RESEARCH PAPER

Tracking Pharmaceuticals and Illicit Drugs in Urban Wastewater: Drug Consumption Trends in Patras, Greece

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Abstract

Urban wastewater surveillance is a crucial tool for public health monitoring and environmental epidemiology. This study investigates the presence of pharmaceutical compounds and illicit drugs in the wastewater of Patras, Greece, aiming to identify consumption patterns and health indicators within the population. A plethora of antidepressants, antipsychotics, antibiotics, antidiabetics, and illicit drugs were detected and quantified using liquid chromatography-tandem mass spectrometry (LC–MS/MS). Key findings show significant levels of various substances such as venlafaxine, valsartan, metformin, rosuvastatin, acetaminophen, levetiracetam, gabapentin, cimetidine, caffeine, and nicotine. The results highlight prevalent public health trends and challenges. Additionally, the detection of illicit drugs such as cocaine (average 169.71 mg/day/1000 people) and methamphetamine (average 13.15 mg/day/1000 people) underscore the ongoing illicit drug circulation in the city, warranting closer public health monitoring. This research demonstrates the value of Wastewater-Based Epidemiology (WBE) as an efficient, non-invasive method for tracking population health, lifestyle behaviors, and environmental contamination, offering critical insights for public health interventions and sustainable wastewater management strategies. Ultimately, this study reinforces the role of wastewater surveillance as a vital decision-support tool for protecting public health and shaping effective policy responses.

Highlights

- Urban wastewater in Patras was analyzed for pharmaceuticals and illicit drugs.
- 108 compounds detected, including antidepressants, antidiabetics, and illegal drugs.
- Cocaine and MDMA levels indicate notable illicit drug use.
- Wastewater-based epidemiology (WBE) revealed seasonal drug and pharmaceuticals consumption trends.
- Findings support WBE as a public health and environmental monitoring tool.

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Introduction

Wastewater-based epidemiology (WBE) has emerged as a vital method for monitoring public health and environmental exposure across various countries. Urban wastewater monitoring offers valuable insights by detecting a range of pathogens, viruses, bacteria, pharmaceuticals, drugs, and other pollutants. This continuous, cost-effective surveillance provides reliable data on long-term trends and seasonal changes, offering a snapshot of a population's health, diet, lifestyle, and environmental exposure (O'Keeffe 2021). By analyzing food residues and chemicals, wastewater

can reveal dietary habits, environmental health risks, and the spread of viruses and bacteria, enabling early outbreak detection and ongoing disease monitoring. It also highlights medication usage patterns and trends in drug consumption, including illegal substances (Anand, et al. 2021; World Health Organization 2024). WBE allows for large-scale, population-normalized data collection, bypassing ethical and legal constraints, regardless of socioeconomic status or healthcare access, and facilitates rapid, targeted public health interventions (Balboa et al. 2021; Sharara et al. 2021; Sweetapple et al. 2023).

WBE has a wide range of applications, including estimating illicit drug use. First introduced in 2001 to monitor cocaine consumption in Milan (Daughton 2001; Zuccato et al. 2005), WBE is now widely used for real-time tracking of drug use trends to support public health and law enforcement efforts. The European Union Drugs Agency (EUDA) publishes annual drug consumption data based on WBE, with international studies from Europe, Australia, Canada, and the U.S. showing its effectiveness. The SCORE network ensures quality control and comparability across international studies, helping to shape public health strategies (https://score-network.eu/). A broad spectrum of drugs, including cocaine, amphetamines, and MDMA, can be detected through wastewater. Cocaine is identified via its metabolite benzoylecgonine, while THC-COOH serves as a marker for cannabis. However, heroin's metabolite 6-monoacetylmorphine is unstable and undetectable (McCall et al. 2016). Recent studies from the SCORE network and EUDA reveal a growing use of cocaine and methamphetamine in European cities. The drug consumption patterns of residents, from Copenhagen to Valencia and Nicosia to Lisbon, were examined by analyzing daily wastewater samples (EUDA 2023). Wastewater analysis has also been used to assess drug use trends especially during the COVID-19 pandemic (Psichoudaki et al. 2023; Galani et al. 2022). However, the increasing presence of new psychoactive substances (NPS) in wastewater presents challenges due to limited knowledge of their chemical properties and metabolism, requiring further investigation (Bruni et al. 2022).

Urban wastewater analysis can also estimate the consumption of legal substances, like alcohol and nicotine, uncovering consumption trends and potential illicit trade (Mastroianni et al. 2014). Comparative studies between nicotine measurements and tobacco sales can indicate illegal tobacco trade. A meta-analysis of 21 studies, which analyzed 2,250 wastewater samples and covered a population of 275.3 million people, found that nicotine consumption was highest in Portugal and lowest in Vietnam (Asadi et al. 2023). Wastewater analysis also assesses population exposure to various chemicals, such as pesticides, preservatives, and personal care products (Carneiro et al. 2024). Moreover,

the parent compound-to-metabolite ratio helps evaluate metabolism and exposure levels, guiding interventions to reduce risks (Rousis et al. 2021).

The detection of pharmaceuticals in urban wastewater has become an important method for monitoring the prevalence of diseases within a population (Ceolotto et al. 2025). As pharmaceutical products consumed by humans end up in wastewater through bodily excretions, researchers can estimate the consumption of specific drugs and, by extension, the prevalence of related diseases. During the COVID-19 pandemic, changes in concentrations of antiviral drugs and other therapeutic substances reflected shifts in disease incidence (Tomsone et al. 2024). Similarly, measuring metformin concentrations in wastewater has been used to estimate the prevalence of type II diabetes, as the drug is excreted in urine and can be tracked in wastewater. This approach enables real-time monitoring, facilitates the rapid detection and monitoring of epidemics and supports the design of more effective intervention programs (Yavuz Guzel et al. 2024). Likewise, oxypurinol, a metabolite of allopurinol, helps estimate gout prevalence (Ahmed et al. 2021). Comparative studies between wastewater and prescription data highlight discrepancies in drug use, as seen in the Netherlands, where wastewater levels of sildenafil exceeded prescription estimates, providing valuable insights into trends of misuse (Venhuis et al. 2014).

