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Wastewater-based epidemiology and enantiomeric profiling for drugs of abuse in South African wastewaters

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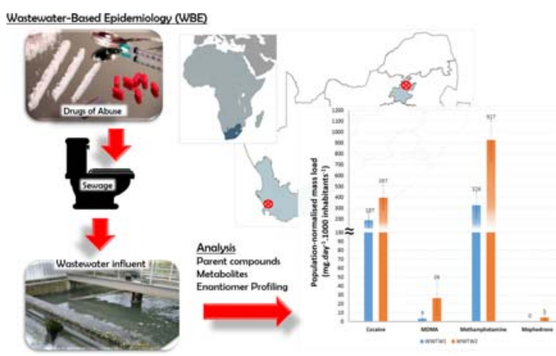
HIGHLIGHTS

- Wastewater-based epidemiology (WBE) was done for the first time in Africa.
- The chiral signature and metabolite ratios suggest predominant consumption.
- Methamphetamine was the primary drug of abuse.
- Cocaine and MDMA were shown as secondary drugs of abuse.
- Mephedrone and Heroin use was qualitatively confirmed within one study area.

GRAPHICAL ABSTRACT



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ABSTRACT

The current study is aimed to introduce a wastewater-based epidemiology (WBE) approach for the first time on the African continent where substance abuse data is limited. The study included the quantification of several drugs of abuse (DOA) in raw wastewater samples. Quantification of urinary metabolites as drug target residues (DTR), as well as enantiomeric profiling of chiral DOA was performed to distinguish between consumption and direct disposal into sewage. Monitoring campaigns were undertaken at two South African wastewater treatment works (WWTWs) located within two provinces of the country. The presence of non-racemic 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine, as well as the metabolite of cocaine, benzoylecgonine (BEG), confirmed their consumption within the areas investigated. Enantiomeric profiling further pointed to the abuse of methamphetamine as the primary DOA with use estimates calculated between 181.9 and 1184.8 $mg \cdot day^{-1} \cdot 1000$ $inhabitants^{-1}$. Population-normalised mass loads for MDMA and cocaine confirmed their status as secondary DOA within the study sites. Use estimates for the new psychoactive substance (NPS) mephedrone were performed for one WWTW. The minor metabolite of heroin, *O*-6-monoacetylmorphine (*O*-6-MAM), was also detected at one WWTW and served as a qualitative indicator for heroin abuse within the area. These findings provide a novel comparison of the WBE approach in a developing-country with other global studies, with the aim to strengthen this approach as a tool to inform drug prevention strategies in countries where substance abuse data is limited due to financial constraints and lack of government structures to facilitate conventional monitoring.

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1. Introduction

Drug use and abuse have notable socio-economic consequences that globally impede sustainable development among communities (UNODC, 2016). Such drugs of abuse (DOA) are not only limited to illicit substances, but also include prescription and over-the-counter (OTC) medications, which have the potential to cause addiction through their designed physiological mechanisms of action. However, the methods to collate information on drug use are largely limited to substance abuse treatment centres and law enforcement reports, which may lead to inaccurate or underestimations of drug abuse. Wastewater-based epidemiology (WBE) has shown great promise to assist with such constraints by providing a near-real time profile of substance abuse (Castiglioni et al., 2016).

As with any consumed product, DOAs are excreted in sewage either as its parental form, or as primary and secondary metabolites, depending on their metabolic pathways in the body. These substances are then transported through the connected sewage network to wastewater treatment works (WWTWs). Apart from their role in the degradation of a large variety of organic pollutants, WWTWs may thus also serve as composite sampling sites for the chemical profiling of wastewater as a non-intrusive tool to estimate drug use and abuse within the communities connected to the sewer system (Castiglioni et al., 2014; Daughton, 2001). However, several discrepancies to this approach have been discussed (Castiglioni et al., 2016), which include the fate of the parent drug in wastewater, as well as the distinction between drug consumption and direct disposal into the recipient waters (Kasprzyk-Hordern and Baker, 2012a). For this reason, the inclusion of metabolic breakdown products as drug target residues (DTR) were proposed to address this limitation by serving as more stable DTRs for consumption estimates, as well as confirming whether the drug has undergone metabolic breakdown due to consumption (Petrie et al., 2016).

