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WASTEWATER ANALYSIS OF ILLICIT SUBSTANCES AS A MEANS OF DETECTING SUBSTANCE ABUSE

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Abstract

Wastewater-Based Epidemiology (WBE) is a rapidly developing discipline with the potential to provide near real-time data on regional and temporal variations in the use of various legal substances, including nicotine, alcohol, caffeine, and illicit drugs. Since the early 2000s, wastewater analysis has been increasingly utilized to gauge the prevalence of illicit drug use within society. Reasons for this adoption include the method's efficiency, the ease of sample acquisition, and the cost-effectiveness of analysis. Wastewater analysis yields estimated consumption data at the provincial level by detecting target substances within the influent of provincial wastewater treatment plants (WWTPs) and subsequently performing back-calculations. WWTP selection for analysis involves a careful evaluation of factors such as the basins/populations served, infrastructure compatibility and the feasibility of 24-hour composite sampling. Following the collection of both influent and effluent water samples from designated WWTPs, a solid-phase extraction (SPE) method is employed for sample preparation. Liquid chromatography-tandem mass spectrometry (LC/MS-MS) is then used to identify and quantify legal and illicit substances and their respective metabolites. The detected concentrations of either the target substance or its primary metabolite form the basis for calculating the estimated amounts of substances consumed within the region. Consequently, WBE has emerged as a valuable tool for the real-time assessment of substance use patterns across various populations, encompassing legal and illicit substances. Moreover, WBE demonstrates promise in novel application areas, including monitoring biomarkers relevant to forensic investigations. These biomarkers may provide insights into lifestyle factors, disease prevalence within a population, and exposure to environmental pollutants.

Keywords: Wastewater, Illicit substance, Wastewater-based epidemiology, Consumption, LC/MS-MS

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1. Introduction

Wastewater represents a complex matrix containing a diverse array of chemical and biological markers (Choi et al., 2018). Within wastewater-based epidemiology (WBE), a particular focus lies in the detection of drug residues, illicit substances, and biomarkers of tobacco and alcohol use (Daglioglu et al., 2019; Gracia-Lor et al., 2017; Guzel et al., 2020; Rice et al., 2020; Van Hal, 2019; van Wel et al., 2016; Zarei et al., 2020). Additionally, WBE holds the potential for expansion into numerous other areas of forensic interest, including investigations into human health, exposure to industrial or agricultural chemicals, the presence of infectious diseases or pathogens, and the development of antibiotic resistance. Furthermore, WBE facilitates the monitoring of exposure to environmental pollutants (Rousis et al., 2017a, b, 2020). The initial demonstration of this method's utility in assessing the population-level use of illicit substances and potentially addictive therapeutic drugs dates back to 2001 (Daughton, 2001). Today, WBE has evolved into a vital tool for estimating the consumption of both legal and illicit substances. This estimation is achieved through the detection and quantification of both unchanged excreted drugs and their uniquely human metabolites within wastewater (Feng et al., 2018).

WBE offers advantages over traditional epidemiological techniques such as retrospective data analysis, surveys, and direct monitoring of large cohorts. Specifically, it enables rapid, near real-time data collection, demonstrating potential as an early warning system for emergent trends (Vitale et al., 2021). This non-invasive approach offers several strengths, including identifying substance use patterns while maintaining individual anonymity, improved detection of illicit substances, and circumvention of biases inherent in survey-based research. Nonetheless, it is essential to acknowledge that the WBE approach does present some uncertainties, which will be discussed separately in the following section. Despite these uncertainties, there has been observed a growth in the number of WBE applications in recent years regarding the estimation of legal and illicit substance consumption (Daglioglu et al., 2021; Daughton, 2001), the assessment of human exposure to environmental pollutants (Gracia-Lor et al., 2018; Guzel et al., 2023), and the monitoring of novel psychoactive substances (Atasoy Aydin et al., 2023).

2. Wastewater-Based Epidemiology Approach

Wastewater analysis enables estimating substance use, consumption, or exposure rates within a population by correlating detected concentrations with relevant metrics. This approach provides valuable qualitative and quantitative insights into population-level activities within a defined wastewater catchment (Choi et al., 2018). Often called chemical information mining in wastewater, WBE represents a highly collaborative field, integrating expertise from diverse scientific disciplines. These include, but are not limited to, environmental science, anthropology, biology, chemistry, epidemiology, engineering, medicine, public health, and sociology (Vitale et al., 2021). This multidisciplinary approach is essential to maximizing the potential of WBE in forensic investigations.