Wastewater analysis has been used, alongside other indicators such as survey data, socioeconomic factors, and environmental conditions, to reveal correlations and offer a multidimensional analysis of public health. It can reveal how socioeconomic conditions affect access to and use of pharmaceuticals or how demographic trends are linked to specific health needs and consumption patterns (O'Keeffe 2021). For example, elevated metformin concentrations in wastewater have been linked to lower per capita disposable income (Vieira et al. 2023), while salbutamol concentrations (used to treat asthma) have been associated with higher levels of air pollution (Fattore et al. 2016). This multidimensional approach allows for the development of more targeted and effective health and environmental policies.

In recent years, WBE has been applied in Greek urban centers such as Athens, Ioannina, and Thessaloniki, providing key insights into pharmaceutical use, illicit drug trends, and behavioral responses to societal stressors, especially during the COVID-19 pandemic (Gago-Ferrero et al. 2020; Galani et al., 2021; Papageorgiou et al. 2016). For example, recent studies in Athens have revealed notable increases in the consumption of antidepressants, antipsychotics, and recreational substances, reflecting the population's psychological and behavioral responses to lockdown conditions (Alygizakis et al. 2021; Galani et al. 2022). These studies also demonstrated the value of WBE in capturing real-time,

community-level public health trends through the analysis of untreated wastewater. However, despite these efforts, data from Patras, Greece's third-largest city, remain limited or absent.

Patras, with a population of approximately 168,000, is a key urban and economic hub in western Greece. As a major port city and university center, it hosts a large transient and youth population, which may significantly influence pharmaceutical use patterns, mental health trends, and recreational drug consumption. These demographic and socio-cultural characteristics make Patras an especially relevant case study for wastewater analysis. Conducting WBE in Patras not only fills a critical geographic and epidemiologic gap in national surveillance but also provides valuable localized data to inform public health and environmental policy at both municipal and national levels. As WBE continues to be adopted globally, this study is the first one implemented in the urban wastewater of Patras city, the third largest city in Greece and aims to detect both legal and illegal pharmaceutical and drug substances, and further proceed to analyze consumption trends, provide insights into community health, and assess the effectiveness of this approach in informing public health decisions. In that sense, the present study provides both a localized assessment of community health in Patras and a methodological model for implementing WBE in similar urban settings.

Materials and Methods

Sample Collection

The present study was conducted in the city of Patras, Greece, aiming to monitor the presence and monthly or seasonal variations of pharmaceuticals and illicit drugs in urban wastewater. The Wastewater Treatment Plant (WWTP) of the Municipality of Patras, being selected as the sampling site, has an average influent flow rate of 1,500 m^3/h and serves a theoretical population of 168,034 inhabitants. Over a twelve-month period, 36 time-proportional 24-h composite influent wastewater samples were collected. Sampling occurred three times per week during the first week of each month, from April 2023 to March 2024. These three weekly samples of 500 mL each, were pooled to one sample representative for each month. Consequently, 12 monthly samples of influent wastewater were analyzed. Sampling was conducted on regular days, excluding holidays and carnival periods, to ensure comparability of the data. The decision to pool weekly samples into monthly composites was made to enhance the representativeness and reliability of the dataset, minimizing potential short-term variability in wastewater composition. Additionally, this approach

was adopted in accordance with the available budget and analytical resources for the study, ensuring a balanced compromise between sample representativeness and feasibility. This systematic approach ensured that the dataset provided an accurate and representative overview of fluctuations in pharmaceutical and drug residues across different months and seasons. The samples were stored at − 20 °C until further analysis. The freezing conditions were maintained to prevent degradation or transformation of the target compounds, ensuring sample integrity for subsequent laboratory procedures.

Chemical Analysis and Identification of the Compounds

Quantitative data were obtained using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). Analysis of samples conducted at the Laboratory of Analytical Chemistry, National and Kapodistrian University of Athens (Thomaidis et al. 2016; Gago-Ferrero et al. 2020) Wastewater samples (50 mL) were filtered using Glass Microfiber Filters (Whatman 47 mm, Maidstone England) and adjusted to pH 6.5±0.2 by the gradual addition of 0.1 N HCl, with continuous monitoring using a calibrated pH meter. A 40 mL aliquot of each sample was spiked with a surrogate internal standard mixture to a final concentration of 25 ng/mL (Gago-Ferrero et al. 2020). Solid-phase extraction (SPE) was performed using in-house mixed-mode cartridges containing four different materials (StrataX-WAX, StrataX-WCX, Envi+, and Strata-X, Phenomenex, Torrance, USA). After conditioning the cartridge with 3 mL methanol and 3 mL water, sample was loaded, and cartridges were washed with 3 mL water and dried under vacuum for 1 h. Elution was performed using 6 mL basic solution (2% NH_4OH in methanol: ethyl acetate 50:50) and 4 mL acidic solution (1.7% formic acid in methanol: ethyl acetate 50:50). Extracts were evaporated under a gentle stream of nitrogen at 40 °C and the residue was reconstituted in 200 µL of methanol: water (1:1) and filtrated through 0.45 µm RC filters.

A chromatographic system, AB Sciex Exion LC, coupled with an AB Sciex QTrap 5500+mass spectrometer, was used for the analysis. Chromatographic separation was achieved on an Atlantis T3 C18 (100 mm×2.1 mm, 3.0 µm) column from Waters Corporation (Milford, MS, USA) with a SecurityGuard pre-column (AQ C18 4×3.0 mm, Phenomenex). The mobile phase contained acetonitrile and 0.1% formic acid (Mobile Phase A) and a buffer containing 0.1% formic acid (Mobile Phase B). The gradient program lasted 14 min, with flow rate maintained at 0.4 mL/min and injection volumes of 10 µL. The mass spectrometer operated in positive/negative ionization modes with the following parameters:

ion spray voltage of +5500 V/-4500 V, source temperature of 550 °C, and gases GS1 and GS2 were set at 55 psi. Acquisition mode set to scheduled multiple reaction monitoring (sMRM) with window width 180 s; transitions for targeted compounds are referred at Supplementary Table S3. Data acquisition and analysis were performed using Analyst 1.7 and SCIEX OS software. The identification criteria for the determination of compounds were the retention time (±0.2 min) and the ion ratio of each analyte compared to the standard solution (±30%).

