fig. 3, Table S6). Chengdu had the highest consumption, followed by Lanzhou (241.1 ± 85.7 mg/1000 in./d). In total, there were 3 cities (Kunming, Guiyang and Xi'an) with consumptions between 100 mg/1000 in./d to 200 mg/1000 in./d, and the consumptions were below 100 mg/1000 in./d in the others.

4. Discussion

4.1. Sources of biomarkers in influents

The ratio of EDDP/methadone measured across wastewater treatment plants is relatively stable around 2, which is similar to that reported by Thai et al. (2016, 2019). This ratio indicated that those drug residues in wastewater samples highly likely originated from human consumption. Significant correlation between influent methadone and EDDP loads again confirmed that methadone and EDDP found in wastewater of cities in China are from human metabolism of methadone rather than direct disposal (Fig. 2). The high ratio of morphine/codeine in across wastewater treatment plants (Fig. S1) also confirm that morphine found in wastewater was predominantly from street heroin use, as reported in our previous study (Du et al., 2017).

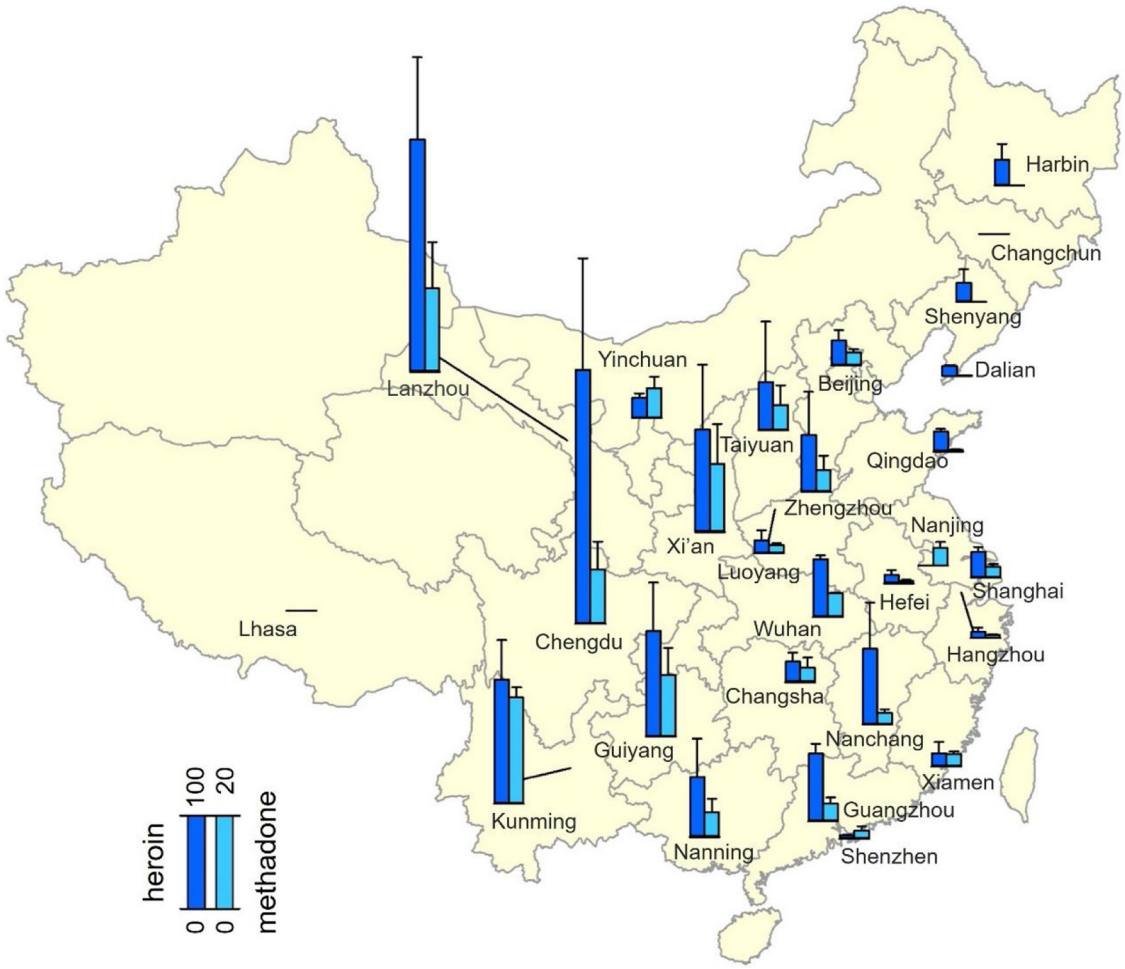


Fig. 3. Average consumption of methadone and heroin (mg/1000 in./d, population-weighted average) in major cities of mainland China.

4.2. Geographical variation of methadone and heroin use in China

The methadone and EDDP loads of sampled wastewater treatment plants in all seven regions of China followed normal distributions (K-S test, $p > 0.05$). One-way ANOVA reveals that methadone and EDDP loads in wastewater display a clear geographical pattern ($p < 0.05$). The methadone and EDDP loads in Northwest China and Southwest China were significantly higher than North China, Central China, South China, East China and Northeast China (Fig. 2). East China and Northeast China had the lowest methadone and EDDP loads. In general, methadone and EDDP loads decreased from the west to the east of the country, although there was an exception for Lhasa (in Southwest China). The findings were similar in morphine and codeine loads (K-S test, $p > 0.05$; One-way ANOVA, $p < 0.05$), with higher loads in the western inland regions than in the eastern regions (Fig. S1). These results indicated the fact that both methadone and heroin are more prevalently in west regions of China. This geographical pattern of methadone and heroin use are mainly attribute to opioids input from the "Golden Crescent" and "Golden Triangle" areas to Northwest and Southwest China. Differences in average daily methadone dose levels, purity of street heroin, and ways of drug use (such as injecting and smoking) in different cities could be also causing the geographical pattern. In addition, the cities with the highest/lowest methadone or heroin consumption indicated that there was relatively high/low methadone or purity of heroin dose level.

4.3. Comparison of MTD and heroin consumptions

A significant positive correlation ($p < 0.05$) between the consumptions of methadone and pure heroin in China can be derived from the results of this study (Fig. 3). This correlation implies that more methadone was used for treatment of heroin dependence where the heroin use was more prevalent, which in turn indicates a good response (based on the correlation between methadone and heroin consumption) of public health system toward heroin dependence problem in cities across China. In addition, data of methadone consumptions from methadone maintenance treatment clinics were collected and compared with WBE data in Kunming and Shenzhen. The total consumptions of methadone maintenance treatment were 3,380,091 mg in May 2017 at the urban areas of Kunming (4.88 million inhabitants) and 609,680 mg in December 2016 in Shenzhen (11.91 million inhabitants), which equate to 23.1 mg/1000 in./d in Kunming and 1.7 mg/1000 in./d in Shenzhen. According to the WBE estimates, methadone consumption of Kunming was 22.0 ± 2.1 mg/1000 in./d and that at of Shenzhen was 1.7 ± 0.9 mg/1000 in./d, which matched very well with the data from methadone maintenance treatment clinics mentioned above. The result indicated that methadone and EDDP found in influent wastewater, no matter high or low, were predominantly from methadone maintenance treatment source rather than the abuse of methadone in the two cities. This finding was further supported by the lack of detection of methadone and EDDP in both Harbin and Lhasa where there was no methadone clinic (Heilongjiang and Tibet provinces). Additionally, methadone is rarely seized by Chinese law enforcement authorities (OCNNCC, 2017), indicating that methadone is not prevalent on the illicit drug

market. But, it didn't mean that the methadone dose level was high enough to prevent withdrawal, or the patients withdrawn from methadone. Meanwhile, the result couldn't indicate that there was no diversion or supplemental supplies of methadone available, or no patients go looking for other sources to prevent withdrawal in other Chinese cities.

5. Conclusions

In summary, as the first study to report concentrations of methadone and EDDP in wastewater on a national scale in China, this paper fills a data gap in the understanding of methadone use in China. The results of this study suggested that methadone, EDDP, morphine and codeine in wastewater of the majority of cities in China comes from human consumption of methadone and heroin. Consumption of methadone was primarily from methadone maintenance treatment in most major Chinese cities. The average consumptions of methadone and heroin in China ranging from < 0.2 to 22.0 ± 2.1 mg/1000 in./d and from 0 to 263.9 ± 115.9 mg/1000 in./d, respectively. Methadone and heroin consumptions in Northwest and Southwest China were much higher than that in other regions.

Role of funding source

This work is supported by the National Natural Science Foundation of China (Grant No. 41701543 and 41877508) and the Fundamental Research Funds for the Central Universities (Grant No. 2018NTST20 and 2017XTCX02).

Contributors

Peng Du and Xiqing Li conceived the study. Peng Du, Ya Bai, Zilei Zhou and Zeqiong Xu conducted the sampling campaign and analysis. Jing Wang and Congbin Zhang collected the data of methadone consumptions in methadone maintenance treatment (Kunming and Shenzhen). Peng Du performed the data analysis and generated the figures and tables. Peng Du, Phong K. Thai, Xiqing Li, Xuan Zhang, Congbin Zhang, Jing Wang and Fanghua Hao wrote and reviewed the manuscript.

Declaration of Competing Interest

Authors declare no competing interests.

Acknowledgements

The authors extremely grateful to all the personnel at the 53 wastewater treatment plants for their assistance in wastewater sampling. We are also wish to thank Workgroup of Kunming Methadone Maintenance Treatment and Clean Syringe Exchange and Workgroup of Guangdong Detoxification Medicine Maintenance Treatment for supported the methadone consumption in methadone maintenance treatment.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drugalcdep.2019.06.034.

References

ACIC (Australian Criminal Intelligence Commission), 2018. National Wastewater Drug Monitoring Program—Report 6. . December 2018, Australia, https://www.acic.gov.au/publications/intelligence-products/national-wastewater-rug-monitoring-program-report.

Bade, R., White, J.M., Gerber, C., 2018. Qualitative and quantitative temporal analysis of licit and illicit drugs in wastewater in Australia using liquid chromatography coupled to mass spectrometry. Anal. Bioanal. Chem. 410, 529–542. https://doi.org/10.1007/s00216-017-0747-2.

Baker, D.R., Kasprzyk-Hordern, B., 2011. Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1218, 7901–7913. https://doi.org/10.1016/j.chroma.2011.08.092.

Baker, D.R., Barron, L., Kasprzyk-Hordern, B., 2014. Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A: chemical analysis and drug use estimates. Sci. Total Environ. 487, 629–641. https://doi.org/10.1016/j.scitotenv.2013.11.107.

Berset, J.D., Brenneisen, R., Mathieu, C., 2010. Analysis of llicit and illicit drugs in waste, surface and lake water samples using large volume direct injection high performance liquid chromatography–electrospray tandem mass spectrometry (HPLC-MS/MS). Chemosphere 81, 859–866. https://doi.org/10.1016/j.chemosphere.2010.08.011.

Boleda, M.A., Galceran, M.A., Ventura, F., 2009. Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. Water Res. 43, 1126–1136. https://doi.org/10.1016/j.watres.2008.11.056.

Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R., Bagnati, R., 2006. Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry. Anal. Chem. 78, 8421–8429. https://doi.org/10.1021/ac061095b.

Cosenza, A., Maida, C.M., Piscionieri, D., Fanara, S., Di Gaudio, F., Viviani, G., 2018. Occurrence of illicit drugs in two wastewater treatment plants in the South of Italy. Chemosphere 198, 377–385. https://doi.org/10.1016/j.chemosphere.2018.01.158.

Du, P., Li, K., Li, J., Xu, Z., Fu, X., Yang, J., Zhang, H., Li, X., 2015. Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology. Water Res. 84, 76–84. https://doi.org/10.1016/j.watres.2015.07.025.

Du, P., Zhou, Z., Bai, Y., Xu, Z., Gao, T., Fu, X., Li, X., 2017. Estimating heroin abuse in major Chinese cities through wastewater-based epidemiology. Sci. Total Environ. 605–606, 158–165. https://doi.org/10.1016/j.scitotenv.2017.05.262.

Du, P., Zhou, Z., Huang, H., Han, S., Xu, Z., Bai, Y., Li, X., 2018. Estimating population exposure to phthalate esters in major Chinese cities through wastewater-based epidemiology. Sci. Total Environ. 643, 1602–1609. https://doi.org/10.1016/j.scitotenv.2018.06.325.

EMCDDA (European Monitoring Centre for Drugs and Drug Addiction), 2016. Assessing Illicit Drugs in Wastewater: Advances in Wastewater-based Drug Epidemiology, European. https://www.drugsandalcohol.ie/25301/.

Foppe, K.S., Hammond-Weinberger, D.R., Subedi, B., 2018. Estimation of the consumption of illicit drugs during special events in two communities in Western Kentucky, USA using sewage epidemiology. Sci. Total Environ. 633, 249–256. https://doi.org/10.1016/j.scitotenv.2018.03.175.

Gao, T., Du, P., Xu, Z., Li, X., 2017. Occurrence of new psychoactive substances in wastewater of major Chinese cities. Sci. Total Environ. 575, 963–969. https://doi.org/10.1016/j.scitotenv.2016.09.152.

Khan, U., van Nuijs, A.L.N., Li, J., Maho, W., Du, P., Li, K., Hou, L., Zhang, J., Meng, X., Li, X., Covaci, A., 2014. Application of a sewage-based approach to assess the use of ten illicit drugs in four Chinese megacities. Sci. Total Environ. 487, 710–721. https://doi.org/10.1016/j.scitotenv.2014.01.043.

Krizman-Matasic, I., Senta, I., Kostanjevecki, P., Ahel, M., Terzic, S., 2019. Long-term monitoring of drug consumption patterns in a large-sized European city using wastewater-based epidemiology: comparison of two sampling schemes for the assessment of multiannual trends. Sci. Total Environ. 647, 474–485. https://doi.org/10.1016/j.scitotenv.2018.07.441.

Lai, F.Y., Bruno, R., Leung, H.W., Thai, P.K., Ort, C., Carter, S., Thompson, K., Lam, P.K.S., Mueller, J.F., 2013. Estimating daily and diurnal variations of illicit drug use in Hong Kong: a pilot study of using wastewater analysis in an Asian metropolitan city. Forensic Sci. Int. 233, 126–132. https://doi.org/10.1016/j.forsciint.2013.09.003.

Li, J., Hou, L., Du, P., Yang, J., Li, K., Xu, Z., Wang, C., Zhang, H., Li, X., 2014. Estimation of amphetamine and methamphetamine uses in Beijing through sewage-based analysis. Sci. Total Environ. 490, 724–732. https://doi.org/10.1016/j.scitotenv.2014.05.042.

Li, J., Zhang, R., Zhang, B., zhang, C., 2013. Human resource development research on methadone maintenance treatment in China. Chin. J. Drug Depend. 22, 64–68 (In Chinese).

Mackul'ak, T., Birosova, L., Gal, M., Bodik, I., Grabic, R., Ryba, J., Skubak, J., 2016. Wastewater analysis: the mean of the monitoring of frequently prescribed pharmaceuticals in Slovakia. Environ. Monit. Assess. 188, 18.

Martínez Bueno, M.J., Ucles, S., Hernando, M.D., Fernandez-Alba, A.R., 2011. Development of a solvent-free method for the simultaneous identification/quantification of drugs of abuse and their metabolites in environmental water by LC-MS/MS. Talanta 85, 157–166. https://doi.org/10.1016/j.talanta.2011.03.051.

Monnelly, V.J., Hamilton, R., Chappell, F.M., Mactier, H., Boardman, J.P., 2018. Childhood neurodevelopment after prescription of maintenance methadone for opioid dependency in pregnancy: a systematic review and meta-analysis. Dev. Med. Child Neurol. https://doi.org/10.1111/dmcn.14117.

Nefau, T., Karolak, S., Castillo, L., Boireau, V., Levi, Y., 2013. Presence of illicit drugs and metabolites in influents and effluents of 25 sewage water treatment plants and ma of drug consumption in France. Sci. Total Environ. 461–462, 712–722. https://doi.org/10.1016/j.scitotenv.2013.12.001.

OCNNCC (Office of China National Narcotic Control Committee), 2015. Report of Narcotics Control of China, Beijing.