WBE calculations involve normalizing measured biomarker concentrations in wastewater influent to determine per capita mass loads. This normalization utilizes both daily runoff data and the population served by the wastewater treatment plant (WWTP) (Figure 1). WBE provides a powerful tool for assessing population-scale trends within designated watersheds, facilitating community-level evaluations of per

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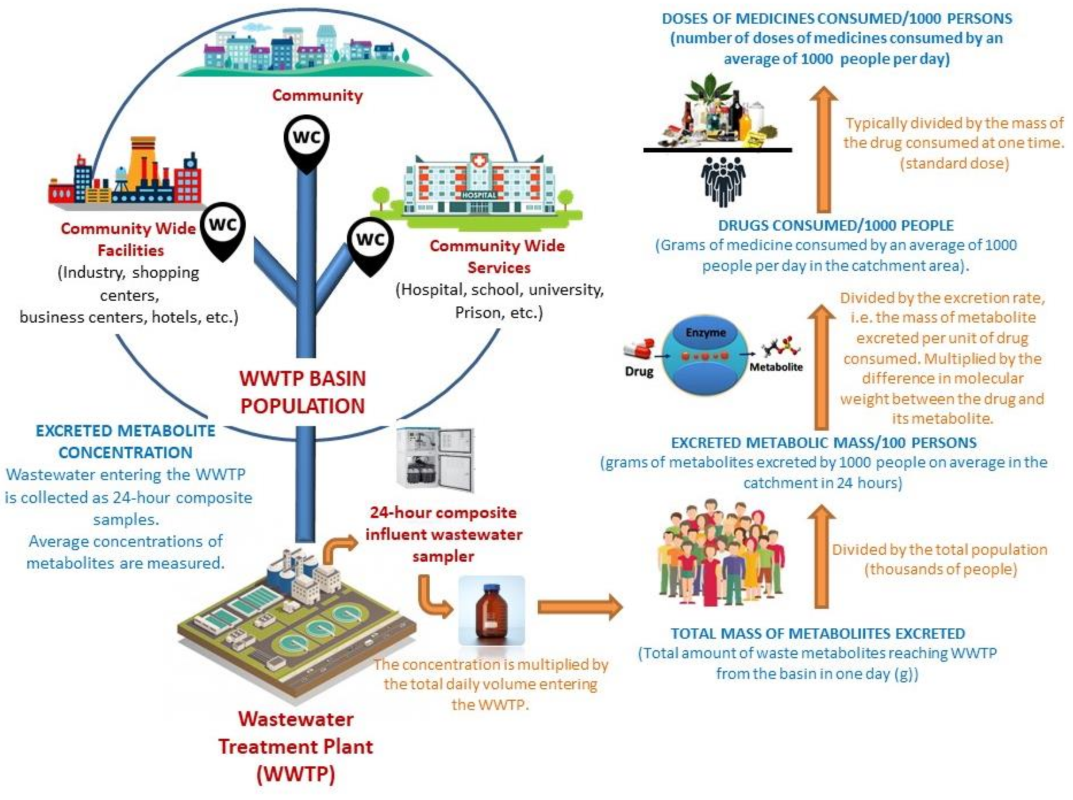
capita consumption, use, exposure, or release of various chemical or biological agents. Crucially, WBE studies must consider the solubility of target substances in water and their partitioning into the aqueous phase. This is essential for obtaining accurate and representative wastewater samples. The following formula (Formula 1) is used to calculate the daily per capita consumption of a parent compound within a given wastewater catchment (Choi et al., 2018):

$$\text{Daily substance consumption}_i\ \mathbf{(mass/day/1000people)} = \frac{C_i * V_{total} * \frac{R_i}{E_i}}{N} \qquad \textit{(formula 1)}$$

In this formula (Formula 1), C_i represents the measured concentration of the analyte residue (either the parent compound or its metabolite) within wastewater-influent samples. V_{total} denotes the total volume of wastewater that enters the wastewater treatment plant (WWTP) throughout the sampling period (typically 24 hours). N signifies the total population served within the wastewater catchment area. R_i is the ratio between the molar mass of the parent substance and the molar mass of its corresponding metabolite. E_i represents the average excretion rate of the drug residue. This value accounts for the proportion of the parent compound or metabolite excreted in a detectable form. Notably, the accuracy of WBE calculations depends on reliable estimates for parameters such as excretion rates (E_i) and the stability of target analytes during wastewater transport.