Prior to LC–MS/MS analysis, a surrogate internal standard (IS) mixture consisting of isotopically labeled analogs was added to each wastewater sample. The internal standards included MDMA-d5, benzoylecgonine-d3, morphine-d3, THC-d3, venlafaxine-d6, caffeine-d9, ibuprofen-d3, and carbamazepine-d10. These compounds were selected to represent a wide range of physicochemical properties and ionization behaviors comparable to the target analytes, enabling effective correction for matrix effects and procedural losses.

Quantification was based on the ratio of the peak area of each analyte to that of the corresponding internal standard, using a seven-point calibration curve (concentration range: 25–2500 ng/L) prepared in matrix-matched wastewater. This approach, validated in previous WBE studies, enables accurate and reproducible quantification even in complex environmental matrices.

To ensure data quality, quality assurance and control (QA/QC) procedures were rigorously applied. An evaluation of the performance characteristics of the methods was previously conducted for the Attica wastewater matrix (Thomaidis et al. 2016; Gago-Ferrero et al. 2020). However, in order to evaluate alterations in recovery due to minor matrix differentiations, a spiked sample at 5 ng/L was prepared in a Patra's wastewater sample. Furthermore, each analytical batch consisted of procedural blank samples, to monitor contamination and evaluate carry-over. Additionally, standard solutions were analysed within each batch to assess instrumental signal drift.

Data Analysis

The concentration (ng/L) of each individual compound detected in the influent wastewater samples was normalized per 1,000 residents based on the average daily flow rate (m³/d) of the WWTP and the estimated real-time population served by the plant. Ammonium loads were used as an anthropogenic marker to estimate the size of the real-time population served (Been et al. 2014). A population equivalent (PE) of 7.0 g NH_4-N per day per person was applied, as this is the value estimated for Greek cities and used by the Greek National Wastewater Epidemiology Network (https://apth.mnss.eu/).

The real-time population served was calculated by dividing the concentration of NH_4-N that was determined in the influent samples with the average NH_4-N excreted daily per resident, according to Eq. 1

$$Real-TimePopulation\,(people)$$
$$= \frac{C\,of\,NH_4-N\left(\frac{g}{m^3}\right)*Average\,daily\,flowrate\left(\frac{m^3}{d}\right)}{7\frac{g\,NH_4-N}{day*person}} \quad (1)$$

Population normalized daily loads (PNDL) of the individual compounds expressed as mg/day/1000 people were calculated as depicted in Eq. 2. Influent concentrations expressed in PNDL reflect the collective excretion rates (mg/day/1000 people) for the detected substances, assuming no losses of wastewater along the sewage system (Zuccato et al. 2008).

$$C_{norm}\left(\frac{mg}{1,000\,people}\right)$$
$$= \frac{C\left(\frac{ng}{L}\right)*inlet\,flow\left(\frac{m^3}{d}\right)*1,000\left(\frac{L}{m^3}\right)*1,000\,(people)}{10^6\left(\frac{ng}{mg}\right)*real\text{-}time\,population\,(people)} \quad (2)$$

Estimation of Drug Use

Specific drugs of abuse, namely cocaine, heroin, amphetamine, methamphetamine (MA), MDMA (ecstasy), THC (cannabis), and codeine, were selected to estimate their consumption rates within the studied population. These substances have well-characterized primary active metabolites, which are excreted in urine in known proportions following human consumption. By adjusting the measured concentrations of these metabolites in wastewater using a correction factor—accounting for the fraction of the parent drug excreted as its metabolite, and the molar mass ratio between the parent drug and the metabolite, the consumption of the parent drug can be back-calculated. The formula used to back-calculate the consumption rate of the illicit drugs follows Eq. 3.

$$Drug\,consumption\,rate\left(\frac{mg}{day*1,000\,people}\right)$$
$$= PNDL\left(\frac{mg}{day*1,000\,people}\right)*\left(\frac{\frac{molar\,mass\,of\,drug}{molar\,mass\,of\,metabolite}}{excreted\,fraction\,of\,metabolite}\right) \quad (3)$$

The correction factors applied in this study were derived from the literature and represent the ratio of the excreted metabolite to the consumed parent drug, adjusted for their respective molar masses. These factors are essential for back-calculating drug consumption from wastewater concentrations (Zuccato et al. 2008). These correction factors

Table 1 Correction factors applied for each illicit substance

Parent drug	Measured metabolite	Correction factor
Cocaine	Benzoylecgonine	2.33
Heroin	Morphine	3.07
Amphetamine	Ampletamine	3.3
Cannabis (THC)	THC-COOH	152
Ecstasy (MDMA)	MDMA	1.5

Table 2 Qualitative characteristics of wastewater inlet

Physicochemical parameters of wastewater samples	Mean	Range
Average daily supply (m^3/day)	36,328	35,200–36,500
Conductivity (µS/cm)	1,572	1,282–1,730
COD (mg/L O$_2$)	334	261–498
BOD (mg/L O$_2$)	172	106–279
Total Nitrogen (mg/L N)	48.3	35.3–65.9
Suspended Solids (mg/L)	140	66–308
Ammonium (mg/L NH$_4$-N)	29.9	16.5–43.6
Phosphorus (mg/L P)	2.5	0.2–3.96

are based on average human excretion rates derived from pharmacokinetic studies. While metabolic rates may vary slightly among individuals and populations, existing literature indicates that these variations are generally within acceptable margins (10–15%) for WBE applications. Additionally, these factors have been used extensively in multi-national WBE campaigns (e.g., SCORE network, EUDA reports) across various European populations, including Mediterranean regions with similar dietary, genetic, and lifestyle characteristics. As such, their application to the Greek population, including that of Patras, is considered appropriate. The specific correction factors applied for each illicit substance, along with its associated metabolite, are presented in Table 1.