OCNNCC (Office of China National Narcotic Control Committee), 2017. Report of

Narcotics Control of China, Beijing.
Östman, M., Fick, J., Nasstrom, E., Lindberg, R.H., 2014. A snapshot of illicit drug use in Sweden acquired through sewage water analysis. Sci. Total Environ. 472, 862–871. https://doi.org/10.1016/j.scitotenv.2013.11.081.
Skees, A.J., Foppe, K.S., Loganathan, B., Subedi, B., 2018. Contamination profiles, mass loadings, and sewage epidemiology of neuropsychiatric and illicit drugs in wastewater and river waters from a community in the Midwestern United States. Sci. Total Environ. 631–632, 1457–1464. https://doi.org/10.1016/j.scitotenv.2018.03.060.
Thai, P.K., Lai, F.Y., Bruno, R., van Dyken, E., Hall, W., O'Brien, J., Prichard, J., Mueller, J.F., 2016. Refining the excretion factors of methadone and codeine for wastewater analysis - combining data from pharmacokinetic and wastewater studies. Environ. Int. 94, 307–314. https://doi.org/10.1016/j.envint.2016.05.033.
Thai, P.K., O'Brien, J.W., Tscharke, B., Mueller, J.F., 2019. Analyzing wastewater samples collected during Census to determine the correction factors of drugs for wastewater-based epidemiology: the case of codeine and methadone. Environ. Sci. Technol. Lett. 6, 265–269. https://doi.org/10.1021/acs.estlett.8b00673.
van Nuijs, A.L.N., Mougel, J.F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2011. A one year investigation of the occurrence of illicit drugs in wastewater from Brussels, Belgium. J. Environ. Monitor. 13, 1008–1016. https://doi.org/10.1039/c0em00686f.
Vuori, E., Happonen, M., Gergov, M., Nenonen, T., Jarvinen, A., Ketola, R.A., Vahala, R., 2014. Wastewater analysis reveals regional variability in exposure to abused drugs and opioids in Finland. Sci. Total Environ. 487, 688–695. https://doi.org/10.1016/j.scitotenv.2013.11.010.
Xu, Z., Du, P., Li, K., Gao, T., Wang, Z., Fu, X., Li, X., 2017. Tracing methamphetamine and amphetamine sources in wastewater and receiving waters via concentration and enantiomeric profiling. Sci. Total Environ. 601–602, 159–166. https://doi.org/10.1016/j.scitotenv.2017.05.045.
Yargeau, V., Taylor, B., Li, H., Rodayan, A., Metcalfe, C.D., 2014. Analysis of drugs of abuse in wastewater from two Canadian cities. Sci. Total Environ. 487, 722–730. https://doi.org/10.1016/j.scitotenv.2013.11.094.

water

MDPI

Article

Occurrence and Fate of Heavy Metals in Municipal Wastewater in Heilongjiang Province, China: A Monthly Reconnaissance from 2015 to 2017

Peng Du [1], Lingrong Zhang [1], Yuntao Ma [2], Xinyue Li [3], Zhenglu Wang [4], Kang Mao [5], Na Wang [6], Ying Li [6], Jia He [1], Xuan Zhang [1], Fanghua Hao [1], Xiqing Li [3], Maodian Liu [3,*] and Xuejun Wang [3,*]

1 Beijing Key Laboratory of Urban Hydrological Cycle and Sponge City Technology, College of Water Sciences, Beijing Normal University, Beijing 100875, China
2 Longjiang Environmental Protection Group CO., Ltd., Harbin 150 000, Heilongjiang, China
3 Laboratory of Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China
4 College of Oceanography, Hohai University, Nanjing 210098, Jiangsu, China
5 State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, China
6 Heilongjiang Kerui Testing Technology Co., Ltd., Harbin 150 000, Heilongjiang, China
* Correspondence: maodian.liu@pku.edu.cn (M.L.); xjwang@urban.pku.edu.cn (X.W.)

Received: 7 January 2020; Accepted: 4 March 2020; Published: 6 March 2020

Abstract: As one of the major sources of pollutions in the environments, effluents from municipal wastewater recently became a hot topic. This study quantified monthly county-level releases of five heavy metals, i.e., lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and mercury (Hg), from municipal wastewater into the environment in the Heilongjiang Province of China, based on sampling, measurement, and modeling tools. Wastewater samples were collected from 27 municipal wastewater treatment plants (MWTPs) in 15 county-level cities of Heilongjiang every month from 2015 to 2017. The concentrations of five heavy metals were analyzed in both influents (Pb: 160 ± 100 μg/L; Cd: 15 ± 9.0 μg/L; Cr: 170 ± 64 μg/L; Hg: 0.67 ± 1.5 μg/L; As: 6.2 ± 4.8 μg/L) and effluents (Pb: 45 ± 15 μg/L; Cd: 5.2 ± 5.1 μg/L; Cr: 57 ± 13 μg/L; Hg: 0.28 ± 0.12 μg/L; As: 2.6 ± 1.4 μg/L). The removal ratios of the five heavy metals ranged from 50% to 67%. Inflow fluxes of Pb, Cr, and Cd displayed increasing trends first then decreased after reaching a maximum value, whereas those of Hg and Pb remained stable. Material flow analysis reveals that constructions of MWTPs are conducive to significantly reduce the releases of heavy metals from urban areas into the aquatic environment in the study area. Additionally, municipal wastewater sludge (used as fertilizer or spread on the land) could be a significant source of heavy metals in the land.

Keywords: heavy metals; municipal wastewater; wastewater treatment plants; flow-analysis; aquatic environment

1. Introduction

Excessive heavy metals accumulation perturbs the environment and causes serious adverse health effects to organisms, including humans [1]. Arsenic (As), cadmium (Cd), and their compounds are classified as human carcinogens by several regulatory agencies [2–4]. The adverse effects of lead (Pb) and mercury (Hg) in humans are shown in mental development, causing neurological and cardiovascular diseases, especially in children [5,6]. Although a low dose of chromium (Cr) is essential to mammals, a high dose of Cr could induce significant renal damage, DNA strand breaks

in peripheral lymphocytes, and respiratory cancers [7,8]. Human activities (industrial, domestic, agricultural, medical, etc.) significantly accelerate release of the naturally existing heavy metals into the environment [9]. For instance, human activities directly emitted 2500 tons/year of Hg into the atmosphere in recent years, accounting for 31% of the total emissions (including natural background and legacy sources) [10]. Dramatically, a total of 28,600 tons of As is emitted into the atmosphere annually, 14-times greater than emissions from natural sources [11]. Contamination by heavy metals has spread globally, including to all environment matrixes, such as the atmosphere, soil, sediment, fresh water, sea water, etc. [12]. Thus, an exact estimation of the amounts of heavy metals released into environmental systems and their fate is crucial for health risk assessment and policymaking.

While researchers have quantified the release of heavy metals from different sources, most previous studies focused on the atmospheric emission of heavy metals. For instance, Kristensen quantified the atmospheric Pb emission from leaded petrol consumption in Australia from 1933 to 2002, and the total emission was around 240,510 tons [13]. Wang et al. assessed the emission and mass balance of Hg in China's coal-fired power plants [14]. Han et al. explained the effects of reaction conditions on the emission behaviors of heavy metals during wastewater sludge pyrolysis [15]. Some studies have quantified the flux of heavy metals into other environmental systems on a large scale [12,16,17]. However, due to the relatively scarce observation data, these processes have been rarely updated or refined in the past several decades. Additionally, information on the contributions from other sources is relatively lacking.

Municipal wastewater, a complex mixture including a large variety of pollutants from both domestic and industrial sources, has become an important anthropogenic source of pollution in aquatic environments [18–20]. Previous studies have focused on the release of some pollutants associated with municipal wastewater to aquatic environments (such as illicit drugs, phthalate esters, photoinitiators, chlorinated paraffins, polycyclic aromatic hydrocarbons, antibiotics, etc.) [21–23]. Liu et al. performed the first estimation of total Hg (including all forms of Hg) and methylmercury (MeHg) releases from municipal wastewater into the environment and found that municipal wastewater could be a significant source based on analyses of total Hg and MeHg in both influents and effluents of sewage across China [24]. A snapshot picture of the release of Hg from municipal wastewater in China was obtained: 160,000 kg of total Hg and 280 kg of MeHg to different environmental systems, respectively. While the study yielded useful insights into Hg release, it did not provide information on temporal variations, and the release of other heavy metals from municipal wastewater in China is still unknown.

The aim of this study was to quantify, for the first time, the monthly county-level release of heavy metals from municipal wastewater into the environment in a particular Province in China. The research was conducted in Heilongjiang Province, China, from 2015 to 2017. Each month, municipal wastewater samples of influent and effluent were collected at 27 municipal wastewater treatment plants (MWTPs) in 15 prefectural and county-level cities that cover all the geographic regions of the province. The concentrations of Pb, Cd, Cr, Hg, and As in the wastewater samples were analyzed to examine the temporal variations in flux and removal. Material flow analysis was applied to provide a comprehensive understanding of the release of target heavy metals from municipal wastewater into various sinks. This study is motivated by our recognition of the contribution of municipal wastewater to heavy metal contamination in the environment, and it is intended to support a further nationwide estimation and policymaking in China.

2. Materials and Methods

2.1. Sample Collection

Wastewater samples were collected from 27 MWTPs in 15 prefectural and county-level cities of Heilongjiang Province, China. In each of these cities, one or two MWTPs were selected for wastewater sample collection, based on the population distribution in Heilongjiang Province (Figure 1). For some major cities in the province with large populations, such as Harbin (9.6 million inhabitants in 2017) [25],

wastewater samples were collected from four MWTPs. According to a recent census, the total population of all the selected cities was more than 25 million in the year 2017 [25], representing about 70% of the entire population of Heilongjiang Province. The methods of sample collection and preservation were according to a previous publication [26] and MEE (Ministry of Ecology and Environment of China) methods (HJ/T 91-2002). Time-proportional composite influent and effluent wastewater samples were collected every month from January to December of 2015 to 2017. Days that experienced heavy precipitation were avoided for sampling purposes to minimize the dilution. All samples were carried back to the laboratory and stored at 4 °C for less than 7 days until analysis. Details of the MWTPs and sampling information are listed in the Supplementary Materials (Table S1).

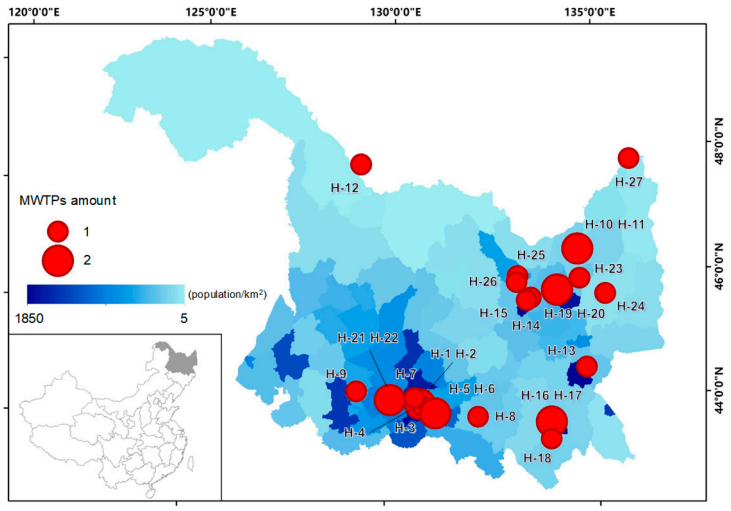


Figure 1. Locations of the municipal wastewater treatment plants (MWTPs) for this study. The date on the distribution of the population is from the census information in 2018 [25].

2.2. Analytical Methodology

Standard solutions of Pb, Cr, Cd, Hg, and As were obtained from the Institute for Environmental Reference Materials of Ministry of Environmental Protection (Beijing, China). Hydrochloric acid (HCl), perchloric acid ($HClO_4$), and hydrogen peroxide (H_2O_2) were purchased from Kemiou Chemical Reagent Co. (Tianjin, China), and nitric acid (HNO_3) was purchased from Jingrui Chemical Co., LTD. (Suzhou, China). Ultrapure water was prepared using a Milli-Q ultrapure system (Millipore, MA, USA).

Determination of the heavy metals in wastewater samples was performed based on methods that were commonly used in previous research and on MEE (Ministry of Ecology and Environment of China) methods (HJ 694-2014, HJ 757-2015, and GB/T 7475-1987) with minor modifications [27,28]. Sample pretreatment and determination were performed at the Heilongjiang Kerui Testing Technology Co., LTD (Harbin, China). For As, heating digestion of samples was conducted using nitric–perchloric acid (5 mL, 1:1, v/v) and hydrochloric acid solution (5 mL, 1:1, v/v), then samples were analyzed using an AFS-8220 atomic fluorescence spectrometer (Beijing Titian Instruments Co., LTD, Beijing, China). For Hg, water bath heating (100 °C) digestion (1 h) of samples was conducted using hydrochloric–nitric acid solution (1 mL, 3:1:4, v/v/v), then samples were analyzed via an AFS-8220 atomic fluorescence spectrometer. For Pb and Cd, nitric acid (5 mL) and hydrogen peroxide (10 mL) were employed for sample heating digestion, palladium nitrate (10 mL) was added, then samples were analyzed using a GF-AAS (ASC-990, Beijing Purkinje General Instrument Co., LTD., Beijing, China). For Cr, nitric acid (5 mL) and hydrogen peroxide (3 mL) were employed for sample heating digestion (180/95 °C), ammonium chloride (5 mL) and hydrochloric acid were added, then samples were analyzed using a TAS-990F atomic absorption spectrophotometer (Beijing Purkinje General Instrument Co., LTD., Beijing, China). Influent and effluent wastewater samples were taken in triplicate for analysis. The limits of

detection (LOD) for As, Hg, Pb, Cd, and Cr were 1.0 µg/L, 0.10 µg/L, 5.0 µg/L, 0.50 µg/L, and 15 µg/L, respectively. The spike recoveries of the standards of As ranged from 92% to 109%, Hg ranged from 92% to 104%, Pb ranged from 92% to 107%, Cd ranged from 83% to 105%, and Cr ranged from 95% to 107%. All the measurement results were adjusted by individual internal standard spike recoveries.

2.3. Material Flow Analysis

Material flow analysis is widely used as a tool to provide a system-oriented view of the sources, sinks, and relative processes of contaminant transport, such as carbon, nutrients, organic pollutants, and trace elements, in both natural and industrial systems [29–32]. In the present study, we specifically used this effective tool to quantitatively understand the transport and fate of Pb, Cd, Cr, Hg, and As embodied in municipal wastewater in Heilongjiang province. Estimates of material flows for all the metals considered direct releases into the environments, influent and effluent wastewater of MWTPs, accumulation in wastewater sludge, and a final step of release into the terrestrial ecosystem and landfill [24]. Calculations performed in the analysis were made based on the mass balance of each heavy metal to ensure that the amount of each heavy metal in the source was equal to the amount in the sinks as below:

$$Source_{ij} = Untreated_i + Influent_{ij}\ , \tag{1}$$

where $Source_{ij}$ is the amount of source of heavy metal i (kg/month) in the service area of municipal treatment plant j; $Untreated_i$ and $Influent_{ij}$ are the amounts of heavy metal i (kg/month) discharged into the aquatic environment and into the treatment plant j, respectively. In Equation (1), $Untreated_i$ and $Influent_{ij}$ could be further calculated as follows:

$$Untreated_i = C_{untreated,\ i} \times V_{untreated} \times K, \tag{2}$$

$$Influent_{ij} = C_{influent,\ ij} \times V_{influent,j} \times K\ , \tag{3}$$

where $C_{influent,\ i}$ is the concentration of heavy metal i (µg/L) in the untreated wastewater; $V_{untreated}$ is the volume of municipal wastewater (m^3/month) directly discharged to the aquatic environment; $C_{influent,\ ij}$ is the concentration of heavy metal i (µg/L) in the influent wastewater of municipal treatment plant j that is measured in the present study; $V_{untreated,j}$ is the volume of municipal wastewater (m^3/month) discharged into the aquatic environment in the service area in municipal treatment plant j; $V_{influent,j}$ is the volume of municipal wastewater (m^3/month) discharged into the municipal treatment plant j; and K in Equations (1) and (2) is a unit conversion coefficient. Similarly, the amount of heavy metal i released from municipal treatment plant j can be calculated as follows:

$$Effluent_{ij} = C_{effluent,\ ij} \times V_{effluent,j} \times K, \tag{4}$$

where $Effluent_{ij}$ is the amount of heavy metal i (kg/month) released from municipal treatment plant j into the aquatic environment; $C_{effluent,\ ij}$ is the concentration of heavy metal i (µg/L) in the effluent wastewater of municipal treatment plant j that is measured in the present study; $V_{effluent,j}$ is the volume of municipal wastewater (m^3/month) discharged from the municipal treatment plant j into the aquatic environment in the service area.