(Adapted from Choi et al., 2018)

Figure 1. Schematic representation of workflow diagram for Wastewater-Based Epidemiology (WBE): Conversion of measured substances to daily mass loads or per capita consumption

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3. Using WBE for Detection of Substances

In general, four groups of illicit drugs are used in the market which include amphetamine-type stimulants, cocaine, heroin and cannabis and the most legally consumed substances are alcohol and tobacco. This article reports community consumption (mg/d/1000 people) of legal and illicit substances evaluated using WBE.

3.1. Amphetamine-type Stimulants

Amphetamine-type stimulants (ATS) include amphetamine, methamphetamine, MDA (3,4-methylenedioxyamphetamine), MDMA (3,4-methylenedioxymethamphetamine), and MDEA (methyldiethanolamine). These substances are frequently targets of forensic investigations due to their illicit use.

Amphetamine, methamphetamine, MDMA (Ecstasy), and MDA are among the most widely analyzed substances in WBE studies (Asicioglu et al., 2021; Daglioglu et al., 2021; Zuccato et al., 2008). The global prevalence of these illicit substances drives intensive research into their detection in wastewater. Generally, MDMA and methamphetamine levels are monitored by quantifying the parent compounds. This approach is favored because these substances undergo extensive urinary excretion in their unchanged form, and their metabolites tend to lack specificity. For instance, methamphetamine is metabolized into amphetamine, potentially confounding efforts to estimate amphetamine loads accurately. Similarly, MDMA is metabolized into MDA, itself a controlled substance, which can complicate the estimation of MDA consumption (Khan & Nicell, 2011).

MDMA, amphetamine, and methamphetamine exhibit chirality. This structural characteristic can significantly impact their potency and pharmacokinetic profiles (including excretion patterns based on the route of administration) and potentially provide clues about their origin. Due to differences in the synthesis pathways of illicit and licit sources (e.g., pharmaceuticals for attention deficit hyperactivity disorder), the chiral profile (ratio of enantiomers) can be used to distinguish between amphetamine derived from licensed or illicit sources (Castiglioni et al., 2008; Kasprzyk-Hordern & Baker, 2012). This application underscores the value of chiral analysis as a powerful tool in forensic investigations.

3.2. Cocaine

Most cocaine consumed by humans is rapidly metabolized by carboxylesterase. Metabolized cocaine is excreted in the urine as benzoylecgonine and ecgonine methyl ester. Only 1 to 9% of cocaine is excreted unchanged (Castiglioni et al., 2008). Therefore, cocaine consumption is generally monitored in wastewater through its selective metabolite, benzoylecgonine (Daglioglu et al., 2021). Moreover, studies have found that cocaine is fairly unstable in wastewater, while benzoylecgonine is relatively more stable, which has led to a focus on benzoylecgonine within wastewater-based epidemiology studies (Chen et al., 2013).

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3.3. Heroin

Heroin undergoes rapid metabolism primarily into morphine, with only a small fraction (approximately 1.3%) converted to 6-monoacetylmorphine (6-MAM) (Asicioglu et al., 2021; Daglioglu et al., 2021; Khan & Nicell, 2011). For the selective monitoring of heroin consumption in wastewater, 6-MAM serves as the target biomarker. However, a significant challenge arises due to the susceptibility of 6-MAM to degradation after sampling. To mitigate this, immediate preservation measures are required, such as the addition of sodium metabisulfite or acidification to pH 2 (Du et al., 2020). Furthermore, this instability of 6-MAM introduces uncertainties regarding its degradation within the sewer system, particularly in locations with long or unknown sewage residence times (McCall et al., 2016). These analytical challenges highlight the complexities faced in forensic applications of WBE. On the other hand, an alternative approach to estimating heroin use involves subtracting prescribed quantities of codeine and morphine from the total amounts detected in wastewater. This method accounts for the partial metabolism of codeine into morphine (Daglioglu et al., 2021). However, it also faces challenges due to the over-the-counter availability of codeine in some regions and the complexities of compiling accurate drug prescription data from various healthcare providers (hospitals, pharmacies, general practitioners, etc.) overall, which can lead to further uncertainties in the estimation process.