This methodology assumes that the parent drug is predominantly and consistently excreted as the metabolite in question, a premise that holds true for all examined substances, except for morphine. While morphine is the active metabolite of heroin, it is also excreted following the consumption of morphine and codeine (albeit in negligible amounts) (Zuccato et al. 2008). As a result, the back-calculation of heroin consumption as estimated by morphine concentration may be subject to overestimation.

Furthermore, by applying typical daily doses consumed by drug users, it is possible to assess the prevalence of drug use within the studied city, according to Eq. 4.

$$\text{Estimated number of dosages}$$
$$= \frac{\frac{\text{Drug consumption rate} \left(\frac{\text{mg}}{\text{day}*1,000\text{people}}\right)}{1,000 \text{ people}} * \text{Population served (people)}}{\text{Drug typical dose} \left(\frac{\text{mg}}{\text{day}* \text{people}}\right)} \quad (4)$$

However, determining the precise amount of a drug dose involves considerable uncertainty, as illicit drugs may be consumed through various routes, and the quantities ingested can vary widely across different user groups and at different stages of an individual's drug use history (Cohen and Sas 1994). Notwithstanding these challenges, the average content of pure active drug in a typical dose for the most common route was derived from the literature (Zuccato et al. 2008). It was assumed to be: 100 mg for intranasal cocaine, 30 mg for oral amphetamine and methamphetamine, 100 mg for oral ecstasy, 30 mg for intravenous heroin, and 125 mg for smoked THC (based on high-potency cannabis containing 14% THC in hashish/marijuana). The total daily doses

consumed in the city were then estimated by dividing the drug consumption rates (mg/day/1000 people) by the corresponding average dose amounts.

Limitations

One limitations of the study is the lack of more representative samples per month. Taking more samples, each week of each month would give more comprehensive results and outcomes. Furthermore, environmental factors, such as rainfall or leakage from industrial sources, may affect the concentration of substances in wastewater and can alter the estimated consumption. Although efforts have been made to exclude periods, such as public holidays and carnival, the possibility of external interferences still remains. Even though the study aims to map trends in use, the lack of sufficient data on new psychoactive substances (NPS), which are constantly changing in chemical composition, leads to possible omissions in the identification and estimation of the actual use of these substances.

Results

Wastewater Influent Characteristics

Physicochemical parameters of the influent wastewater samples were measured and are shown in Table 2.

Groups of Substances

A total of 108 chemical compounds were detected from 226 targeted substances by LC–MS/MS. The detected compounds and metabolites were classified according to the Anatomical Therapeutic Chemical (ATC) Classification System, which categorizes medicinal products into five hierarchical levels: (1) anatomical main group, (2) therapeutic subgroup, (3) pharmacological subgroup, (4) chemical subgroup, and (5) specific chemical substance. This system

allows for consistent grouping and comparison of pharmaceutical use patterns. At the first level, the system includes 14 main anatomical/pharmacological groups, e.g. 'A' for alimentary tract and metabolism, or 'R' for the respiratory system. Each ATC main group is divided into 2nd levels, which could be either pharmacological or therapeutic groups. The 3rd and 4th levels are chemical, pharmacological or therapeutic subgroups and the 5th level is the individual chemical substance. The 'N' category, encompassing substances for nervous system conditions and drugs of abuse, was the largest group in this study (46 compounds). It was further divided into pharmacological and therapeutic sub-groups, including antidepressants (N06A), antipsychotics (N05A), analgesics (N02A, N02B), and antiepileptics (N03). Substances prescribed for treatment of cardiovascular diseases (n=24) comprised vasoprotectives (C05), diuretics (C03), beta blockers (C07). Thirteen compounds were grouped in ATC code 'J' for anti-infectives (antibacterials J01, antifungals J02, and antivirals J05), 8 under 'M' for the musculoskeletal system, 7 under 'A' for alimentary tract and metabolism, while smaller groups concerned respiratory, blood, and antineoplastic agents. Nine substances were assigned to more than one ATC group, including a) Orphenadrine, that was detected in low levels, and is a centrally acting muscle relaxant (M03) and an anticholinergic agent to improve motor function in Parkinson's disease (N04), b) Lidocaine that exerts local anesthetic properties (N01B), but it is also used to treat ventricular tachycardia and fibrillation (C01B) and a vasoprotective (C05) agent, and c) Paraxanthine, a stimulant found in coffee, tea, and chocolate, that

is used as both a diuretic (C03) and a drug for obstructive airway diseases (R03).

A list of the 108 detected compounds along with their measured concentrations (ng/L) in the pooled wastewater samples and their population normalized daily loads (mg/day/1000 people) is provided in the Supplementary Material (Tables S1 and S2), respectively. The 25% more abundant compounds detected in the WWTP of Patras are depicted in Fig. 1.