Substantial amounts of heavy metal that are discharged into MWTPs might potentially be stored in wastewater sludge during the treatment process. Following the published literature [24], the amounts of heavy metals that are accumulated in the wastewater sludge of municipal treatment plants were estimated as follows:

$$Sludge_{ij} = Influent_{ij} - Effluent_{ij}\ , \tag{5}$$

where $Sludge_{ij}$ represents the amount of heavy metal i (kg/month) accumulated in the wastewater sludge of municipal treatment plant j. In the present study, the fate of wastewater sludge from MWTPs in Heilongjiang province was investigated, and landfill, cropland, and incineration plants

were identified. Based on our investigation in the study area, the fate of the heavy metals in wastewater sludge can be further described as follows:

$$Sludge_{ij} = Landfill_{ij} + Cropland_{ij} + Incineration_{ij}\ , \quad (6)$$

where $Landfill_{ij}$ is the amount of heavy metal i released from municipal treatment plant j that is stored in a landfill (kg/month); $Cropland_{ij}$ is the amount of heavy metal i from municipal treatment plant j that is transported to cropland as a fertilizer (kg/month); and $Incineration_{ij}$ is the amount of heavy metal i from municipal treatment plant j that is transported to incineration plants (kg/month). According to our investigation, there is no other application of wastewater sludge in the study area.

Finally, the amounts of heavy metal transported into sinks are equal to the amounts from their sources were ensured, which can be further described by the following equation:

$$Sink_{ij} = Landfill_{ij} + Cropland_{ij} + Incineration_{ij} + Effluent_{ij} + Untreated_{ij}, \quad (7)$$

Large or minor amounts of heavy metals might be emitted into the air from the incineration of municipal wastewater sludge. The amounts of metal emissions from incineration were further estimated. According to previous studies, 1%–20%, 40%–60%, <1%, 2%–40%, and 48% of Pb, Cd, Cr, As, and Hg, respectively, might be emitted into the atmosphere during the incineration process [24,33,34].

In the present study, the standard deviation (95% confidence interval) of fluxes of each heavy metal in the material flow analysis was calculated to characterize the uncertainty of the results. The variation of the measured concentration of each heavy metal was considered in the uncertainty analysis. Uniform distribution with a fixed coefficient of deviation (5%) was assumed for the statistical data of the volume of municipal wastewater generation, based on the published literature [24,35].

2.4. Data Sources

Census and incineration data were collected from Heilongjiang Bureau of Statistics [25]. The respective data of influent/effluent flows, power consumption, and produced and disposed sludge were provided by each MWTP.

2.5. Statistical Analysis

Statistical analysis was performed by SPSS 20 (IBM Co., Armonk, NY, USA), and significance levels were determined at $p < 0.05$ and $p < 0.01$. Pearson correlation analysis was used to assess the correlation between removal ratios of the five heavy metals and power consumption. Student's t-test and One-NOVA were used to compare the differences in removal ratios and heavy metals flux in different years.

3. Results and Discussion

3.1. Occurrence of Heavy Metals in Municipal Wastewater

Pb, Cd, and Cr were detected in all the raw influent wastewater samples analyzed, with concentrations ranging from 44 μg/L to 940 μg/L (mean ± STD, 160 ± 100 μg/L), from 7.0 μg/L to 78 μg/L (mean ± STD, 15 ± 9.0 μg/L), and from 88 μg/L to 650 μg/L (mean ± STD, 170 ± 64 μg/L), respectively, in Heilongjiang from 2015 to 2017 (Table 1). Hg and As concentrations were below the LOD in only one and two raw influent wastewater samples, respectively, and above the LOD in the others. The maximum Hg and As concentrations in influent samples were 38 μg/L (mean ± STD, 0.67 ± 1.5 μg/L) and 95 μg/L (mean ± STD, 6.2 ± 4.8 μg/L), respectively (Table 1). The high detection frequencies of the mentioned targets in raw influents indicated that domestic releases are ubiquitous in Heilongjiang, which, in turn, indicated potentially high exposure levels to heavy metals for local inhabitants. Nevertheless, since the influent municipal wastewater in China may partly contain urban

surface runoff and industrial wastewater discharge, it is not currently possible to use the heavy metals contained in influent water as an indicator of inhabitant exposure in the study area [24].

Table 1. Statistics of heavy metals concentration in municipal wastewater samples.

Heavy Metals		n	DF [a] (%)	Min	Median	Max	Mean ± STD
				μg/L			
Influent	Pb	641	100.0	44	140	940	160 ± 100
	Cd	641	100.0	7.0	13	78	15 ± 9.0
	Cr	649	100.0	88	160	650	170 ± 64
	Hg	651	99.8	<LOD	0.54	38	0.67 ± 1.5
	As	650	99.7	<LOD	5.6	95	6.2 ± 4.8
Effluent	Pb	656	99.5	<LOD	45	170	45 ± 15
	Cd	656	99.5	<LOD	4.5	56	5.2 ± 5.1
	Cr	656	99.7	27	58	130	57 ± 13
	Hg	657	99.5	<LOD	0.26	1.0	0.28 ± 0.12
	As	657	99.7	<LOD	2.4	15	2.6 ± 1.4

[a] Detection frequency.

The five heavy metals were detected in most effluent samples, with detection frequencies of 99.5% (Pb), 99.5% (Cd), 99.7% (Cr), 99.5% (Hg), and 99.7% (As). Among the five analyzed heavy metals in effluents, Cr (57 ± 13 μg/L) had the highest mean concentration, followed by Pb (45 ± 15 μg/L). The average effluent concentrations of Cd, As, and Hg were all below 10 μg/L. The range, median, arithmetic mean concentrations, and standard deviations (STD) are presented in Table 1. The average influent and effluent concentrations of As, Cd, and Hg in this study were similar to those in other previous studies conducted in Italy, UK, Poland, Chungking, etc., but much lower than those in Spain. For Cr and Pb, the average influent and effluent concentrations were higher than those in most other countries and Chinese cities, but lower than those in Turkey for Cr and in the UK for Pb (Table 2). Compared with other countries (Table 2), the higher Pb concentration found in this study is consistent with the high blood Pb levels of Chinese citizens [36]. There is a relatively large variability in the concentrations of heavy metals in municipal wastewater in China. For instance, the average influent Pb concentrations in different cities in China were found to range from 4.1 to 480 μg/L [37–40]. The average influent Cd concentrations also ranged from 1.3 to 61 μg/L [37–40]. A similar situation was also found for the other three metals (Table 1). This reflects that MWTPs in different regions in China might receive heavy metals from different sources (for example, different types of industrial sources) [40]. Further investigation of the sources of heavy metals in municipal wastewater is needed.

Table 2. Comparison of influents and effluents concentrations (μg/L) and removal ratios (%) of five heavy metals in different countries.

Country	No. of MWTPs	As			Cd			Cr			Hg			Pb		
		In [a]	Ef [b]	Re [c]	In	Ef	Re	In	Ef	Re	In	Ef	Re	In	Ef	Re
Italy [41]	5	4.3	2.2	44.5	7.5	0.10	73	41	10	64	1.6	0.90	33	7.1	7.6	36
France [42]	1	n.d. [d]	n.d.	n.d.	0.60	<0.20	>67	9.0	20	50	n.d.	n.d.	n.d.	18–20	<1	>95
Greece [43]	1	n.d.	n.d.	n.d.	3.3	1.5	55	40	20	50	n.d.	n.d.	n.d.	39	27	31
Poland [44]	1	n.d.	n.d.	n.d.	10–20	n.d.	10–20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	~50	n.d.	30–40
UK [45–47]	1	11–13	2.0–2.5	81	1.5–2.0	0.25–0.50	79	35	10	71	2.5	0.3	90	600	25	96
France [48]	9	3.0–10	n.d.	20	≤3.0	n.d.	40–75	10–100	n.d.	≥75	n.d.	n.d.	n.d.	10–100	n.d.	≥75
Indian [49]	3	1.1	0.8	30	n.d.	n.d.	n.d.	3.5	3.1	14.00	n.d.	n.d.	n.d.	0.20	0.10	34
Turkey [50]	2	n.d.	n.d.	n.d.	11	5	55	1500	240	77	n.d.	n.d.	n.d.	50	21	58
Spain [51]	1	n.d.	n.d.	n.d.	5000	5000	n.d.	n.d.	n.d.	n.d.	370	370	n.d.	5.21	6.1	n.d.
Jiaozuo, China [37]	1	15	12.7	14	1.3	n.d.	n.d.	18.2	0.4	98	n.d.	n.d.	n.d.	4.1	n.d.	n.d.
Chungking, China [38]	1	1.1	0.70	33	1.2	0.58	53	n.d.	n.d.	n.d.	0.55	0.13	76	18	13	30
Xi'an China [39]	1	0.90	0.030	97	n.d.	n.d.	n.d.	92	63	31	0.43	0.06	86	n.d.	n.d.	n.d.
Yantai, China [40]	1	n.d.	n.d.	n.d.	61	6.1	90	560	46	92	1.5	0.11	93	480	48	90
This study (Heilongjiang, China)	27	6.2 ± 4.8	2.6 ± 1.4	55	15 ± 9.0	5.2 ± 5.1	64	170 ± 64	57 ± 13	64	0.67 ± 1.5	0.28 ± 0.12	50	160 ± 100	45 ± 15	67

[a] Influent concentration; [b] Effluent concentration; [c] Removal ratio; [d] no data.

3.2. Removal Ratios

The removal ratio was derived by dividing the difference between the influent and effluent concentrations at a MWTP by the influent concentrations. The average removal ratios of Pb (67 ± 9 %), Cd (64 ± 11%), and Cr (64 ± 7%) were higher than 60% in Heilongjiang, while Hg (50 ± 9%) and As (55 ± 9%) had lower removal ratios than the other three heavy metals. This means that more Hg and As compared to other target heavy metals entered receiving waters. The removal ratios of all five heavy metals in both 2016 and 2017 were significantly higher than those in 2015 ($p < 0.05$), and the lowest value of each target was found in 2015 (Figure 2). However, there was no significant difference in the removal ratio between 2016 and 2017 ($p > 0.05$). These results indicated that there were increasing trends in the removal ratios for all targets between 2015 and 2016; after 2016, they maintained a stable level (Figure 2). Additionally, the power consumption per ton of municipal wastewater in 2015 was significantly lower than those in 2016 and 2017 ($p < 0.05$), and no significant difference was found between the latter two years. This trend was similar to that of the removal ratios of the heavy metals. A significant positive correlation was found between the power consumption and removal ratios of all five heavy metals (Figure S1). In general, the removal ratio increases with power consumption. Thus, this correlation implies that the increasing trends of the removal ratios were potentially due to increases in treating efficiencies in these MWTPs. Due to the limitation of the sampling campaign (once a month), no several consecutive days of influent and effluent samples were obtained in a month, removal ratios of heavy metals in different treatment techniques subjected to large variation and cannot be compared in this study.

Compared with previous studies, the removal ratios of the five heavy metals in this study were at a moderate level. Overall, a large variation has been found in the removal ratios of the five heavy metals in different countries (Table 2). For instance, the average removal ratios range from 20% to 97% for As, 10% to 79% for Cd, 33% to 98% for Hg, 50% to 77% for Cr, and 30% to 96% for Pb. This is attributed to the fact that MWTPs are not designed for removing heavy metals, and the apparent removal in most of the cases is the result of the metals partitioning to the solid phase of the treatment systems [52]. Thus, the release of heavy metals from MWTPs into the environment should not be ignored.

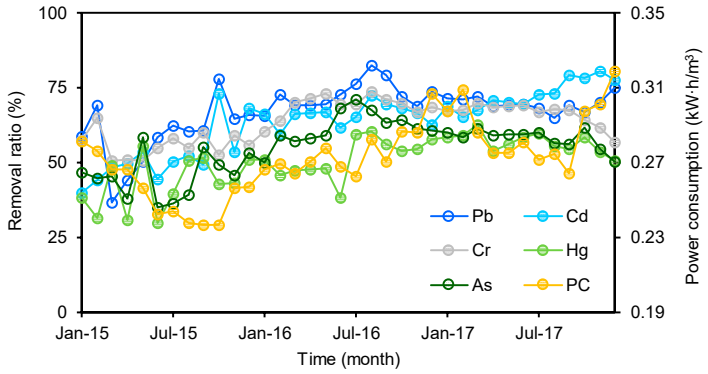


Figure 2. Apparent removal ratios of five heavy metals and power consumption (PC) at sampled MWTPs.

3.3. Variations of Heavy Metals Flux

The average inflow flux of Pb was 1100 ± 350 kg/month in the sampled MWTPs; the highest value was 2000 kg/month, occurring in August 2016, followed by 1700 kg/month (March 2016) and 1600 kg/month (March 2017), and the lowest value was 530/month kg (January 2015) (Figure 3). One-NOVA showed that there were no significant differences between Pb flux in different seasons ($p > 0.05$). Similar results were also found for Cd (average ± STD, 100 ± 17 kg/month), Cr (average ± STD, 1200 ± 250 kg/month), Hg (average ± STD, 5.0 ± 2.6 kg/month), and As flux (average ± STD, 45 ± 15 kg/month) (Figure 3). Although the temperature varies greatly in different seasons (average temperature ranged from −15 °C to 17 °C), there were no significant variations in the inflow flux of

heavy metals in the study area. The total flux was 40,000 kg for Pb, 3600 kg for Cd, 44,000 kg for Cr, 180 kg for Hg, and 1600 kg for As from 2015 to 2017. For Pb, Cd, and Cr, the average flux in 2016 was significantly higher than those in 2015 and 2017 ($p < 0.05$) (Figure 3). The Pb, Cd, and Cr flux during the sampling period displayed an increasing trend initially, then decreased after a maximum value was reached. The flux variations of Hg and As were relatively stable compared with those of the other heavy metals between 2015 and 2017.

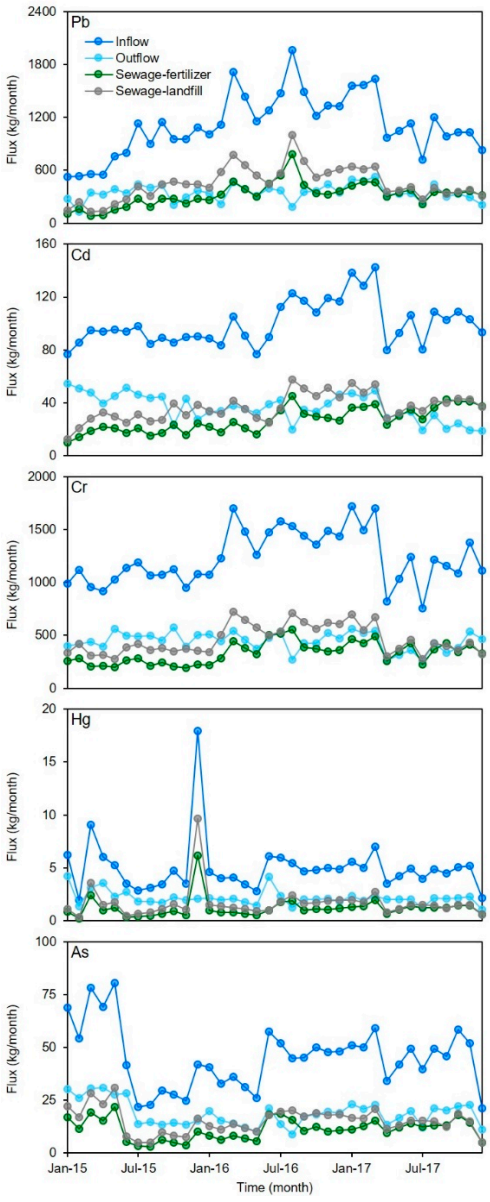


Figure 3. Temporal variations of five heavy metals flux at sampled MWTPs.