3.4. Cannabis

Marijuana, with its primary psychoactive component THC (tetrahydrocannabinol), ranks among the most frequently abused substances. Its excretion pattern is predominantly fecal (approximately 65%), with a smaller portion (approximately 20%) excreted in urine. THC-COOH (carboxylic acid), the primary glucuronide conjugate of THC, is the major urinary metabolite, while 11-OH-THC (hydroxylated THC) is the predominant form found in feces (Sharma et al., 2012).

Analysis of the THC metabolite THC-COOH is the standard biomarker used for estimating cannabis consumption in WBE studies (Asicioglu et al., 2021; Causanilles et al., 2017; Daglioglu et al., 2021; Feng et al., 2018). Nonetheless, the very low urinary excretion rate of THC-COOH (less than 1%) raises concerns about its reliability as a representative marker. Additionally, potential losses due to the adsorption of THC-COOH to particles and surfaces within the sewer system introduce further uncertainty into load estimations. Consequently, these limitations underline the need for continued research and refinement of sampling techniques to ensure accurate assessments in forensic applications (Causanilles et al., 2017). While accurately quantifying cannabis use through WBE presents significant challenges, its status as the most widely consumed illicit drug worldwide underscores the continued importance of refining this technique. Monitoring drug consumption trends via WBE holds particular value in countries where legalization or decriminalization policies have been implemented. This approach offers a means of assessing the impact of such policy changes on substance use patterns within a population.

3.5. Alcohol

Alcohol (ethanol) consumption can be indirectly assessed through the detection of its minor metabolites, ethyl sulfate (EtS) and ethyl glucuronide (EtG) (Reid et al., 2011). Due to its significantly

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greater stability in wastewater, EtS is the preferred biomarker for estimating ethanol consumption in WBE studies (Daglioglu et al., 2020; Reid et al., 2011; Yavuz Guzel et al., 2021). Despite the low urinary excretion rate of EtS following ethanol consumption (approximately 0.012% of the initial dose), the substantial volumes of ethanol consumed at the population level enable its detection in wastewater using sensitive LC-MS/MS methods. Studies have successfully back-calculated EtS concentrations to estimate ethanol consumption in liters per 1000 people per day, which can be expressed in standard beverage units (Daglioglu et al., 2020). Given the significant public health burden associated with alcohol use, including numerous health conditions and mortality, WBE offers a valuable tool for monitoring alcohol consumption patterns and informing public health interventions.

3.6. Tobacco

Nicotine is the primary biomarker for assessing tobacco consumption in wastewater-based epidemiology due to its well-understood pharmacokinetics (Castiglioni et al., 2015). It is important to note that this measurement encompasses various sources of nicotine, including nicotine gum, patches, and e-cigarettes. Nicotine’s primary metabolites, cotinine, and hydroxycotinine, can also be monitored in wastewater. Analyzing these metabolites makes it possible to back-calculate the estimated number of cigarettes consumed per 1000 people (Yavuz Guzel et al., 2021). Additionally, research has explored the use of other tobacco biomarkers, such as anabasine and anatabine, for wastewater monitoring purposes. (Tscharke et al., 2016)

4. Limitations of Wastewater Based Epidemiology

The main uncertainties within WBE stem from several key factors: issues in obtaining representative wastewater samples, the potential instability of target analytes and their metabolites within the sewer environment, the necessity of reliable analytical measurements, and the need for robust back-calculation methods to estimate substance use accurately (Castiglioni et al., 2013; Lai et al., 2011; Ort et al., 2010; Shimko et al., 2021). Furthermore, variations in sampling techniques, flow measurement, and population size estimations can introduce further uncertainties. The potential for degradation or transformation of target chemicals within the sewer system presents a significant challenge, highlighting the importance of conducting stability studies for selected biomarkers (Castiglioni et al., 2013; Shimko et al., 2021; Sirén & El Fellah, 2017).