Consumption Patterns of Illicit Drugs

A rough estimate of drug use prevalence in the studied city, with a population of 168,034 residents served by the WWTP, was calculated for certain illicit substances using Eq. 4. The findings indicate an average daily consumption of 397 doses of heroin, 356 doses of cannabis, 272 doses of cocaine, 55 doses of amphetamine, and 14 doses of ecstasy. Monthly fluctuations in these doses are illustrated in Fig. 2. For certain months, data points for cannabis and ecstasy are missing; this is because the concentrations of the corresponding metabolites (THC-COOH and MDMA) were below the method's limit of quantification (LOQ). In such cases, consumption could not be reliably estimated and values were omitted to avoid misinterpretation.
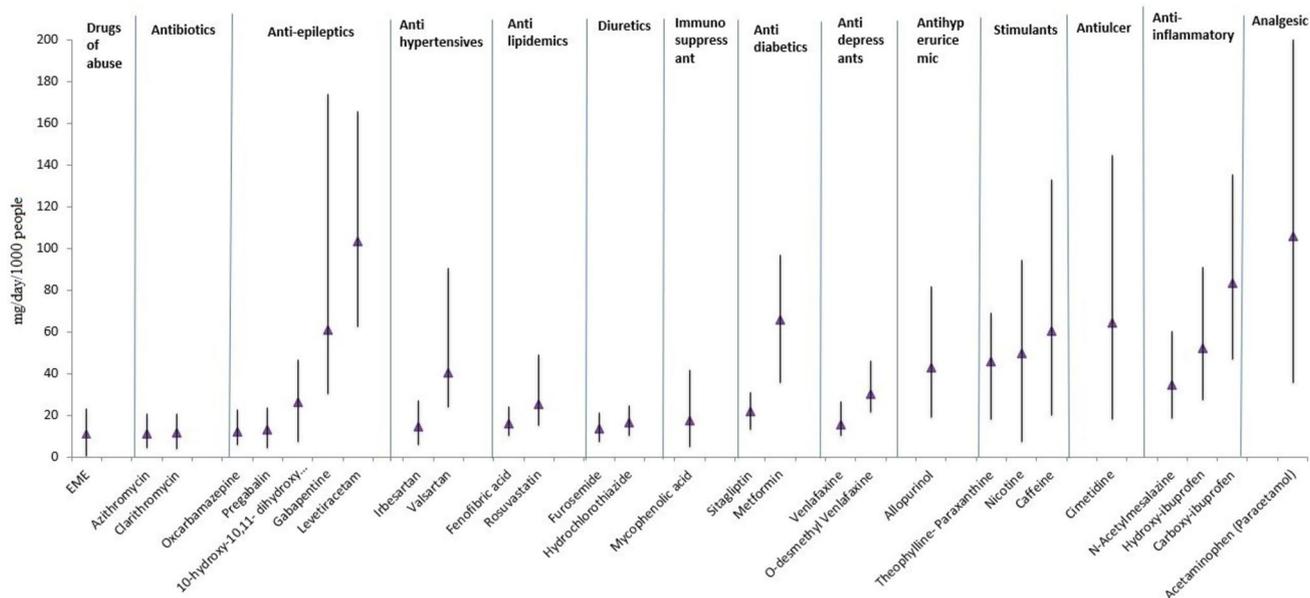

Fig. 1 Average population-normalized daily loads (PNDLs) of the 25% more abundant compound detected in the inlet of the WWTP of Patras. Values are expressed in mg/day/1000 people. Lines represent the range from minimum to maximum values, while arrows indicate the mean values

Fig. 2 Monthly fluctuations of estimated doses consumed per day.in Patras, for certain illicit substances
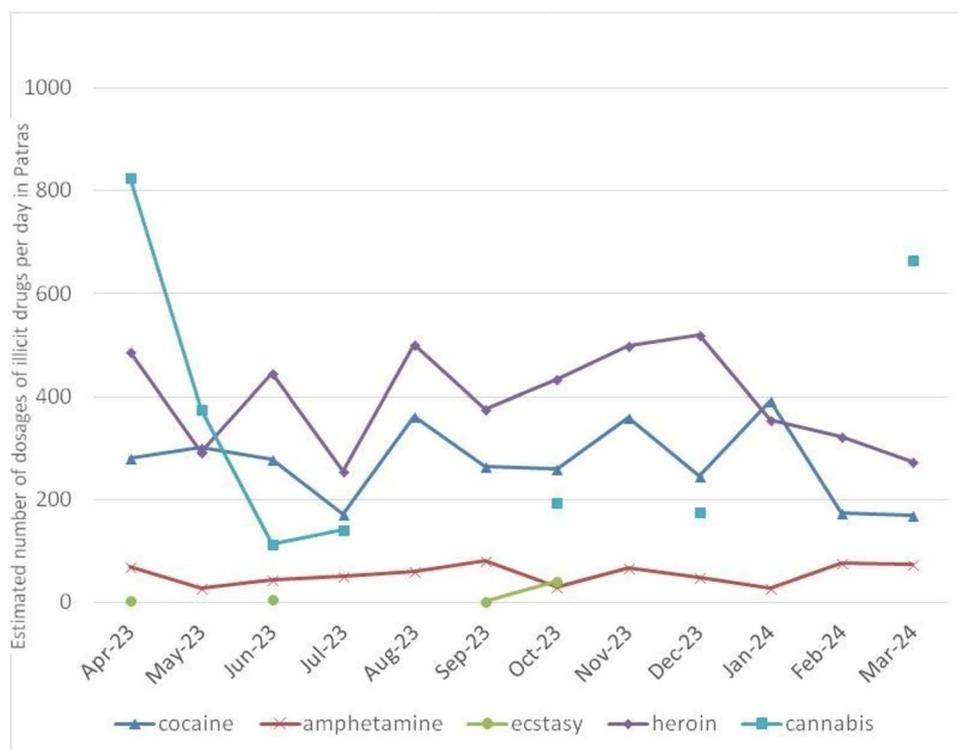
Discussion

Abundance of Compounds in Wastewater

Unsurprisingly, the most abundant substance observed in the WWTP of Patras was the analgesic acetaminophen (paracetamol), found at average levels of 4.4 µg/L and ranging from 1.7 µg/L (January 2024) to 7.4 µg/L (November 2023). This level is comparable to those observed in the municipal influents of other Greek cities: Athens (mean 4.8 µg/L) (Gago-Ferrero et al. 2020), Ioannina (range 4.7–52.5 µg/L) (Kosma et al. 2010), Volos 1.422 µg/L (Papageorgiou et al. 2016), but also in the WWTP influent samples throughout the world (range: 1.746–43.223 µg/L) (Al-Kaf et al. 2017). For example, in Beijing, China it was detected in a range of 4.2–11.2 µg/L during a surveillance campaign in 2018–2019 (Duan et al. 2022).