The average effluent fluxes of the five heavy metals ranged from 2.1 ± 0.69 kg/month (Hg) to 440 ± 86 kg/month (Cr) from 2015 to 2017 (Figure 3). The variations in effluent flux of the five heavy metals were more stable than those in influent flux due to the high removal ratios. The average Pb, Cd, Cr, Hg, and As fluxed for sludge-landfill were 320 ± 140 kg/month, 27 ± 9.4 kg/month, 330 ± 100 kg/month, 1.2 ± 0.98 kg/month, and 11 ± 5.0 kg/month from 2015 to 2017, respectively. The average fluxes for sludge-fertilizer were 450 ± 190 kg/month (Pb), 37 ± 10 kg/month (Cd), 460 ± 140 kg/month (Cr), 1.7 ± 1.5 kg/month (Hg), and 15 ± 6.0 kg/month (As) in the same period. In total, there were 2800 kg of Pb, 2300 kg of Cd, 28,000 kg of Cr, 100 kg of Hg, and 950 kg of As released

from sludge from 2015 to 2017 in the study area. The variations in the five heavy metals' fluxes in both landfill and fertilizer sludge were similar to those in influents. This means that most of the heavy metal was transferred to sludge during the municipal wastewater treatment process.

County-level inventories of Pb, Cd, Cr, Hg, and As released from municipal wastewater in Heilongjiang, China, in 2017, were constructed based on the fitting models (Figure 4). The results showed that the release of all five heavy metals was mainly concentrated in the cities and counties in the southwestern region of Heilongjiang Province. In this region, Harbin (the provincial capital) was the top contributor to the release of Pb, Cd, Cr, Hg, and As from municipal wastewater, followed by the cities Daqing and Qiqihar, and nearly one order of magnitude higher than other cities and counties. This was attributed to their higher population densities compared to other cities and counties. This result was similar to those of previous studies, which indicated that other anthropogenic emissions, such as black carbon, phosphorus, and antibiotics, are also high in Heilongjiang, China [35,53,54].

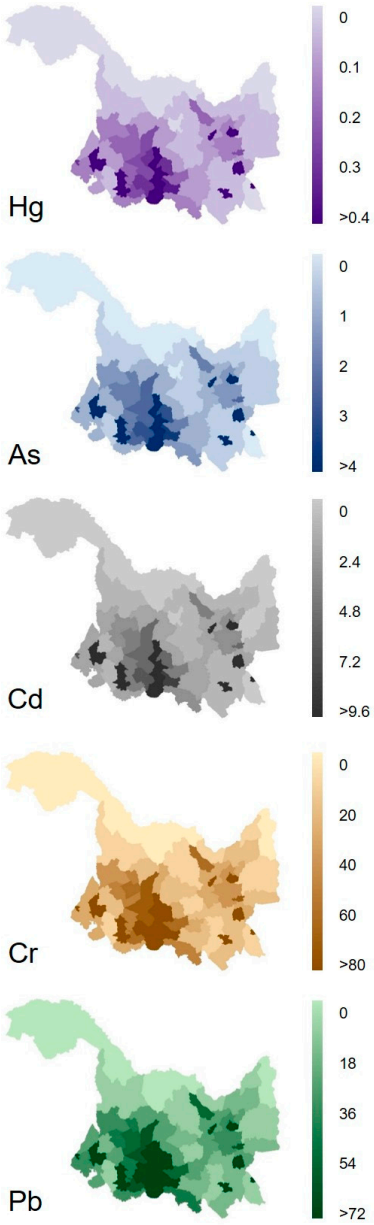


Figure 4. Distributions of five heavy metals released (g/km^2·yr) from municipal wastewater in Heilongjiang province in 2017.

3.4. Material Flows from Municipal Wastewater to Sinks

The results of the material flow analysis indicated that substantial amounts of Pb, Cd, Cr, Hg, and As were released from municipal wastewater in Heilongjiang province; these amounts were 15,000, 1400, 16,000, 62, and 610 kg, respectively, in 2017. Meanwhile, relatively small amounts of these heavy metals were directly released into the aquatic environment without any treatment: 1600, 150, 1700, 6.4, and 63 kg of Pb, Cd, Cr, Hg, and As, respectively, in 2017 (Figure 5). On the other hand, 14,000, 1300, 15,000, 56, and 550 kg of Pb, Cd, Cr, Hg, and As, respectively, were discharged into MWTPs in 2017 (Figure 5). Subsequently, 4900, 370, 4900, 24, and 230 kg of Pb, Cd, Cr, Hg, and As, respectively, were released from municipal treatment plants into the aquatic environment in Heilongjiang province in 2017; these values are equal to 35%, 28%, 33%, 43%, and 42% of their discharges into MWTPs (Figure 5). Total amounts of 6500, 520, 6600, 30, and 290 kg of Pb, Cd, Cr, Hg, and As, respectively, were released from municipal wastewater into the aquatic environment in Heilongjiang province in 2017; these values were significantly decreased from the amounts released from municipal wastewater as shown above (Figure 5). These results suggest that constructions of MWTPs are conducive to significantly reduce the releases of heavy metals from urban areas into the aquatic environment in the study area. The results suggest that the overall municipal wastewater management strategy in the study area could serve as a valuable reference, particularly for developing regions that are struggling with inland water contaminations. Nevertheless, it shows that wastewater sludge is an important temporary sink for these five heavy metals from municipal wastewater.

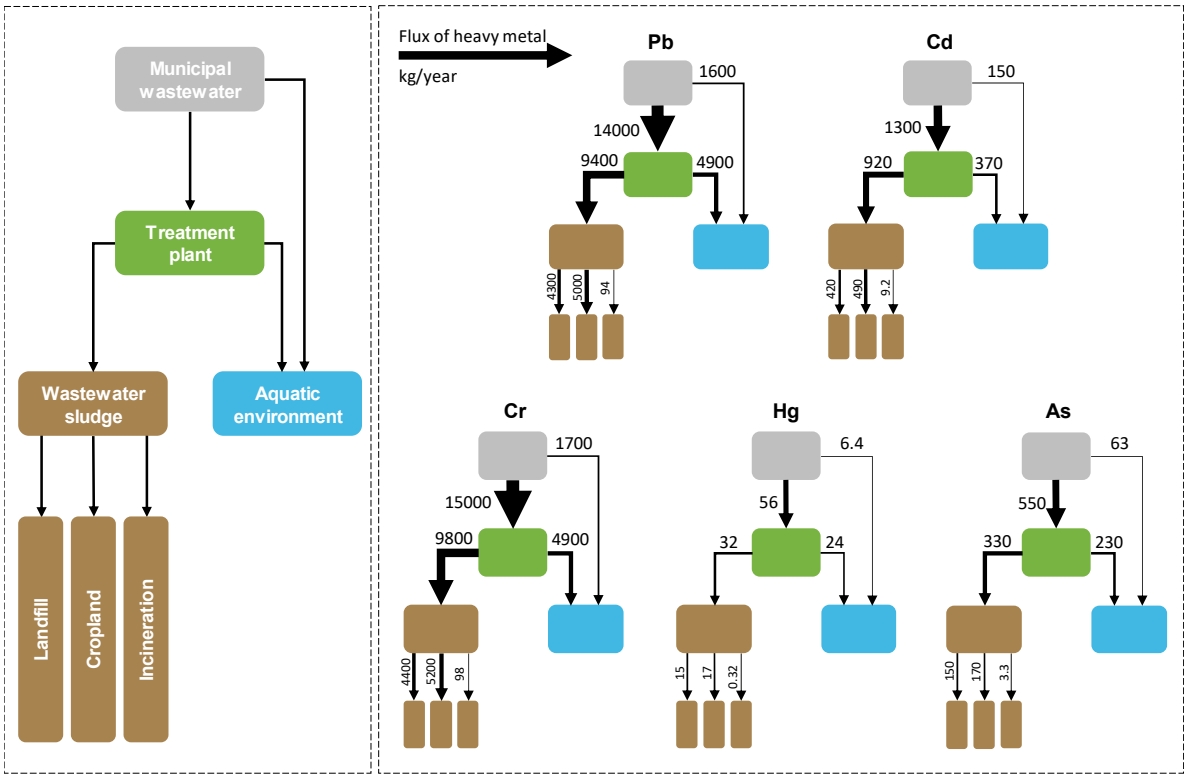


Figure 5. Material flow analysis of five heavy metals from municipal wastewater to sinks (the flow from municipal wastewater to treatment plant represents influent heavy metal of the wastewater treatment plant, while the flow from municipal wastewater to aquatic environment represents the metal discharged from untreated wastewater to aquatic environment directly).

Material flow analysis showed that municipal wastewater sludge could be a significant source of heavy metal pollution in the land. In total, 9400, 920, 9800, 32, and 330 kg of Pb, Cd, Cr, Hg, and As, respectively, were generated in wastewater sludge in 2017 (Figure 5). Approximately half of

these heavy metals were stored in landfill, which seems a good choice as a reservoir for heavy metals. Meanwhile, substantial amounts of heavy metals were transported to cropland in wastewater sludge that was used as a fertilizer for crop plants; these amounts were 5000, 490, 5200, 17, and 170 kg for Pb, Cd, Cr, Hg, and As, respectively, in 2017 (Figure 5). This might potentially pose a health risk to humans. For example, previous studies suggested that Hg released into paddy soil could be methylated and accumulated in rice grains, which could subsequently become a significant dietary source for inhabitant Hg exposure [55,56]. Similarly, As, Cd, and Cr can also enter the food chain and become widely distributed throughout plants and animals, and thereby pose a risk to humans and wildlife [57–59]. Many publications in the literature also suggest that the application of wastewater sludge to cropland can enhance the accumulation of heavy metals in certain crop plants [60–62]. However, inconsistent results are still reported [63–65], and the reason for the difference is not well understood. The density of organic carbon in crop soil in Heilongjiang (average: 26 kg/m^2) is substantially higher than those in most other regions in China (average in China: 16 kg/m^2) [66,67]. Meanwhile, the soil pH value in Heilongjiang (5.5–7.2) is higher than that in Southern China (4.5–5.5) [68]. This might reduce the availability of soil heavy metals in Heilongjiang [69–71]. Further investigation of the accumulation of heavy metals from wastewater sludge into crop plants is desirable.

The results from the material flow analysis suggest that relatively small amounts of heavy metal were transported to incineration plants (i.e., 94, 9.2, 98, 0.32, and 3.3 kg of Pb, Cd, Cr, Hg, and As, respectively, in 2017) (Figure 5). Accordingly, 0.94–19, 3.7–5.5, <0.98, 0.15, and 0.066–1.3 kg of Pb, Cd, Cr, As, and Hg were emitted into the atmosphere during the incineration process. This means that the atmosphere is not a significant sink for these heavy metals from municipal wastewater. According to our investigation in Heilongjiang, only 1.0% of the municipal wastewater sludge was transported to incineration plants in 2017 [25]. This value is lower than that reported by the government in 2014 (3.5%, provincial average) [72]. Meanwhile, 65% and 63% of municipal wastewater sludge in Jiangsu and Zhejiang Provinces were transported to incineration plants in 2014, while the proportions in Anhui, Fujian, and Shandong exceeded 20% [72]. From the angle of controlling atmospheric heavy metal emissions, burying wastewater sludge in landfills might be a better option for Heilongjiang and other regions in China [73]. However, landfill leachate might also lead to heavy metal contamination of the surrounding soil, groundwater, and plants, particularly in developing countries [74–77]. In the study area, no heavy metal releases were identified in other parts of the terrestrial ecosystem besides the aquatic environment and cropland.

4. Conclusions

In this study, monthly county-level monitoring of five heavy metals in municipal wastewater was conducted in a Chinese province, which could fill a data gap in the understanding of the release of heavy metals from municipal wastewater into the environment in Heilongjiang. The five heavy metals in influents and effluents were detected with concentrations of up to 940 μg/L and 170 μg/L, respectively. Inflow fluxes of Pb, Cr, and Cd from 2015 to 2017 displayed an increased trend initially, then decreased after reaching a maximum value. Material flow analysis provided quantified evidence that the construction of MWTPs is conducive to significantly reducing the discharge of heavy metals into the aquatic environment in the study area. In addition, municipal wastewater sludge (used as fertilizer or spread on the land) was shown to be a significant source of heavy metal to the land.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/3/728/s1, Table S1: Municipal wastewater treatment plants and sampling information in Heilongjiang Province, Figure S1: Relationship between removal ratios of five heavy metals and power consumption.

Author Contributions: P.D. and M.L. conceived the study. Y.M., N.W., and Y.L. conducted the sampling campaign and analysis. M.L., P.D., L.Z., and X.L. (Xinyue Li) performed the data analysis and generated the figures and tables. P.D., M.L., X.W., Z.W., K.M., J.H., X.Z., F.H., and X.L. (Xiqing Li) wrote and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by the National Natural Science Foundation of China (Grant No. 41701543, 41977311, and 41630748), the Fundamental Research Funds for the Central Universities (Grant No. 2018NTST20, 2019B06614, and 2017XTCX02), and the 111 Project (B18006).

Acknowledgments: The authors are extremely grateful to all the personnel at the sampled MWTPs for their assistance in wastewater sampling.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rai, P.K.; Lee, S.S.; Zhang, M.; Tsang, Y.F.; Kim, K.-H. Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environ. Int.* **2019**, *125*, 365–385. [CrossRef] [PubMed]
2. IARC (International Agency for Research on Cancer). *Monographs—Cadmium*; IARC: Lyon, France, 1993.
3. Waalkes, M.P.; Misra, R.R.; Chang, L.W. *Toxicology of Metals*; CRC Press: Boca Raton, FL, USA, 1996.
4. El-Kady, A.A.; Abdel-Wahhab, M.A. Occurrence of trace metals in foodstuffs and their health impact. *Trends Food Sci. Technol.* **2018**, *75*, 36–45. [CrossRef]
5. Al-Saleh, I.; Al-Rouqi, R.; Elkhatib, R.; Abduljabbar, M.; Al-Rajudi, T. Risk assessment of environmental exposure to heavy metals in mothers and their respective infants. *Int. J. Hyg. Environ. Health* **2017**, *220*, 1252–1278. [CrossRef] [PubMed]
6. Zhou, H.; Yang, W.; Zhou, X.; Liu, L.; Gu, J.; Wang, W.; Zou, J.; Tian, T.; Peng, P.; Liao, B. Accumulation of heavy metals in vegetable species planted in contaminated soils and the health risk assessment. *Int. J. Environ. Res. Public Health* **2016**, *13*, 289. [CrossRef] [PubMed]
7. Costa, M. Toxicity and carcinogenicity of Cr (VI) in animal models and humans. *Crit. Rev. Toxicol.* **1997**, *27*, 431–442. [CrossRef]
8. Soghoian, S.; Sinert, R. Heavy Metal Toxicity. Available online: http://emedicine.medscape.com/article/814960-overview (accessed on 24 August 2018).
9. Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K.; Sutton, D.J. Heavy metals toxicity and the environment. *EXS* **2012**, *101*, 133–164.
10. Outridge, P.M.; Mason, R.P.; Wang, F.; Guerrero, S.; Heimburger-Boavida, L.E. Updated global and oceanic mercury budgets for the united nations global mercury assessment 2018. *Environ. Sci. Technol.* **2018**, *52*, 11466–11477. [CrossRef]
11. Wai, K.; Wu, S.; Li, X.; Jaffe, D.A.; Perry, K.D. Global atmospheric transport and source-receptor relationships for arsenic. *Environ. Sci. Technol.* **2016**, *50*, 3714–3720. [CrossRef]
12. Nriagu, J.O.; Pacyna, J.M. Quantitative assessment of worldwide contamination of air, water and soils by trace-metals. *Nature* **1988**, *333*, 134–139. [CrossRef]
13. Kristensen, L.J. Quantification of atmospheric lead emissions from 70 years of leaded petrol consumption in Australia. *Atmos. Environ.* **2015**, *111*, 195–201. [CrossRef]
14. Wang, S.; Zhang, L.; Li, G.; Wu, Y.; Hao, J.; Pirrone, N.; Sprovieri, F.; Ancora, M. Mercury emission and speciation of coal-fired power plants in China. *Atmos. Chem. Phys.* **2010**, *10*, 1183–1192. [CrossRef]
15. Han, H.; Hu, S.; Syed-Hassan, S.S.A.; Xiao, Y.; Wang, Y.; Xu, J.; Jiang, L.; Su, S.; Xiang, J. Effects of reaction conditions on the emission behaviors of arsenic, cadmium and lead during sewage sludge pyrolysis. *Bioresour. Technol.* **2017**, *236*, 138–145. [CrossRef] [PubMed]
16. Han, F.X.X.; Su, Y.; Monts, D.L.; Plodinec, M.J.; Banin, A.; Triplett, G.E. Assessment of global industrial-age anthropogenic arsenic contamination. *Naturwissenschaften* **2003**, *90*, 395–401. [CrossRef] [PubMed]
17. Mason, R.P.; Fitzgerald, W.F.; Morel, F.M.M. The biogeochemical cycling of elemental mercury—Anthropogenic influences. *Geochim. Cosmochim. Acta* **1994**, *58*, 3191–3198. [CrossRef]
18. Boxall, A.B.; Kolpin, D.W.; Halling-Sørensen, B.; Tolls, J. Are veterinary medicines causing environmental risks? *Environ. Sci. Technol.* **2003**, *37*, 286A–294A. [CrossRef]
19. Chen, W.; Li, Y.; Chen, C.-E.; Sweetman, A.J.; Zhang, H.; Jones, K.C. DGT passive sampling for quantitative in situ measurements of compounds from household and personal care products in waters. *Environ. Sci. Technol.* **2017**, *51*, 13274–13281. [CrossRef]
20. Jones, L.; Sullivan, T.; Kinsella, B.; Furey, A.; Regan, F. Occurrence of selected metals in wastewater effluent and surface water in Ireland. *Anal. Lett.* **2016**, *50*, 724–737. [CrossRef]