Apart from the benefits of establishing DOA metabolite loads in wastewater, the enantiomeric profiling of chiral DOA may also be used to distinguish between direct disposal, consumption, and manufacturing (Camacho-muñoz et al., 2016; Emke et al., 2014; Kasprzyk-Hordern and Baker, 2012a, 2012b; Petrie et al., 2016). Some DOA, such as 3,4-methylenedioxymethamphetamine (MDMA) and mephedrone are manufactured in their racemic form, from which the enantiomers will follow different metabolic pathways and excretion patterns within the body, which leads to a non-racemic mixture in sewage (Castrignanò et al., 2017a; Kasprzyk-Hordern and Baker, 2012a). Therefore, if the enantiomeric composition of the drug is racemic in the wastewater sample, it might indicate direct disposal of the drug rather than its consumption (Emke et al., 2014). In contrast, the manufacturing of some chiral illicit drugs, such as methamphetamine, is primarily enantioselective (Castrignanò et al., 2017b; Xu et al., 2017), as the potency and desired physiological effects differ between the chiral isoforms. For methamphetamine, the *S*-enantiomer is the predominant form to represent an illicit origin, which has also been confirmed during WBE (Castrignanò et al., 2017b; Xu et al., 2017). However, it has been reported that both enantiomers may also be associated with illicit methamphetamine use, depending on the method of synthesis and trafficking. For example, a racemic mixture of methamphetamine was detected in Norwegian wastewater samples in contrast to other European countries where wastewater was predominantly enriched with the S-enantiomer (Castrignanò et al., 2017b). The authors highlighted that this occurrence was due to the known differences in manufacturing and trafficking of the drug between countries. Establishing the enantiomeric signature of chiral DOA in wastewater therefore provide an added value to WBE for improved drug enforcement strategies, substance abuse estimates, and information to social services.

WBE has been applied in many countries to date (Castiglioni et al., 2014; Devault et al., 2017; Emke et al., 2014; Evans et al., 2016; Lai et al., 2017; Ort et al., 2014; Petrie et al., 2016; Subedi and Kannan, 2014; Xu et al., 2017). Ironically, the value of implementing such an overarching approach to monitor drug abuse in African countries are lacking, where it may arguably provide an effective means to fill a void left by a chronic shortages in funding and human capacity. Given the current state of substance abuse within developing countries, as well as limited drug use statistics, assessment tools such as WBE are needed to assist with future drug use prevention strategies. Recent reports have highlighted an increase in the abuse of illicit drugs in South Africa (Dada et al., 2017; USDS, 2017), with these substances shown to be present in wastewater (Archer et al., 2017). The aim of the current study was therefore (i) to monitor the loads of common illicit drugs (cocaine, methamphetamine, MDMA and heroin), as well as the new psychoactive substance (NPS) mephedrone at two South African WWTWs in order to estimate the drug use patterns within communities serviced by the sewage systems, (ii) to lay a foundation for local drug monitoring programmes and (iii) to adopt this approach that should facilitate a common 'language' with nascent programmes in developed countries.

2. Methods

2.1. Sampling locations

Two WWTWs were selected for a consecutive 7-day sampling campaign during 2017 (Fig. 1). WWTW1 is Gauteng Province serving one city in the East Rand district adjacent to the city of Johannesburg, and WWTW2 located in the Western Cape Province of South Africa serving several suburbs around the city of Cape Town (Fig. 1). The information for the plants are shown in the Supplementary information (Table S1). The current *de facto* population estimate (PE) for the WWTWs were estimated from population growth projections since the last national census campaign in 2011, which resulted in a PE of 200,000 for WWTW1 and 470,000 for WWTW2.

2.2. Chemicals and consumables

The study included the multi-residue quantification for 16 DTRs (cocaine, benzoylecgonine, cocaethylene, (±)-amphetamine, (±)-methamphetamine, (±)-mephedrone, (±)-ephedrine, (±)-pseudoephedrine, norephedrine, (±)-MDMA, (±)-HMMA, (±)-HMA, heroin, *O*-6-monoacetylmorphine, morphine and normorphine) using analytical methods described elsewhere (Castrignanò et al., 2016), which is summarised in the supplementary information (Fig. S1). The following internal standards were included in the water samples to assist with quantification: cocaine-d3, benzoylecgonine-d8, cocaethylene-d3, rac-amphetamine-d5, rac-methamphetamine-d5, rac-mephedrone-d3, rac-MDMA-d5, heroin-d9, *1S,2R*-(+)-ephedrine-d3, morphine-d6 and PCP-d5. Methanol (MeOH, HPLC-grade; Sigma) and ultra-pure water (Millipore) were used for cleaning glassware and for solid phase extraction (SPE). All glassware were deactivated using 5% dimethyldichlorosilane (DMDCS) in toluene, followed by two wash steps in toluene, and three wash steps in MeOH. Acetonitrile, DMDCS, MeOH and ammonium acetate were all purchased from Sigma-Aldrich.

2.3. Sample collection and preparation

Raw wastewater samples from the two study sites were taken over a period of seven consecutive days during the month of March 2017. Briefly, composite samples (100 mL every 10 min) were taken over a 24 hour period (9 am to 9 am) using a time-and-volume-proportional composite sampler (Aquacell, Aquamatic Ltd., UK) at the raw sewage inlet after the grit screens. The samples were kept cold during sampling and transportation to the laboratory, from which sample filtration and extraction were completed upon arrival. Duplicate raw wastewater samples (50 mL each) from each sampling locality and day were filtered using 0.7 μm glass microfiber filters (grade GF/F; Whatman®, Sigma-