Other compounds that were detected in 100% of the samples and at mean concentrations greater than 1 µg/L included the anti-epileptics levetiracetam, gabapentin, and 10-hydroxy-10,11-dihydroxy-carbamazepine; stimulants such as nicotine, caffeine, and theophylline; non-steroidal anti-inflammatory drugs (NSAIDs) like hydroxy-ibuprofen and carboxy-ibuprofen; the anti-diabetic drug metformin; the anti-ulcer medication cimetidine; the anti-hyperuricemic agent allopurinol; the antilipidemic rosuvastatin; the antidepressant O-desmethyl Venlafaxine; the anti-hypertensive Valsartan; and the anti-inflammatory N-Acetylmesalazine.

Compounds with average concentrations in the 100–1000 ng/L range, were of various therapeutic classes, such as benzoylecgonine and EME (the main metabolites of cocaine), the NSAID diclofenac, the immunosuppressant mycophenolic acid, the antipsychotic amisulpride, and the antibiotics azithromycin and clarithromycin. Additionally, some compounds ranged between 10 and 100 ng/L, including the primary metabolites of amphetamine (MA, MDA, and MDMA) and the antibiotics clindamycin and trimethoprim.

High paracetamol levels reflect its widespread, over-the-counter use for common ailments such as headaches, fever, and pain. Similarly, elevated concentrations of pharmaceuticals like paracetamol, ibuprofen, and metformin reflect their extensive therapeutic use for pain, inflammation, and chronic conditions, such as diabetes. The notable presence of antidiabetic drugs—including metformin and sitagliptin—in wastewater likely corresponds to Greece's rising prevalence of type 2 diabetes, estimated at about 7–8% of adults based on e-prescription data (e.g., 8.2% in 2014–2015 and 7.9% in 2021) (Liatis et al. 2016). Metformin remains the most frequently prescribed diabetes medication (Siafarikas et al. 2024). The recurrent detection of venlafaxine, pregabalin, and levetiracetam may reflect substantial mental-health and pain-related treatment needs, including off-label use. In particular, venlafaxine and its metabolite align with increasing antidepressant prescriptions in Greece (Nimatoudis et al. 2004). Overall, wastewater findings capture both

pharmaceutical consumption patterns and broader public health trends in the region.

Antidepressants

Among antidepressants prescribed for major depressive disorder, venlafaxine and its metabolite O-desmethylvenlafaxine were the most abundant in Patras' wastewater. Venlafaxine is a serotonin-norepinephrine reuptake inhibitor (SNRI) used primarily for the treatment of major depression, but also for generalized anxiety disorder, social phobia, and panic disorder under the brand name of Effexor (Thase et al. 2001). Venlafaxine averaged 657 ng/L, within the range of 468–989 ng/L, which is higher than levels reported for Ioannina (123.4 ng/L) (Papageorgiou et al. 2016), but lower than those measured in Athens in 2014 (920 ng/L) (Gago-Ferrero et al. 2020). O-desmethylvenlafaxine reached an average of 1,311 ng/L, about four times higher than Ioannina (280.9 ng/L) (Papageorgiou et al. 2016) and roughly 1.5 times higher than Athens (0.89 μg/L) (Gago-Ferrero et al. 2020). This pattern aligns with pharmacokinetic data showing minimal venlafaxine excretion (≈5%) but substantially higher excretion of its metabolite (29–48%), a trend also observed in Italian WWTPs (Boix et al. 2016). The prominence of O-desmethylvenlafaxine is consistent with its widespread prescription in Mediterranean countries (Boix et al. 2016). In contrast, both compounds appeared at much lower concentrations in Beijing (venlafaxine: 8.86–76.55 ng/L; O-desmethylvenlafaxine: 38.47–400.39 ng/L) (Duan et al. 2022). Mirtazapine, commonly used for depression accompanied by insomnia or weight loss (Avena-Woods and Hilas 2012), was detected in Patras at 113 ng/L, compared with 330 ng/L in Athens (Gago-Ferrero et al. 2020) and 6.7 ng/L in Ioannina (Papageorgiou et al. 2016).

Antipsychotics and Opiates Analgesics

Amisulpride was the dominant antipsychotic in Patras' wastewater (mean: 250.8 ng/L), followed by norquetiapine (58.7 ng/L). Amisulpride appeared at lower levels in Athens in 2014 (70 ng/L; Gago-Ferrero et al. 2020) and in three WWTPs in northwest Italy (18–120 ng/L; Massano et al. 2023). Amisulpride, a benzamide dopamine antagonist, is primarily prescribed for schizophrenia and also shows efficacy in dysthymia due to its dual mechanism of action at low and high doses (Leucht et al. 2009). Quetiapine and its metabolite norquetiapine, used for schizophrenia, bipolar disorder, and adjunctive depression therapy (Young et al. 2010). were detected in Italy at 11–39 ng/L, comparable to Patras (17.6–44.5 ng/L;; Massano et al. 2023).

Tramadol, a synthetic opioid analgesic frequently prescribed for the management of moderate to severe pain, acts as a weak agonist at the μ-opioid receptor while also inhibiting the reuptake of norepinephrine and serotonin, contributing to its analgesic effects. Tramadol undergoes primary metabolism in the liver to form O-desmethyl-tramadol, which exhibits a higher affinity for the μ-opioid receptor and is largely responsible for the opioid activity associated with tramadol (Grond & Sablotzki 2004).In the wastewater of Patras, tramadol was consistently detected at notable levels, with a mean concentration of 308.6 ng/L and a range between 222 ng/L and 415 ng/L. Comparatively, lower concentrations were observed in Italian WWTPs, with values ranging from 41 to 215 ng/L (Massano et al. 2023). Given that tramadol is commonly prescribed for moderate to severe pain, its presence in wastewater may reflect an increased rate of prescriptions within the population.