21. Du, P.; Thai, P.K.; Bai, Y.; Zhou, Z.; Xu, Z.; Zhang, X.; Wang, J.; Zhang, C.; Hao, F.; Li, X. Monitoring consumption of methadone and heroin in major Chinese cities by wastewater-based epidemiology. *Drug Alcohol Depend.* **2019**, *205*, 107532. [CrossRef]
22. Du, P.; Zhou, Z.; Huang, H.; Han, S.; Xu, Z.; Bai, Y.; Li, X. Estimating population exposure to phthalate esters in major Chinese cities through wastewater-based epidemiology. *Sci. Total Environ.* **2018**, *643*, 1602–1609. [CrossRef]
23. Lei, K.; Zhu, Y.; Chen, W.; Pan, H.-Y.; Cao, Y.-X.; Zhang, X.; Guo, B.-B.; Sweetman, A.; Lin, C.-Y.; Ouyang, W.; et al. Spatial and seasonal variations of antibiotics in river waters in the Haihe river catchment in China and ecotoxicological risk assessment. *Environ. Int.* **2019**, *130*, 104919. [CrossRef]
24. Liu, M.; Du, P.; Yu, C.; He, Y.; Zhang, H.; Sun, X.; Lin, H.; Luo, Y.; Xie, H.; Guo, J.; et al. Increases of total mercury and methylmercury releases from municipal sewage into environment in China and implications. *Environ. Sci. Technol.* **2018**, *52*, 124–134. [CrossRef] [PubMed]
25. HBS (Heilongjiang Bureau of Statistics). *Heilongjiang Statistical Yearbook 2018*; China Statistics Press: Beijing, China, 2018.
26. Du, P.; Li, K.; Li, J.; Xu, Z.; Fu, X.; Yang, J.; Zhang, H.; Li, X. Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology. *Water Res.* **2015**, *84*, 76–84. [CrossRef] [PubMed]
27. Silalahi, J.; Tampubolon, S.D.R.; Sagala, M.R.M.; Saraswati, I.N.; Silalahi, Y.C.E. Analysis of arsenic in raw and cooked rice by atomic absorption spectrophotometer. In Proceedings of the IOP Conference Series: Earth and Environmental Science, Banda Aceh, Indonesia, 26–27 September 2018; Volume 205.
28. Brombach, C.C.; Chen, B.; Corns, W.T.; Feldmann, J.; Krupp, E.M. Methylmercury in water samples at the pg/L level by online preconcentration liquid chromatography cold vapor-atomic fluorescence spectrometry. *Spectrochim. Acta B* **2015**, *105*, 103–108. [CrossRef]
29. Brunner, P.H.; Rechberger, H. *Handbook of Material Flow Analysis: For Environmental, Resource, and Waste Engineers*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2016.
30. Chen, Y.; Zang, L.; Shen, G.; Liu, M.; Du, W.; Fei, J.; Yang, L.; Chen, L.; Wang, X.; Liu, W.; et al. Resolution of the ongoing challenge of estimating nonpoint source neonicotinoid pollution in the Yangtze River basin using a modified mass balance approach. *Environ. Sci. Technol.* **2019**, *53*, 2539–2548. [CrossRef]
31. Churkina, G. Modeling the carbon cycle of urban systems. *Ecol. Model.* **2008**, *216*, 107–113. [CrossRef]
32. Liu, M.; Xie, H.; He, Y.; Zhang, Q.; Sun, X.; Yu, C.; Chen, L.; Zhang, W.; Zhang, Q.; Wang, X. Sources and transport of methylmercury in the Yangtze River and the impact of the Three Gorges Dam. *Water Res.* **2019**, *166*, 115042. [CrossRef] [PubMed]
33. Beltran, A.; Marce, R.M.; Cormack, P.A.G.; Borrull, F. Synthetic approaches to parabens molecularly imprinted polymers and their applications to the solid-phase extraction of river water samples. *Anal. Chim. Acta* **2010**, *677*, 72–78. [CrossRef]
34. Hu, D.; Zhang, W.; Chen, L.; Chen, C.; Ou, L.; Tong, Y.; Wei, W.; Long, W.; Wang, X. Mercury emissions from waste combustion in China from 2004 to 2010. *Atmos. Environ.* **2012**, *62*, 359–366. [CrossRef]
35. Wang, R.; Tao, S.; Wang, W.; Liu, J.; Shen, H.; Shen, G.; Wang, B.; Liu, X.; Li, W.; Huang, Y.; et al. Black carbon emissions in china from 1949 to 2050. *Environ. Sci. Technol.* **2012**, *46*, 7595–7603. [CrossRef]
36. Li, M.M.; Gao, Z.Y.; Dong, C.Y.; Wu, M.Q.; Yan, J.; Cao, J.; Ma, W.J.; Wang, J.; Gong, Y.L.; Xu, J.; et al. Contemporary blood lead levels of children aged 0–84 months in China: A national cross-sectional study. *Environ. Int.* **2020**, *134*, 105288. [CrossRef]
37. Li, L.; Mao, Y.; Li, Y.; Ma, Y.; Wang, X.; Song, D. Fate of heavy metals in the carrousel oxidation ditch wastewater treatment process. *J. Saf. Environ.* **2014**, *14*, 217–223. (In Chinese)
38. Lu, J.; Chen, L.; Zhao, X. Study on the variation of heavy metals in the traditional municipal activated sludge wastewater treatment process. *Environ. Pollut. Control* **2008**, *30*, 29–32. (In Chinese)
39. Luo, L.; Kang, D.; Wang, X. Removal and fate of typical heavy metals in municipal wastewater treatment process. *J. Saf. Environ.* **2010**, *10*, 52–55. (In Chinese)
40. Yang, Q.; Chang, X.; Liu, Y.; Zhao, M.; Han, J. Analysis of heavy metals removal through A2/O process of municipal wastewater treatment in Yantai City. *Environ. Sci. Technol.* **2009**, *32*, 143–145. (In Chinese)
41. Carletti, G.; Fatone, F.; Bolzonella, D.; Cecchi, F. Occurrence and fate of heavy metals in large wastewater treatment plants treating municipal and industrial wastewaters. *Water Sci. Technol.* **2008**, *57*, 1329–1336. [CrossRef] [PubMed]

42. Buzier, R.; Tusseau-Vuillemin, M.-H.; Meriadec, C.M.D.; Rousselot, O.; Mouchel, J.-M. Trace metal speciation and fluxes within a major French wastewater treatment plant: Impact of the successive treatments stages. *Chemosphere* **2006**, *65*, 2419–2426. [CrossRef] [PubMed]
43. Karvelas, M.; Katsoyiannis, A.; Samara, C. Occurrence and fate of heavy metals in the wastewater treatment process. *Chemosphere* **2003**, *53*, 1201–1210. [CrossRef]
44. Chipasa, K.B. Accumulation and fate of selected heavy metals in a biological wastewater treatment system. *Waste Manag.* **2003**, *23*, 135–143. [CrossRef]
45. Goldstone, M.E.; Atkinson, C.; Kirk, P.W.W.; Lester, J.N. The behavior of heavy-metals during waste-water treatment. III. Mercury and arsenic. *Sci. Total Environ.* **1990**, *95*, 271–294. [CrossRef]
46. Goldstone, M.E.; Kirk, P.W.W.; Lester, J.N. The behavior of heavy-metals during waste-water treatment. I. Cadmium, chromium and copper. *Sci. Total Environ.* **1990**, *95*, 233–252. [CrossRef]
47. Goldstone, M.E.; Kirk, P.W.W.; Lester, J.N. The behavior of heavy-metals during waste-water treatment. II. Lead, nickel and zinc. *Sci. Total Environ.* **1990**, *95*, 253–270. [CrossRef]
48. Choubert, J.M.; Pomies, M.; Ruel, S.M.; Coquery, M. Influent concentrations and removal performances of metals through municipal wastewater treatment processes. *Water Sci. Technol.* **2011**, *63*, 1967–1973. [CrossRef] [PubMed]
49. Joshua, D.I.; Abeykoon, S.; Watanabe, I.; Paszek, L.; Balakrishna, K.; Akiba, M.; Guruge, K.S. Seasonal movement of trace-element discharge in a typical south-Indian suburban community. *Water Sci. Technol.* **2018**, *77*, 1035–1047. [CrossRef] [PubMed]
50. Üstün, G.E. Occurrence and removal of metals in urban wastewater treatment plants. *J. Hazard. Mater.* **2009**, *172*, 833–838. [CrossRef] [PubMed]
51. Teijon, G.; Candela, L.; Tamoh, K.; Molina-Diaz, A.; Fernandez-Alba, A.R. Occurrence of emerging contaminants, priority substances (2008/105/CE) and heavy metals in treated wastewater and groundwater at Depurbaix facility (Barcelona, Spain). *Sci. Total Environ.* **2010**, *408*, 3584–3595. [CrossRef] [PubMed]
52. Cantinho, P.; Matos, M.; Trancoso, M.A.; Correia dos Santos, M.M. Behaviour and fate of metals in urban wastewater treatment plants: A review. *Int. J. Environ. Sci. Technol.* **2016**, *13*, 359–386. [CrossRef]
53. Zhang, Q.Q.; Ying, G.G.; Pan, C.G.; Liu, Y.S.; Zhao, J.L. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: Source analysis, multimedia modeling, and linkage to bacterial resistance. *Environ. Sci. Technol.* **2015**, *49*, 6772–6782. [CrossRef]
54. Liu, X.; Sheng, H.; Jiang, S.; Yuan, Z.; Zhang, C.; Elser, J.J. Intensification of phosphorus cycling in China since the 1600s. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 2609–2614. [CrossRef]
55. Liu, M.; Chen, L.; He, Y.; Baumann, Z.; Mason, R.P.; Shen, H.; Yu, C.; Zhang, W.; Zhang, Q.; Wang, X. Impacts of farmed fish consumption and food trade on methylmercury exposure in China. *Environ. Int.* **2018**, *120*, 333–344. [CrossRef]
56. Rothenberg, S.E.; Windham-Myers, L.; Creswell, J.E. Rice methylmercury exposure and mitigation: A comprehensive review. *Environ. Res.* **2014**, *133*, 407–423. [CrossRef]
57. Hans, M.; Oscar, R. *Cadmium in the Environment*; Birkhäuser: Basel, Switzerland, 1974.
58. Mandal, B.K.; Suzuki, K.T. Arsenic round the world: A review. *Talanta* **2002**, *58*, 201–235. [CrossRef]
59. Shanker, A.K.; Cervantes, C.; Loza-Tavera, H.; Avudainayagam, S. Chromium toxicity in plants. *Environ. Int.* **2005**, *31*, 739–753. [CrossRef] [PubMed]
60. Muchuweti, A.; Birkett, J.W.; Chinyanga, E.; Zvauya, R.; Scrimshaw, M.D.; Lester, J.N. Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: Implications for human health. *Agric. Ecosyst. Environ.* **2006**, *112*, 41–48. [CrossRef]
61. Singh, R.P.; Agrawal, M. Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of Beta vulgaris plants. *Chemosphere* **2007**, *67*, 2229–2240. [CrossRef] [PubMed]
62. Singh, R.P.; Agrawal, M. Variations in heavy metal accumulation, growth and yield of rice plants grown at different sewage sludge amendment rates. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 632–641. [CrossRef] [PubMed]
63. Borjesson, G.; Kirchmann, H.; Katterer, T. Four Swedish long-term field experiments with sewage sludge reveal a limited effect on soil microbes and on metal uptake by crops. *J. Soils Sediments* **2014**, *14*, 164–177. [CrossRef]
64. Frost, H.L.; Ketchum, L.H. Trace metal concentration in durum wheat from application of sewage sludge and commercial fertilizer. *Adv. Environ. Res.* **2000**, *4*, 347–355. [CrossRef]

65. McBride, M.B. Toxic metal accumulation from agricultural use of sludge—Are usepa regulations protective. *J. Envrion. Qual.* **1995**, *24*, 5–18. [CrossRef]
66. Li, Z.P.; Han, F.X.; Su, Y.; Zhang, T.L.; Sun, B.; Monts, D.L.; Plodinec, M.J. Assessment of soil organic and carbonate carbon storage in China. *Geoderma* **2007**, *138*, 119–126. [CrossRef]
67. Tang, H.; Qiu, J.; Van Ranst, E.; Li, C. Estimations of soil organic carbon storage in cropland of China based on DNDC model. *Geoderma* **2006**, *134*, 200–206. [CrossRef]
68. Shangguan, W.; Dai, Y.; Liu, B.; Zhu, A.; Duan, Q.; Wu, L.; Ji, D.; Ye, A.; Yuan, H.; Zhang, Q.; et al. A China data set of soil properties for land surface modeling. *J. Adv. Model. Earth Syst.* **2013**, *5*, 212–224. [CrossRef]
69. Kashem, M.A.; Singh, B.R. Metal availability in contaminated soils: I. Effects of floodingand organic matter on changes in Eh, pH and solubility of Cd, Ni and Zn. *Nutr. Cycl. Agroecosyst.* **2001**, *61*, 247–255. [CrossRef]
70. Yin, Y.J.; Allen, H.E.; Li, Y.M.; Huang, C.P.; Sanders, P.F. Adsorption of mercury(II) by soil: Effects of pH, chloride, and organic matter. *J. Environ. Qual.* **1996**, *25*, 837–844. [CrossRef]
71. Zeng, F.; Ali, S.; Zhang, H.; Ouyang, Y.; Qiu, B.; Wu, F.; Zhang, G. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environ. Pollut.* **2011**, *159*, 84–91. [CrossRef] [PubMed]
72. NBS (National Bureau of Statistics). *China Environment Yearbook 2015*; National Bureau of Statistics: Beijing, China, 2015.
73. Deng, C.; Xie, H.; Ye, X.; Zhang, H.; Liu, M.; Tong, Y.; Ou, L.; Yuan, W.; Zhang, W.; Wang, X. Mercury risk assessment combining internal and external exposure methods for a population living near a municipal solid waste incinerator. *Environ. Pollut.* **2016**, *219*, 1060–1068. [CrossRef] [PubMed]
74. Bakis, R.; Tuncan, A. An investigation of heavy metal and migration through groundwater from the landfill area of Eskisehir in Turkey. *Environ. Monit. Assess.* **2011**, *176*, 87–98. [CrossRef]
75. Kanmani, S.R.G. Assessment of heavy metal contamination in soil due to leachate migration from an open dumping site. *Appl. Water Sci.* **2013**, *3*, 193–205. [CrossRef]
76. Oluyemi, E.A.; Feuyit, G.; Oyekunle, J.A.O.; Ogunfowokan, A.O. Seasonal variations in heavy metal concentrations in soil and some selected crops at a landfill in Nigeria. *Afr. J. Environ. Sci. Technol.* **2008**, *2*, 89–96.
77. Pastor, J.; Hernandez, A.J. Heavy metals, salts and organic residues in old solid urban waste landfills and surface waters in their discharge areas: Determinants for restoring their impact. *J. Environ. Manag.* **2012**, *95*, S42–S49. [CrossRef]

污水中新精神活性物质的分析方法优化及验证

陈培培, 杜鹏, 周子雷, 徐泽琼, 高婷婷, 李喜青*
(北京大学城市与环境学院, 地表过程分析与模拟教育部重点实验室, 北京 100871)

摘要: 随着对传统毒品打击力度的增大, 各种为规避现行法律管制而合成的新精神活性物质(new psychoactive substances, NPS)层出不穷. NPS 被滥用后, 经人体新陈代谢所生成的代谢产物和未被代谢的原药随尿液进入生活污水, 从而汇入污水处理厂, 经处理后被排入自然水体. 因此在国内外以往相关研究的基础上, 应用固相萃取和 UPLC-MS/MS 技术, 建立并优化了污水中 11 种常见 NPS 的前处理及检测方法. 比较了污水前处理条件(如 SPE 柱、样品 pH 值、淋洗和复溶等)对目标物回收率的影响. 结果表明最优前处理条件为:选用 Oasis MCX 柱, 在 pH = 2 的条件下加载样品, 用 2 mL pH = 2 的超纯水和 2 mL 甲醇淋洗 SPE 柱, 400 μL 20% 的甲醇水溶液复溶. 通过对目标物的保留时间、回收率、基质效应、检出限和定量限、精密度等指标的评价, 说明优化后的前处理方法和 C18-UPLC-MS/MS 检测方法高效可靠. 应用优化后的方法对北京市 11 家污水处理厂的进、出水样进行了分析, 验证了方法的可靠性, 为进一步开展 NPS 的污水流行病学研究及健康风险评价提供了参考依据.