Likewise, several factors significantly influence the outcomes of WBE analyses. Primarily, the human metabolism and excretion patterns of target drugs or substances play a crucial role. Also, characteristics of the sewer system itself, such as the contributing population size and wastewater volumes, must be considered (McCall et al., 2016; Ort et al., 2018). Accurate population size estimation is essential in WBE, as it enables normalization of daily biomarker loads (often using endogenous markers like cortisol or cotinine) to facilitate comparisons between cities. While population fluctuations due to tourism or commuting may introduce uncertainties in smaller communities, these effects are less pronounced in WWTPs serving large populations exceeding 100,000 people (Chen et al., 2014; Ort et al., 2014; Sims & Kasprzyk-Hordern, 2020).

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Another factor is the complexity of the wastewater matrix presenting a significant challenge in WBE analysis. A crucial task is determining whether human excretion is the primary source of detected metabolites or if contributions from external sources, such as hospitals, agricultural, or industrial activities, are present. Contamination from external sources can distort results, making them unreliable indicators of population-level substance use. Additionally, errors in estimating the population served by the WWTP, leaks or infiltration within the collection system, and the type of sewage system (separate or combined) can further skew estimations. These factors can lead to either overestimation or underestimation of substance concentrations, compromising the accuracy of WBE for forensic applications (Aghaei et al., 2023; González-Mariño et al., 2020; Zarei et al., 2020).

Accurate flow rate measurement during sampling is crucial for reliable back-calculations in WBE. Variations in sampling volume introduce uncertainty. Additionally, the co-occurrence of multiple drugs within a region can potentially affect universal correction factors based on urinary excretion rates. This interaction highlights a potential limitation of WBE, particularly in complex forensic investigations involving polydrug use (Huizer et al., 2021).

Uncertainties arising from sample collection include variations in sample volume due to fluctuating wastewater flow rates, inconsistent sampling frequencies, and potentially limited monitoring periods. These factors can introduce variability into WBE analyses, compromising the reliability of results for forensic investigations (González-Mariño et al., 2020; Zarei et al., 2020).

The WBE approach has several inherent limitations as well. First, it cannot provide insights into the prevalence or frequency of substance use, routes of administration, or the purity of substances within a population. Moreover, accurately determining the average number of doses consumed based on total substance amounts presents challenges due to variations in individual dosage and differences in purity levels (Been et al., 2016; EMCDDA, 2016; McCall et al., 2016; Ort et al., 2018). These limitations must be carefully considered when interpreting WBE data within forensic investigations. Besides, the WBE approach cannot yield data on demographics such as age groups, frequency of use, or routes of exposure. Given these limitations, WBE is best utilized as a complementary tool alongside established biomonitoring methods rather than as a standalone replacement. Still, though WBE does not provide individual-level exposure information, the analysis of pooled urine samples offers a cost-effective alternative to extensive biological monitoring studies, even for large populations (Aghaei et al., 2023), which outlines WBE as a valuable addition to forensic analyses.

5. Conclusions and Future Perspectives

To sum up, WBE offers a promising approach for the real-time assessment of various substances, including nicotine, alcohol, illicit substances, and drugs of abuse within populations. Beyond its established applications, WBE holds significant potential for developing novel biomarkers that reveal population-level health status, disease incidence, lifestyle factors, and exposure to environmental pollutants. Looking ahead, WBE could become a crucial component of integrated human biomonitoring frameworks, providing anonymized data on population characteristics relevant to forensic investigations. By incorporating food, diet, and health biomarkers, WBE could offer more direct insights into population well-being. Overall,

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WBE studies represent a valuable tool for accessing previously inaccessible information, particularly within public health and forensic investigations.

6. Recommendations for the Northern Cyprus

Illicit substance, alcohol and nicotine can be monitored in wastewater samples taken from the influent of wastewater treatment plants in Northern Cyprus. Hence, it is recommended that the authorities concerned study WBE as an effective complementary tool to other evaluation tools in helping to detect substance abuse as a means to help in the fight to deter substance abuse.

Declaration of Conflicts Interests

The authors declare that they have no conflict of interest to disclose.

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