Antiepileptics

Among the antiepileptics detected, levetiracetam, 10-hydroxy-10,11-dihydroxy-carbamazepine, and gabapentin were the most abundant. Their elevated levels may reflect higher local prevalence of epilepsy, seizures, neuropathic pain, or an aging population, though firm conclusions are limited by scarce literature. Levetiracetam, widely used for epilepsy and other neurological disorders, is largely excreted unchanged (≈66%; Steinhoff & Staack 2019), which may explain its prominence. It was detected at a mean concentration of 4,470 ng/L (range: 3,006–5,382 ng/L). Gabapentin, a GABA analogue used for neuropathic pain and epilepsy (Fink et al. 2002), ranged from 1,610–6,450 ng/L (mean: 2,585 ng/L), comparable to concentrations reported in a Greek hospital effluent (~2,000 ng/L; Arvaniti et al. 2023).

Oxcarbazepine, a sodium-channel blocker used in seizure control (Rogawski and Löscher 2004) was found at 270–1,111 ng/L (mean: 522 ng/L), while its active metabolite 10-hydroxy-10,11-dihydroxy-carbamazepine averaged 1,120 ng/L (range: 340–1,739 ng/L). Compared with Northwest Italy, where oxcarbazepine concentrations averaged 380 and 200 ng/L (Massano et al. 2023) levels in Patras appear nearly 80% higher.

Antihypertensive and Antilipidemics

A multitude of antihypertensive substances were detected in the city's urban wastewater. Valsartan was found to be at a higher rate compared to all antihypertensives, with a constant presence throughout the year. It is an angiotensin II receptor blocker (ARB), selectively blocks the AT1 receptor, reduces vasoconstriction and aldosterone secretion, and is widely used for hypertension and heart failure (Bissessor and White 2007). Valsartan ranged from 1,088 to 3,365 ng/L in Patras, with a mean concentration of 1,766 ng/L. As a

widely prescribed antihypertensive, it is commonly detected in urban wastewater at comparable levels; for instance, Athens reported a similar mean concentration of 1,660 ng/L (Gago-Ferrero et al. 2020).

Irbesartan, an angiotensin II receptor blocker (ARB), was detected with concentrations ranging from 275 to 1008 ng/L, and a mean concentration of 637 ng/L, with a notable peak observed in November 2023. Compared to Athens, where the mean concentration was 200 ng/L (Gago-Ferrero et al. 2020), the levels in this study represent a significantly higher load of this pharmaceutical in Patras.

Rosuvastatin was the most abundant antilipidemic detected, with a mean value of 1,116 ng/L, and levels ranging from 617 to 1,819 ng/L, notably higher than the mean concentration observed in Athens (170 ng/L) (Gago-Ferrero et al. 2020). Rosuvastatin is known for its high prescription rate and, like other statins, persists in wastewater at higher levels compared to other antilipidemic agents (Sulaiman et al. 2015).

Antidiabetics

Metformin, a first-line medication for type 2 diabetes, reduces hepatic gluconeogenesis and enhances peripheral insulin sensitivity, leading to improved glycemic control and reduced cardiovascular risk (Bailey and Turner 1996). It was the most abundant antidiabetic drug detected, with a mean concentration of 2,804 ng/L, ranging from 2,175 to 3,300 ng/L. Similarly, other studies highlight the significant presence of metformin in urban wastewater systems across Europe, primarily due to its widespread use in diabetes treatment and its persistence through conventional wastewater treatment processes (Posselt et al. 2018). Metformin has been detected in influent wastewater from various cities worldwide, with concentrations varying widely. In the U.S., the maximum concentration in WWTP influents ranged from 36,100 to 73,300 ng/L (Zheng et al. 2024). In Xian, China, concentrations ranged from 304 to 793 ng/L (Bai 2023). A study in 10 Turkish cities found average metformin concentrations in WWTP influents of 97,810 ng/L in 2019, 75,190 ng/L in 2020, and 69,130 ng/L in 2021 (Yavuz Guzel et al. 2024). Similarly, a national study in Australia detected metformin in all WWTP influent samples, with concentrations ranging from 8,200 to 191,000 ng/L and an average concentration of 58,000 ng/L (Yang et al. 2022).

Antibiotics

The most abundant antibiotics in this study were clarithromycin, azithromycin, and metronidazole, with mean concentrations of 486, 481, and 453 ng/L, respectively. In Athens, clarithromycin appeared at much higher levels (2,700 ng/L), while azithromycin (30 ng/L) and metronidazole (170 ng/L) were detected at substantially lower concentrations. Even lower metronidazole values (ND–64.7 ng/L) and higher levels of lincomycin, sulfamethoxazole, and trimethoprim were reported in Volos (Papageorgiou et al. 2016). In Southern California, sulfamethoxazole showed particularly high concentrations (1,372 and 1,354.29 ng/L across two WWTPs; Phonsiri et al. 2019), exceeding those measured in Patras (Table S1), whereas trimethoprim levels in Patras were similar to those in WWTP2 (59.57 ng/L). These antibiotics are widely prescribed, especially macrolides such as azithromycin and clarithromycin, which frequently appear in wastewater due to substantial excretion of unchanged drug or active metabolites (Omuferen et al. 2022). All detected antibiotics were present in 100% of samples, while sulfadiazine, sulfapyridine, minocycline, doxycycline, isoniazid, roxithromycin, and tetracycline were not detected.

Anti-Inflammatory Agents

N-Acetylmesalazine, also known as N-acetyl-5-aminosalicylic acid, is the primary metabolite of mesalazine (5-aminosalicylic acid), a drug commonly used for the treatment of inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease (Ransford 2002). Its presence found to be quite high in urban wastewater with mean concentration 1,513 ng/L and range 851–2,230 ng/L, which may indicate the high incidence of these autoimmune diseases in the population, as well as possible high prescription rates or incomplete metabolism and excretion of the drug. Regarding its presence in wastewater, specific data on mesalazine concentrations in influent wastewater are limited.