关键词:新精神活性物质; 污水流行病学; 污水; 固相萃取; 超高效液相色谱-串联质谱联用仪
中图分类号: X830.2 **文献标识码**: A **文章编号**: 0250-3301(2018)08-0000-00 **DOI**: 10.13227/j.hjkx.201712251

Optimization and Validation of the Analytical Method to Detect New Psychoactive Substances in Wastewater

CHEN Pei-pei, DU Peng, ZHOU Zi-lei, XU Ze-qiong, GAO Ting-ting, LI Xi-qing*
(Laboratory for Earth Surface Processes, Ministry of Education, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China)

Abstract: New psychoactive substances (NPS) are emerging continuously, as the crackdown against traditional drugs becomes stricter. Metabolites of NPSs and the unchanged drugs enter wastewater through urine and are collected and treated by wastewater treatment plants before being discharged into the aquatic environment. Based on previous research, solid phase extraction (SPE) and ultra-performance liquid chromatography tandem mass spectrometry ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) were used and the detection method of 11 NPSs was optimized. Influences of wastewater pretreatment procedures, including SPE columns; pH values of water samples; and processes of flushing and redissolving on NPS recoveries were compared. It was shown that the best recovery was achieved when Oasis MCX columns were used and the pH value of wastewater samples was adjusted to 2. A flushing process with 2 mL water at pH = 2 followed by 2 mL methanol was needed. Redissolution of the residue after evaporation was best achieved with 400 μL of a 20% methanol water solution. It was indicated by retention times, recoveries, matrix effects, limits of detection, limits of quantification, as well as precision that C18-UPLC-MS/MS and the optimized method are efficient and valid. Influent and effluent samples from 11 municipal wastewater treatment plants in Beijing were analyzed using the optimized method for validation. The validated method can be used as an important reference for monitoring NPSs via wastewater-based epidemiology and for assessing the risk of NPS abuse in China.

Key words: new psychoactive substances (NPS); wastewater-based epidemiology; wastewater; solid phase extraction (SPE); ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS)

新精神活性物质(new psychoactive substances, NPS)是指为了逃避法律对传统毒品的管制或提高毒品的药理活性, 对已有毒品的化学结构加以修饰, 制成的具有类似列管毒品麻醉、兴奋或致幻作用的药物[1]. 与传统毒品相比, NPS 种类更为繁多、更新速度更快. 截至2015 年底, 已发现 NPS 的种类从 2009 年的 166 种增长到了 2015 年的 644 种, 远远超过国际管制的常规违禁药物总数(234 种)[2]. 目前发现的 NPS 主要包括以下七类:合成卡西酮类、哌嗪类、苯乙胺类、合成大麻素类、植物源类物质(如卡痛叶和鼠尾草等)、氯胺酮和其他物质(包括氨基茚满、色胺和苯环己哌啶型物质等)[2,3]. 随着 NPS 滥用的增多以及其危害性逐渐被认识, 部分 NPS 开始被一些国家列为管制药物. 我国也已将部分卡西酮类和哌嗪类物质列入管制药品[3,4].

收稿日期: 2017-12-29; **修订日期**: 2018-02-24
基金项目: 国家自然科学基金项目(41701543)
作者简介: 陈培培(1993~), 女, 硕士研究生, 主要研究方向为毒品滥用监测方法, E-mail:chenpeipei0420@126.com
* 通信作者, E-mail:xli@urban.pku.edu.cn

与人们服用的常规药物类似，NPS 被滥用后，经人体新陈代谢所生成的代谢产物和未被代谢的原药随尿液进入生活污水，从而汇入污水处理厂. 经污水厂处理后，未被去除的 NPS 被排入自然水体. 因此，污水处理厂排放被认为是环境中 NPS 的主要来源之一[5~7]. NPS 具有较强的生物活性，其大量被排入到环境中，不仅对环境造成了污染，而且带来了潜在的健康风险. 因此，NPS 也被环境学家列为一类新型污染物[8~10].

基于 NPS 带来的社会问题和环境问题，对其进行定性定量分析并估算其滥用量对于毒品管控和健康风险评价具有重要意义. 然而，传统方法如社会调查不能准确、客观的估算 NPS 的滥用量，且操作成本较高、不确定性较大. 近年来，一种叫污水流行病学的方法(wastewater-based epidemiology)被提出并用于估算毒品的滥用量. 其原理是通过测定污水处理厂进水中毒品及其代谢物浓度，通过污水厂日处理量、服务区人口及毒品排泄率等参数反算毒品滥用量. 该方法所得数据更为客观，具有时效性高、便于不同区域横向比较等优点，且已在估算传统毒品滥用量等方面发挥了巨大作用[11~14]. 近年来，已有研究团队应用该方法分析测定了污水中卡西酮类和哌嗪类 NPS，认为该方法具有预警 NPS 滥用的潜能[15,16]，并可用于评价禁毒措施的绩效性[17,18]. 但污水中其它常见 NPS(如 4-甲氧基甲基苯丙胺、2-甲基氨基-1-(3,4-亚甲二氧苯基)-1-丙酮、2,5-二甲氧基-4-苯乙胺等)的分析方法仍不完善[19~22].

为进一步完善污水中常见 NPS 的分析方法并提高分析测定效率，经调查，本研究选取国内滥用较多的 11 种 NPS 作为目标化合物，建立污水中目标物前处理及分析测定方法，并通过对北京市主城区 11 家污水处理厂进、出水样品的分析进行方法验证，以期为今后开展 NPS 的污水流行病学研究及健康风险评价奠定基础.

1 材料与方法

1.1 材料

11 种 NPS 标准品(100 μg·mL^{-1}于甲醇，纯度 > 99%)以及各自相应的氘代内标储备液(10 μg·mL^{-1}于甲醇，纯度 > 99%)购于美国 Cerilliant 公司，包括氯胺酮(KET)、甲卡西酮(MC)、麻黄碱(EPH)、4-甲氧基甲基苯丙胺(PMMA)、2-甲基氨基-1-(3,4-亚甲二氧苯基)-1-丙酮(MDMC)、2,5-二甲氧基-4-苯乙胺(2C-I)、甲氧麻黄酮(MEPH)、卡西酮(CA)、1-苄基哌嗪(BZP)、1-(3-三氟甲基苯基)哌嗪(TFMPP)和 1-(3-氯苯基)哌嗪(mCPP)，以及 KET-D4、MC-D3、EPH-D3、MEPH-D3、MDMC-D3、2C-I-^{13}C-D3、CA-D5、BZP-D7、TFMPP-D4 和 mCPP-D8.

主要仪器和耗材：12 位固相萃取装置(德国 CNW 公司)，Oasis MCX 和 Oasis HLB 固相萃取柱(均为 60 mg 3 mL，美国 Waters 公司)，超高效液相色谱-串联质谱联用仪(色谱仪：日本岛津 UFLCXR-LC，质谱仪：美国 AB Sciex API-4000)等.

1.2 质谱和液相色谱条件的优化

根据目标物的性质选择离子源和离子化模式. 根据目标物及其氘代化合物的分子量设置扫描的荷质比 *m/z* 范围，寻找目标物母离子. 利用仪器自动优化去簇电压(DP)得到最大母离子响应. 手动调节和优化碰撞能量(CE)，得到最佳响应的子离子对. 将每种目标物丰度最大的离子对作为定量离子，另一离子对作为定性离子.

采用液相色谱柱 Phenomenex Gemini C18 柱(100 mm × 2 mm，3 μm)，优化流动相 pH 值和所含盐类、洗脱梯度等条件，以获得目标物的理想峰型和高分离度.

1.3 样品前处理方法

采用固相萃取的方法对污水样品进行浓缩和净化，使用 Oasis MCX 作为 SPE 柱的样品前处理方法如图 1. 使用 Oasis HLB 作为 SPE 柱的样品前处理方法与上述基本相同，不同之处在于②Oasis HLB 柱活化：依次加入 6 mL 甲醇和 6 mL 超纯水，流速 1 ~ 2 mL·min^{-1}；⑦洗脱：用 8 mL 甲醇洗脱干燥的 HLB 柱，流速 1 ~ 2 mL·min^{-1}.

1.4 质量控制

1.4.1 回收率

分别取 20、100 和 200 μL 的 100 ng·mL^{-1}混标注入 50 mL pH = 2 的超纯水中配成样品溶液，并设置一组流程空白，同时进行前处理和测定. 根据流程空白和加标样品的测定数据及实际加标浓度计算得到 11 种 NPS 在不同浓度梯度下各自的回收率.

1.4.2 基质效应

选取 BJ-6 污水厂进水水样作为基质效应的供试水样. 对该水样进行前处理，在上机前分别向已处理的样品中加入 20、100 和 200 μL 的 100 ng·mL^{-1}混标和 100 μL 的 200 ng·mL^{-1}内标，得到浓度为 10、50 和 100 ng·mL^{-1}的样品和空白样品

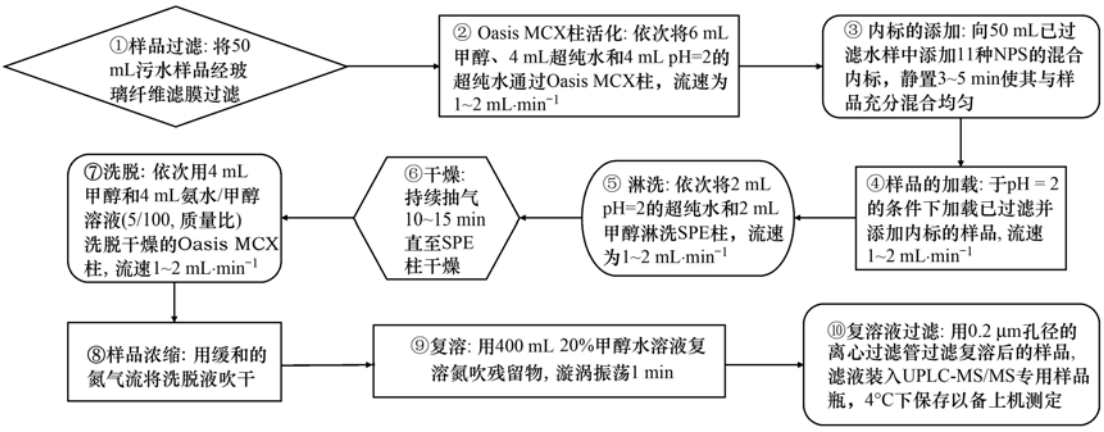


图 1　使用 Oasis MCX 作为 SPE 柱的样品前处理方法

Fig. 1　Pretreatment procedures using Oasis MCX SPE columns

(仅加入内标). 通过比较各目标物在不同浓度梯度下加标水样与空白水样的检测浓度之差和其在相应浓度标准样品中的检测浓度，评价基质对各目标物响应值的抑制和促进作用.

1.4.3　检出限和定量限

将低浓度目标物混合标准溶液上机测定，仪器检出限(ILOD)和仪器定量限(ILOQ)分别以 3 倍信噪比(3S/N)和 10 倍信噪比(10S/N)确定. 方法检出限(MLOD)和方法定量限(MLOQ)分别通过以下公式计算得到：

$$\mathrm{MLOD(或\ MLOQ)} = \frac{\mathrm{ILOD\ 或\ ILOQ} \times 400\ \mu\mathrm{L}}{500\ \mathrm{mL}}$$

式中，400 μL 为上机浓缩液的体积，50 mL 为前处理所取污水样的体积.

1.4.4　标准曲线的线性及范围

配制含甲醇体积 20%、浓度范围为 0.1 ~ 200 $\mathrm{ng\cdot mL^{-1}}$的不同浓度梯度的标准工作溶液，尽可能保证浓度范围覆盖实际污水样品中的水平.

1.4.5　精密度

分别对同一标准样品连续多次测定($n = 3$)和多天重复测定($n = 3$)来衡量仪器的日内精密度与日间精密度，对同一水样连续前处理($n = 3$)和多天重复前处理($n = 3$)来衡量前处理方法和实验操作的日内精密度与日间精密度，结果均用相对标准偏差(RSD)表示.

2　结果与讨论

2.1　优化后的液相色谱条件

本研究采用 Phenomenex Gemini C18(100 mm × 2 mm, 3 μm)液相色谱柱对目标化合物进行分离. 优化后的流动相为：0.12% 甲酸 30 $\mathrm{mmol\cdot L^{-1}}$甲酸铵超纯水溶液(A 相)；甲醇(B 相). 洗脱梯度如表 1 所示，流速为 0.3 $\mathrm{mL\cdot min^{-1}}$，柱温为 35℃，进样量为 5 μL. 优化后的液相色谱条件不仅提高了仪器对目标化合物的灵敏度，而且明显缩短了分析周期，提高了分析效率.

表 1　C18-UPLC-MS/MS 流动相洗脱梯度

Table 1　Elution gradient of the mobile phase in C18-UPLC-MS/MS

时间/min	A 相/%	B 相/%
0.0	95	5
3.0	70	30
5.0	20	80
9.5	5	95
14.0	95	5

2.2　优化后的质谱条件

本研究采用多反应监控模式(MRM)对所有目标化合物进行检测. 离子源为电喷雾离子源(ESI)，离子源电压(IS)为3 500 V，离子源温度(TEM)为 525℃，离子化模式为 ESI(+)；碰撞池气压(CAD)为 10 psi(69 kPa)，气帘气压力(CUR)为 20 psi(138 kPa)，干燥气(GS1)与辅助气(GS2)压力均为 40 psi(276 kPa). 每种目标化合物及其相应内标的母离子和定量、定性离子的核质比(m/z)、去簇电压(DP)、碰撞电压(CE)及保留时间(RT)等质谱参数列于表 2 中.

图 2 为优化后 11 种 NPS 的色谱图. 由表 2 和图 2 可知，不同目标化合物的保留时间均有较大差异，说明优化后的 C18-UPLC-MS/MS 条件能够对所选目标物进行有效分离.

2.3　优化后的样品前处理条件

2.3.1　SPE 柱和样品 pH 值

Oasis HLB 和 Oasis MCX 是环境水样前处理中广泛使用的两种 SPE 柱. 本研究分别考察两种 SPE

表 2 目标物 C18-UPLC-MS/MS 的质谱参数

Table 2 Mass spectrometric parameters of C18-UPLC-MS/MS

物质	母离子	定量离子			定性离子			RT
	m/z	*m/z*	DP/V	CE/V	*m/z*	DP/V	CE/V	/min
KET	238.1	207.1	50	20	125.1	50	38	6.07
KET-D4	242.1	129.1	60	37	—	—	—	6.07
MC	164.0	146.0	40	18	131.2	40	28	3.58
MC-D3	167.4	149.2	45	18	—	—	—	3.57
EPH	166.2	148.0	48	20	133.0	45	25	3.49
EPH-D3	169.3	151.2	40	18	—	—	—	3.48
PMMA	180.2	149.2	50	23	121.2	45	30	4.71
MEPH-D3	181.1	163.0	80	19	—	—	—	4.92
MDMC	208.2	160.0	55	25	190.1	50	16	3.98
MDMC-D3	211.3	162.9	50	23	—	—	—	3.97
2C-I	308.2	291.0	55	20	275.8	50	30	5.95
2C-I-^{13}C-D3	312.3	294.9	53	19	—	—	—	5.95
MEPH	178.1	160.0	80	19	145.2	80	29	4.93
MEPH-D3	181.1	163.0	80	19	—	—	—	4.92
CA	150.0	132.1	80	19	117.1	80	32	3.34
CA-D5	155.3	137.1	50	16	—	—	—	3.30
BZP	177.1	85.1	80	32	91.0	80	22	3.28
BZP-D7	184.1	98.0	80	32	—	—	—	3.11
TFMPP	231.0	188.0	80	33	119.0	80	42	5.84
TFMPP-D4	235.1	190.0	80	32	—	—	—	5.84
mCPP	197.0	154.1	80	27	199.2	80	35	5.47
mCPP-D8	205.1	158.1	80	31	—	—	—	5.45

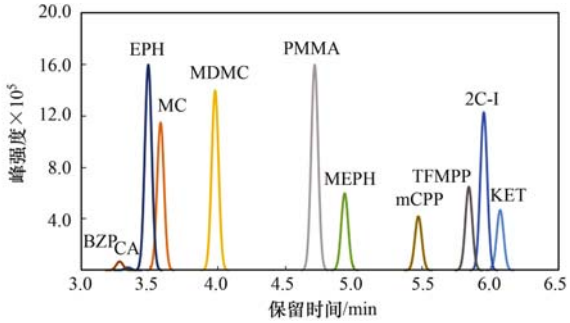


图 2 11 种 NPS 的 C18-UPLC-MS/MS 色谱图

Fig. 2 C18-UPLC-MS/MS chromatograms of the 11 NPSs

柱在不同 pH 条件下加载样品对目标 NPS 回收率的影响. Oasis HLB 柱的加载条件为 pH = 2, 7, 11; Oasis MCX 柱的加载条件为 pH = 2, 7. 按 1.3 节中所述方法进行前处理.

由表 3 可知, HLB 柱在酸性、中性和弱碱性条件下均无法对 CA 进行富集. MC、PMMA、2C-I 和 MEPH 的回收率在样品 pH 值为 2、7、11 时存在显著差异, 且回收率存在低于 80% 和高于 120% 的情况. 可见, 使用 Oasis HLB 柱对样品进行富集时, 样品 pH 值对目标物的回收率有显著影响, 且不同 NPS 的最佳 pH 值存在较大差异. 因此 Oasis HLB 柱不适宜用于测定污水中常见 NPS 的前处理过程. 而使用 Oasis MCX 柱时, 在样品 pH 值为 2 和 7 的条件下, 每种 NPS 均被有效富集, 且目标物的回收率基本在 80% ~120% 之间, 因此 Oasis MCX 柱更适合用于水样的前处理. 综合比较使用 Oasis MCX 柱时的两种样品 pH 值条件, pH = 2 时各种物质的回收率更接近于 100%, 因此本研究认为在 pH 值为 2 的条件下使用 Oasis MCX 柱更适合于含多种 NPS 水样的前处理与同步测定. 在后续实验及今后实际污水样品前处理时, 统一选用 Oasis MCX 柱并将样品 pH 值调至 2.

本研究对于 SPE 柱选择的实验结果与文献[23 ~25]的研究结论具有一致性, 即对于 CA、MC、MEPH 等 NPS 在内的毒品, 用 Oasis HLB 柱前处理的回收率和基质效应均差于 Oasis MCX 柱或不能达到实验要求. 这可能是由于 Oasis MCX 柱的基质为聚苯乙烯-二乙烯基苯高聚物, 与 Oasis HLB 吸附剂相比, 对碱性化合物具有更高的选择性和灵敏度. 而本研究所监测的 NPS 均具有含氮的碱性基团, 因此 Oasis MCX 柱能够更有效满足富集要求.

2.3.2 淋洗步骤

当目标物及其内标受基质干扰严重或者基质对两者的影响程度不一致时, 为保证目标物准确定量, 有必要在污水样品加载之后增加对 SPE 柱的淋洗步骤. 本研究在 Oasis MCX 固相萃取方法的基础上, 在

样品加载结束之后，将淋洗步骤分别改为无淋洗、2 mL pH = 2 的超纯水淋洗和 2 mL 甲醇淋洗这 3 组对照实验，研究淋洗步骤对目标物回收率的影响.

表 4 中的数据显示，无淋洗和仅用 2 mL pH = 2 的超纯水淋洗时，KET 和 MEPH 的回收率降至 80% 以下，CA 的回收率降至不足 50%；而仅用 2 mL 甲醇淋洗时 2C-I 的回收率降至了 80% 以下，CA 的回收率降至不足 55%. 这表明样品加载后先用 2 mL pH = 2 的超纯水、再用 2 mL 甲醇淋洗的两个步骤均不可或缺.

表 3　不同 SPE 柱和水样 pH 值条件下目标物的回收率比较/%

Table 3　Comparison of recoveries with different SPE columns and pH values of water samples/%

物质	Oasis HLB			Oasis MCX	
	pH = 2	pH = 7	pH = 11	pH = 2	pH = 7
KET	81.30 ± 6.90	82.33 ± 2.33	82.47 ± 3.87	81.47 ± 0.47	84.07 ± 2.47
MC	117.50 ± 16.10	94.13 ± 5.33	21.95 ± 2.85	97.07 ± 2.47	119.40 ± 2.60
EPH	85.00 ± 10.80	96.27 ± 3.47	86.80 ± 1.60	94.10 ± 2.10	92.20 ± 1.80
PMMA	88.50 ± 11.90	241.00 ± 21.00	212.00 ± 40.00	88.20 ± 10.00	79.80 ± 10.80
MDMC	109.70 ± 6.30	99.13 ± 5.73	93.80 ± 7.40	89.13 ± 2.73	94.27 ± 0.27
2C-I	50.70 ± 15.50	73.00 ± 2.80	74.87 ± 1.67	80.90 ± 1.30	75.67 ± 1.87
MEPH	84.67 ± 9.67	90.10 ± 2.90	70.90 ± 4.10	90.60 ± 7.60	89.80 ± 5.80
CA	0	0	0	74.80 ± 3.80	70.53 ± 6.13
BZP	83.87 ± 1.27	89.60 ± 2.60	92.10 ± 4.70	91.93 ± 3.73	95.20 ± 1.20
TFMPP	81.50 ± 4.90	104.53 ± 8.13	109.20 ± 1.80	90.10 ± 1.70	101.40 ± 1.20
mCPP	74.60 ± 7.20	82.40 ± 0.20	83.27 ± 4.27	75.67 ± 3.67	92.20 ± 5.00

表 4　不同淋洗和复溶条件下目标物的回收率比较/%

Table 4　Comparison of recoveries under different washing and redissolving conditions/%

物质	淋洗步骤			复溶步骤	
	无	水	甲醇	200 μL 20% 甲醇水溶液	80 μL 甲醇 + 320 μL 水
KET	78.00 ± 4.80	78.20 ± 2.20	82.27 ± 0.67	89.27 ± 2.67	87.47 ± 2.87
MC	92.80 ± 4.60	100.33 ± 5.33	110.90 ± 5.70	94.00 ± 4.00	94.47 ± 1.67
EPH	84.60 ± 8.80	88.60 ± 1.20	92.93 ± 5.53	89.53 ± 2.93	90.33 ± 3.73
PMMA	85.10 ± 8.50	81.00 ± 8.20	99.27 ± 6.27	176.73 ± 17.67	187.33 ± 23.93
MDMC	87.47 ± 6.27	85.53 ± 4.53	93.20 ± 6.00	97.73 ± 2.33	94.13 ± 0.93
2C-I	76.40 ± 1.40	78.90 ± 0.90	76.00 ± 5.80	77.93 ± 1.33	83.20 ± 2.00
MEPH	75.00 ± 1.40	77.13 ± 2.53	86.00 ± 5.40	85.53 ± 2.33	79.67 ± 1.47
CA	49.93 ± 4.93	48.47 ± 7.47	52.87 ± 2.27	94.93 ± 2.53	92.87 ± 9.87
BZP	87.73 ± 2.53	89.00 ± 2.20	87.60 ± 2.00	104.53 ± 3.53	98.00 ± 0.60
TFMPP	88.73 ± 4.13	88.93 ± 5.33	96.93 ± 4.13	100.27 ± 2.27	102.27 ± 1.67
mCPP	71.60 ± 5.00	72.33 ± 3.73	78.07 ± 3.27	95.00 ± 2.00	93.93 ± 1.73

2.3.3　复溶溶剂的量与复溶步骤

为研究复溶条件的改变对目标物回收率的影响，本研究设置两组对照实验，分别为：①200 μL 20% 甲醇水溶液复溶氮吹残留物 1 min，②先用 80 μL 甲醇复溶 30 s，再加入 320 μL 超纯水复溶 30 s.

由表 4 中不同复溶条件下的回收率数据可知，改变复溶溶剂的量和步骤后，PMMA 的回收率均超过 120%，远超可接受范围. 因此本研究认为，在现有的固相萃取和 C18-UPLC-MS/MS 条件下，采用 400 μL 20% 甲醇水溶液复溶氮吹残留物 1 min 最为合适.

2.4　对已优化方法的评价

2.4.1　回收率和基质效应

如表 5 所示，在基本覆盖了实际污水中 NPS 检出浓度的各种条件下，每种目标物的回收率基本上均在 80% ~ 120% 之间，基质效应基本上均在?20% ~20% 之间. 结果总体表明，本研究采用上述方法进行前处理和样品测定是可行的.

2.4.2　检出限、定量限、标准曲线线性及范围

表 6 中的结果显示，各种目标物的 LOD 和 LOQ 均很低，基本满足实际污水样品测定的要求. 所有目标物的标准曲线相关系数均在 0.99 以上，且基本所有浓度梯度下的准确度均在 85% ~115% 之间、RSD < 15%，因此标准曲线可以用于实际污水中 NPS 浓度的测定.

2.4.3　精密度

由表 7 可知，仪器精密度的日内和日间 RSD 均小于 2.5%，方法精密度的日内和日间 RSD 均小于

8%，表明仪器的稳定性和实验方法的可重复性均符合要求. 综上，应用本研究中优化的 SPE 前处理方法和 C18-UPLC-MS/MS 测定方法可以用于污水样品中的多种 NPS 同步分析.

表 5 SPE-C18-UPLC-MS/MS 优化方法的回收率和基质效应/%

Table 5 Recoveries and matrix effects in SPE-C18-UPLC-MS/MS/%

物质	回收率			基质效应		
	10 ng·mL^{-1}	50 ng·mL^{-1}	100 ng·mL^{-1}	10 ng·mL^{-1}	50 ng·mL^{-1}	100 ng·mL^{-1}
KET	82.67 ± 1.27	81.47 ± 0.47	81.73 ± 5.73	−13.20 ± 1.70	−18.40 ± 0.80	−11.63 ± 1.67
MC	119.50 ± 1.50	97.07 ± 2.47	87.45 ± 0.65	−19.90 ± 2.30	−26.60 ± 6.50	−26.30 ± 3.20
EPH	92.87 ± 1.27	94.10 ± 2.10	96.30 ± 2.00	−21.67 ± 16.50	−23.73 ± 6.20	−25.47 ± 5.50
PMMA	103.70 ± 5.30	88.20 ± 10.00	108.33 ± 13.33	2.25 ± 16.88	13.80 ± 12.00	15.40 ± 4.30
MDMC	97.75 ± 1.05	89.13 ± 2.73	94.63 ± 1.23	−16.20 ± 6.20	−12.20 ± 1.60	−11.37 ± 3.73
2C-I	75.95 ± 0.65	80.90 ± 1.30	75.40 ± 5.00	−24.40 ± 5.60	−28.80 ± 2.00	−21.00 ± 0.10
MEPH	87.35 ± 1.45	90.60 ± 7.60	82.27 ± 3.37	−26.70 ± 18.00	−21.00 ± 14.90	−23.10 ± 1.20
CA	80.80 ± 15.10	74.80 ± 3.80	78.60 ± 8.90	−6.70 ± 3.40	−18.10 ± 6.00	−16.30 ± 1.10
BZP	100.45 ± 5.55	91.93 ± 3.73	95.40 ± 1.70	−19.40 ± 3.70	−29.40 ± 6.80	−22.50 ± 1.20
TFMPP	94.80 ± 3.80	90.10 ± 1.70	97.25 ± 0.65	−15.90 ± 6.10	−22.40 ± 3.40	−18.40 ± 8.40
mCPP	79.00 ± 3.50	75.67 ± 3.67	86.90 ± 5.20	−16.30 ± 10.90	−13.87 ± 4.53	−8.17 ± 5.63

表 6 SPE-C18-UPLC-MS/MS 优化方法的检出限、定量限、标准曲线线性及范围

Table 6 LOD, LOQ, linearity and range of the standard curve in SPE-C18-UPLC-MS/MS

物质	检出限 LOD		定量限 LOQ		标准曲线	
	ILOD/ng·mL^{-1}	MLOD/ng·L^{-1}	ILOQ/ng·mL^{-1}	MLOQ/ng·L^{-1}	相关系数(r^2)	线性范围/ng·mL^{-1}
KET	0.05	0.20	0.10	0.40	0.998 3	0.10 ~ 200
MC	0.05	0.20	0.10	0.40	0.992 0	0.10 ~ 200
EPH	0.05	0.20	0.10	0.40	0.997 5	0.10 ~ 200
PMMA	0.05	0.20	0.10	0.40	0.997 7	0.10 ~ 200
MDMC	0.05	0.20	0.10	0.40	0.997 5	0.10 ~ 200
2C-I	0.05	0.20	0.10	0.40	0.996 5	0.10 ~ 200
MEPH	0.05	0.20	0.10	0.40	0.994 9	0.10 ~ 200
CA	0.50	2.00	1.00	4.00	0.997 6	1.00 ~ 200
BZP	0.10	0.40	0.50	2.00	0.996 0	0.50 ~ 200
TFMPP	0.05	0.20	0.10	0.40	0.997 1	0.10 ~ 200
mCPP	0.10	0.40	0.50	2.00	0.996 3	0.50 ~ 200

表 7 仪器精密度和 SPE-C18-UPLC-MS/MS 优化方法的精密度/%

Table 7 Precision of SPE-C18-UPLC-MS/MS and the optimized method/%

物质	仪器精密度/ng·mL^{-1}						方法精密度/ng·mL^{-1}					
	10		50		100		10		50		100	
	日内 RSD	日间 RSD	日内 RSD	日间 RSD	日内 RSD	日间 RSD	日内 RSD	日间 RSD	日内 RSD	日间 RSD	日内 RSD	日间 RSD
KET	1.00	1.00	1.60	1.15	0.30	0.65	1.80	2.00	1.00	1.20	3.20	4.60
MC	1.70	1.60	1.40	1.80	2.40	1.75	3.00	6.90	1.20	3.20	5.00	1.30
EPH	1.40	2.46	2.00	2.35	1.00	2.10	2.60	2.70	4.20	4.30	4.00	3.20
PMMA	1.30	1.30	0.80	1.60	0.70	1.80	1.06	3.99	3.90	3.90	5.00	2.95
MDMC	2.00	1.70	0.60	2.00	0.50	1.00	2.10	1.67	4.60	1.80	1.90	1.80
2C-I	0.80	0.54	1.90	1.40	0.60	1.70	1.30	7.14	6.00	2.60	1.60	6.70
MEPH	1.00	1.00	1.40	0.60	2.40	1.70	2.90	6.42	4.00	7.60	1.90	7.20
CA	1.00	0.50	1.40	0.60	2.40	2.30	2.30	4.40	3.80	4.20	2.10	2.50
BZP	1.00	1.20	2.30	1.00	1.00	2.30	1.40	1.40	3.20	4.80	3.40	3.90
TFMPP	1.00	1.80	1.50	1.70	1.00	0.80	7.60	2.90	3.40	6.50	1.30	2.90
mCPP	2.00	0.70	1.20	1.70	1.00	2.00	5.80	7.00	1.80	7.40	5.00	5.30

3 在实际污水样品中的应用

本研究于 2016 年 12 月对北京市 11 家污水处理厂的进水和出水进行了样品采集，每家污水厂至少采集 2 d 以上的样品，采用全自动水质采样器采集 24 h 混合水样. 全部 11 家污水厂日均污水流量约为 26 万 m^3，服务区总人口达1 096万人，约占北京市常住人口的 50.4%. 采用上述优化的方法对样

品进行前处理和分析测定，根据进水和出水中 NPS 浓度的差别计算各种目标物的去除率.

测定结果显示，MDMC、MEPH、CA、BZP 和 mCPP 在 11 家污水厂的进水和出水样品中均未被检出，表明这几种 NPS 在北京市可能尚无显著滥用趋势. 其余 6 种 NPS 在各污水厂进水样品中的平均浓度如图 3 所示. 其中，EPH 在进水中的浓度水平最高，浓度范围在 84.23 ~ 383.6 ng·L^{-1}；其次为 PMMA，浓度范围在 9.12 ~ 38.48 ng·L^{-1}. 值得注意的是，2C-I 虽然仅在 BJ-4 检出，但其在进水中的浓度高达 92 ng·L^{-1}，说明在该厂服务区内可能存在 2C-I 的滥用或者倾倒现象. 进水中 KET、MC 和 TFMPP 的浓度均低于 8 ng·L^{-1}. 实际污水样品测定结果表明，本研究中的优化方法能够基本满足污水中的 NPS 检测要求.

这 11 家污水处理厂对每种目标物的去除率汇总于表 8 中. 数据显示，BJ-5、BJ-9 和 BJ-11 中目标物去除率较高，而其余污水厂对多数目标物的去除率并不理想，甚至出现了负去除率的情况. 造成负去除率的原因可能是污水在污水处理厂中存在长达数小时的停留时间，因此进、出水中测得的目标物浓度水平无法准确对应. 但总体看来，这一结果仍表明常见的污水处理工艺对 NPS 的去除能力有限，有相当数量的NPS将最终进入河流湖海中，对水环境生态形成潜在的威胁.

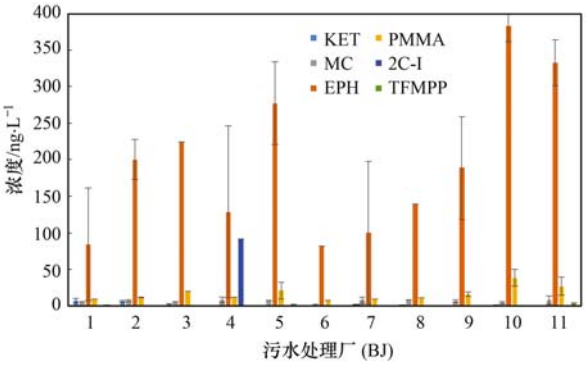


图 3　北京市 11 家污水处理厂进水中的 NPS 浓度

Fig. 3　Concentrations of NPSs in influent wastewaters of 11 wastewater treatment plants in Beijing

表 8　北京市 11 家污水处理厂对 NPS 的去除率/%

Table 8　Removal rates of NPSs in 11 wastewater treatment plants in Beijing/%

污水厂	KET	MC	EPH	PMMA	2C-I	TFMPP
BJ-1	45.08	-31.09	-55.18	-23.68	—	-15.30
BJ-2	69.24	100.00	98.42	100.00	—	—
BJ-3	55.43	-71.47	-14.64	17.48	—	—
BJ-4	—	1.53	-4.94	34.08	-20.87	—
BJ-5	—	86.43	97.99	100.00	—	100.00
BJ-6	—	-84.17	-124.51	-191.18	—	—
BJ-7	27.29	48.90	-25.47	-144.63	—	—
BJ-8	-19.67	36.23	44.40	70.28	—	—
BJ-9	—	100.00	98.79	94.77	—	—
BJ-10	-286.90	42.59	96.42	100.00	—	—
BJ-11	—	100.00	100.00	100.00	—	44.79

4　结论

(1)对污水样品前处理方法进行了优化，表明应选用 Oasis MCX 柱，预先调节样品至 pH = 2，样品加载完后先用 2 mL pH = 2 的超纯水淋洗、再用 2 mL 甲醇淋洗 Oasis MCX 柱，抽干后氮吹，最后用 400 μL 20% 甲醇水溶液复溶残留物 1 min.

(2)11 种 NPS 的保留时间、回收率、基质效应、检出限、定量限、精密度等方法评价指标数据表明，本研究所确定的 SPE 前处理方法和 C18-UPLC-MS/MS 测定方法分离效果好、回收率高、基质效应弱、检出限和定量限低、仪器和方法精密度高，可用于实际污水样品中 NPS 浓度的检测.

(3)对北京市 11 家污水厂进、出水样品中 NPS 浓度的测定结果显示，优化的前处理和测定方法能够基本满足分析要求. EPH、PMMA 和 2C-I 在进水中的浓度较高. 多数目标物在污水厂中的去除率并不理想，NPS 随出水进入河流等生态环境，产生潜在威胁.

参考文献：

[1] 游彦，邓毅，赵敏. 第三代毒品——新精神活性物质(NPS)发展趋势评估、管制瓶颈与应对策略[J]. 四川警察学院学报，2017，**29**(1)：97-102.
You Y，Deng Y，Zhao M. On the growing trend assessment，bottleneck of regulation and countermeasure of NPS，the third generation drug[J]. Journal of Sichuan Police College，2017，**29**(1)：97-102.

[2] United Nations Office on Drugs and Crime. World drug report [R]. New York：United Nations Office on Drugs and Crime，

2016.

[3] 张黎, 张拓. 新精神活性物质的滥用危害与防控问题研究——以构建我国禁毒防控体系为视角[J]. 中国人民公安大学学报(社会科学版), 2013, **29**(4): 88-96.

[4] 王佳宁. 国家禁毒办: 我国列管新精神活性物质达 138 种[EB/OL]. http://news.xinhuanet.com/legal/2017-06/19/c_129636093.htm, 2017-06-195.
Wang J N. Office of China Narcotics Control Commission: the number of new psychoactive substances in the control list reached 138[EB/OL]. http://news.xinhuanet.com/legal/2017-06/19/c_129636093.htm, 2017-06-19.

[5] 张艳, 张婷婷, 陈卫平, 等. 北京市水环境中精神活性物质污染特征[J]. 环境科学, 2017, **38**(7): 2819-2827.
Zhang Y, Zhang T T, Chen W P, *et al*. Distribution characteristics of drugs of abuse and their metabolites in aqueous environment of Beijing, China [J]. Environmental Science, 2017, **38**(7): 2819-2827.

[6] Gao T T, Du P, Xu Z Q, *et al*. Occurrence of new psychoactive substances in wastewater of major Chinese Cities[J]. Science of the Total Environment, 2017, **575**: 963-969.

[7] Fedorova G, Randak T, Golovko O, *et al*. A passive sampling method for detecting analgesics, psycholeptics, antidepressants and illicit drugs in aquatic environments in the Czech Republic [J]. Science of the Total Environment, 2014, **487**: 681-687.

[8] 杨红莲, 袭著革, 闫峻, 等. 新型污染物及其生态和环境健康效应[J]. 生态毒理学报, 2009, **4**(1): 28-34.
Yang H L, Gong Z G, Yan J, *et al*. Ecological and environmental health effects of emerging contaminant of concern [J]. Asian Journal of Ecotoxicology, 2009, **4**(1): 28-34.

[9] Kasprzyk-Hordern B, Dinsdale R M, Guwy A J. Illicit drugs and pharmaceuticals in the environment - Forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities[J]. Environmental Pollution, 2009, **157**(6): 1773-1777.

[10] González Alonso S, Catalá M, Romo Maroto R R, *et al*. Pollution by psychoactive pharmaceuticals in the Rivers of Madrid metropolitan area (Spain) [J]. Environment International, 2010, **36**(2): 195-201.

[11] Lai F Y, Ort C, Gartner C, *et al*. Refining the estimation of illicit drug consumptions from wastewater analysis: Co-analysis of prescription pharmaceuticals and uncertainty assessment [J]. Water Research, 2011, **45**(15): 4437-4448.

[12] Li J, Hou L L, Du P, *et al*. Estimation of amphetamine and methamphetamine uses in Beijing through sewage-based analysis [J]. Science of the Total Environment, 2014, **490**: 724-732.

[13] Khan U, Van Nuijs A L N, Li J, *et al*. Application of a sewage-based approach to assess the use of ten illicit drugs in four Chinese megacities[J]. Science of the Total Environment, 2014, **487**: 710-721.

[14] Ort C, Van Nuijs A L, Berset J D, *et al*. Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis [J]. Addiction, 2014, **109**(8): 1338-1352.

[15] Reid M J, Baz-Lomba J A, Ryu Y, *et al*. Using biomarkers in wastewater to monitor community drug use: A conceptual approach for dealing with new psychoactive substances [J]. Science of the Total Environment, 2014, **487**: 651-658.

[16] Kinyua J, Covaci A, Maho W, *et al*. Sewage-based epidemiology in monitoring the use of new psychoactive substances: Validation and application of an analytical method using LC-MS/MS [J]. Drug Testing and Analysis, 2015, **7**(9): 812-818.

[17] Burgard D A, Banta-Green C, Field J A. Working upstream: how far can you go with sewage-based drug epidemiology? [J] Environmental Science & Technology, 2014, **48**(3): 1362-1368.

[18] Castiglioni S, Thomas K V, Kasprzyk-Hordern B, *et al*. Testing wastewater to detect illicit drugs: state of the art, potential and research needs [J]. Science of the Total Environment, 2014, **487**: 613-620.

[19] González-Mariño I, Gracia-Lor E, Rousis N I, *et al*. Wastewater-based epidemiology to monitor synthetic cathinones use in different European countries[J]. Environmental Science & Technology, 2016, **50**(18): 10089-10096.

[20] Andrés-Costa M J, Andreu V, Picó Y. Analysis of psychoactive substances in water by information dependent acquisition on a hybrid quadrupole time-of-flight mass spectrometer[J]. Journal of Chromatography A, 2016, **1461**: 98-106.

[21] Tscharke B J, Chen C, Gerber J P, *et al*. Temporal trends in drug use in Adelaide, South Australia by wastewater analysis [J]. Science of the Total Environment, 2016, **565**: 384-391.

[22] Andrés-Costa M J, Andreu V, Picó Y. Liquid chromatography-mass spectrometry as a tool for wastewater-based epidemiology: assessing new psychoactive substances and other human biomarkers[J]. TrAC Trends in Analytical Chemistry, 2017, **94**: 21-38.

[23] Baz-Lomba J A, Reid M J, Thomas K V. Target and suspect screening of psychoactive substances in sewage-based samples by UHPLC-QTOF[J]. Analytica Chimica Acta, 2016, **914**: 81-90.

[24] Van Nuijs A L N, Gheorghe A, Jorens P G, *et al*. Optimization, validation, and the application of liquid chromatography-tandem mass spectrometry for the analysis of new drugs of abuse in wastewater[J]. Drug Testing and Analysis, 2014, **6**(7-8): 861-867.

[25] Styszko K, Dudarska A, Zuba D. The presence of stimulant drugs in wastewater from Krakow (Poland): a snapshot[J]. Bulletin of Environmental Contamination and Toxicology, 2016, **97**(3): 310-315.

荣誉证书

杜 鹏

被评选为北京大学二零一七年度优秀博士后，特发此证。

北京大学

2017年12月31日

CCTV. 节目官网

大家都在搜

登录 | 注册 | 客服 返回央视网 >>

PC客户端 移动客户端

首页 直播 节目单 频道大全 栏目大全 片库 4K专区 热榜 看点 主持人

CCTV-13新闻频道 共同关注

[共同关注]缉毒新技术：污水验毒 追查毒源

来源：央视网 2017年06月26日 18:46

CCTV. 节目官网

大家都在搜

登录 | 注册 | 客服 返回央视网 >>

PC客户端 移动客户端

首页 直播 节目单 频道大全 栏目大全 片库 4K专区 热榜 看点 主持人

CCTV-13新闻频道 共同关注

[共同关注]缉毒新技术：污水验毒 追查毒源

来源：央视网 2017年06月26日 18:46


CZ 广州
iQIYI 爱奇艺
污水能验毒！佛山成立污水监测毒情实验室
午间新闻
通过在酒吧 KTV等场所
6月26日 星期二
中央第五环境保护督查组于6月5日至7月5日进驻广东开展环境保护督察“回头看”


腾讯视频
李喜青 北京大学城市与环境学院研究员
采样来分析其中的毒品

查污水就可知毒情 中山成为全国首个“污水验毒”试点

2017年06月24日 14:24
来源：凤凰网广东

原标题：市公安局与北京大学环境法医学联合实验室昨成立　中山成为全国首个“污水验毒”试点

来源：中山日报

本报讯　昨日，市公安局、北京大学城市与环境学院举行了环境法医学联合实验室签约仪式。副市长、市公安局局长郑泽晖和北京大学城市与环境学院党委书记刘耕年签署了建立环境法医学联合实验室框架协议和禁毒合作框架协议。据悉，这是北京大学城市与环境学院与地方公安合作成立的国内第一个毒品环境法医学实验室，标志着中山成为全国首个通过污水验毒监测毒情新模式的试点。

6年前，北京大学城市与环境学院课题组就开始研究“污水验毒”新项目，通过分析环境中的毒品检测毒品滥用以及涉毒的犯罪活动。以前判断一个地方毒情形势，往往是通过问卷调查或是访问，也有的是根据打击查获毒品的结果加以判断，有其局限性，无法知道一座城市或某个区域真实的毒情。课题组提出一种新思路和方法，通过测定污水中的毒品或其代谢产物的浓度，结合污水的流量等来反推区域内毒品的滥用程度。

课题组负责人、北京大学城市与环境学院教授李喜青告诉记者，要了解毒情，传统的方法是通过问卷调查或是访问，有其局限性，主观影响很大，时间长成本高。而从查获的毒品来判断滥用情况，也无法知道真实的毒情。环境科学界提出一种新思路，就是通过测定污水中的毒品或者其代谢产物的浓度。李喜青说，人吸食了毒品就会代谢，代谢产物通过尿液进入下水道，再到生活污水处理厂。课题组就在污水处理厂的入水口采样，然后通过灵敏度极高的液相色谱串联质谱技术分析检测。这种技术检测的速率很高，基本上当时就可以出结果，且接错率极低。

刘耕年表示，课题组在未知毒物鉴定、污染源追溯和污染损害鉴定等环境法医学研究领域处于国内领先水平，率先开创了通过分析污水和地表水中毒品监测毒情的方法，取得了一系列重要进展，部分成果居于国际领先水平。希望与中山的合作，不仅为中山禁毒工作和智慧公安建设作出贡献，也为广东乃至全国禁毒工作树立典范。

郑泽晖说，“6·26”国际禁毒日前夕，市公安局与北京大学城市与环境学院签订环境法医学联合实验室框架协议和禁毒合作框架协议，是我市公安实战部门与专业院校科研技术的一次深度协作，将进一步提升我市追溯环境污染源、鉴定环境损害和毒品滥用情况监测等能力。接下来，市公安局将积极配合北京大学的工作团队，依托与北大签订的禁毒框架协议和环境法医学联合实验室，深化污水检测在禁毒领域的应用。

发明专利证明复印件

复合型纳米零价铁颗粒

申请号：201510176103.8
申请日：2015-04-15

申请(专利权)人 刘骁勇

地址 250013 山东省济南市解放路62号山东职业学院培训基地办公楼108室

发明(设计)人 李静 刘骁勇 杜鹏

主分类号 B22F1/02(2006.01)I

分类号 B22F1/02(2006.01)I B09C1/08(2006.01)I B82Y30/00(2011.01)I

公开(公告)号 104827028A

公开(公告)日 2015-08-12

专利代理机构

代理人