Stimulants

Among the stimulants detected, it is crucial to focus on caffeine and nicotine concentrations, as both are found at high levels and contribute to the stimulation of the central and peripheral nervous systems. While nicotine is primarily associated with addiction, it also has therapeutic applications in smoking cessation therapies (Benowitz 2009). Caffeine levels ranged from 964 to 4,941 ng/L in the wastewaters of Patras, with a mean concentration of 2,665 ng/L, while nicotine levels ranged from 356 to 4,202 ng/L, with a mean concentration of 2,212 ng/L. A study conducted in Volos reported similar caffeine levels, with a mean range of 2,876–4,968 ng/L (Papageorgiou et al. 2016). A systematic review and meta-analysis, that examined global nicotine consumption rates through WBE, revealed that the highest nicotine consumption rates were in Portugal, while the lowest were observed in Vietnam. It also highlighted that

nicotine consumption levels detected through WBE reached "risk" levels, underscoring the need for immediate public health interventions (Asadi et al. 2023). Furthermore, a study analyzing influent wastewater from 13 sewage treatment plants across Italy reported mean caffeine concentrations of 17,600 – 67,600 ng/L and nicotine concentrations of 1,360–6,870 ng/L, showing a significant difference in the magnitude of the consumption of these substances in various countries (Senta et al. 2015).

Drugs of Abuse

The findings of the study indicate a consistent presence of cocaine and its metabolites in the wastewater of Patras, with minor increases observed in August, November, and January. Cocaine, a potent central nervous system stimulant, acts by inhibiting the reuptake of dopamine, norepinephrine, and serotonin, thereby increasing the synaptic concentrations of these neurotransmitters. Upon metabolism in the liver, cocaine is converted into benzoylecgonine and ecgonine methyl ester (EME), with benzoylecgonine being the primary metabolite detected in urine during forensic and clinical toxicology analyses (Bortolotti et al. 2012). Benzoylecgonine, given its extended presence in urine compared to cocaine, is frequently used as a marker of recent cocaine use. The 2024 European Union Drugs Agency (EUDA) report identifies the highest concentrations of benzoylecgonine in the wastewater of western and southern European cities, particularly in Belgium (max: 1,996.98 mg/day/1000 people), the Netherlands (max: 1,206.28 mg/day/1000 people), and Spain (max: 1,482.91 mg/day/1000 people). In contrast, eastern European cities generally exhibit lower levels, with Kokkola, Finland, reporting 3.05 mg/day/1000 people, and Larnaca, Cyprus, 38.39 mg/day/1000 people. Cocaine consumption has been reported to present a pattern of recreational drug use, as its levels are typically elevated on weekends (Friday to Monday) (EMCDDA, 2024). In Athens, the estimated level of cocaine consumption in 2024 was 181.21 mg/day/1000 people, whereas in Patras, the values varied from 110.53 mg/day/1000 people in July 2023 to 265.34 mg/day/1000 people in November 2023, yielding an average of 169 mg/day/1000 people.

The average amphetamine consumption in Patras was 18.54 mg/day/1000 people, with concentrations fluctuating between 6.43 mg/day/1000 people in March and 38.46 mg/day/1000 people in May. In comparison, Athens recorded a daily average of 12.98 mg/day/1000 people in 2024, with historical data ranging from 2.24 mg/day/1000 people in 2017 to 16.84 mg/day/1000 people in 2014. Notably, amphetamine concentrations in northern and eastern European cities, particularly Sweden, where the highest level reached 518.28 mg/day/1000 people, are reported to be substantially higher than those observed in southern Europe. While amphetamine levels in southern European cities remain generally lower, recent data show signs of an increasing trend (EMCDDA, 2024). Amphetamines are central nervous system stimulants that, along with derivatives, like methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA), induce stimulant effects by promoting the release of monoamines. MDMA, commonly known as "Ecstasy" or "Molly," is known for its empathogenic properties, stemming from serotonin release (Green et al. 2003; Bouzoukas et al 2025). MDMA and MDA also have stimulant and hallucinogenic properties. Legal forms of amphetamines are prescribed for conditions such as narcolepsy and Attention Deficit Hyperactivity Disorder (ADHD), but methamphetamine, MDA, and MDMA remain illicit substances. In Greece, cocaine, MDA, and MDMA are classified as narcotics under Law 3459/2006, aligning with international conventions such as the UN Single Convention on Narcotic Drugs (1961) and the EU Framework Decision on drug trafficking (2004/757/JHA). In 2024, the estimated methamphetamine consumption in Patras was 13.15 mg/day/1000 people, slightly lower than the corresponding value of 20.35 mg/day/1000 people in Athens, as per the EUDA report (EUDA, 2024). Although methamphetamine use remains relatively low across most cities, there are indications of an increasing trend in some Central European cities. Historically, Czechia and Slovakia have exhibited the highest consumption levels, with Czechia reaching 595.46 mg/day/1000 people and Slovakia 295.37 mg/day/1000 people. MDMA consumption in Patras was estimated at 2.85 mg/day/1000 people, nearly three times lower than the 7.77 mg/day/1000 people recorded in Athens in 2014. MDMA was sporadically detected in the wastewater of Patras during April, June, September, and October of 2023. According to European data, Amsterdam, the Netherlands, recorded the highest MDMA load in 2024, at 282.51 mg/day/1000 people, continuing an upward trend since 2011 (EUDA, 2024).

Opioids such as codeine and morphine, derived from the opium poppy, exert their effects primarily by binding to μ-opioid receptors. Morphine, a potent analgesic, is metabolized into codeine to exert its therapeutic effects (Smith 2009). In Greece, both substances are regulated under Law 3459/2006, and their possession is strictly controlled. Morphine, however, is also the primary metabolite of heroin. The estimated daily consumption of heroin in Patras, as derived from morphine's levels, ranged from 41.84 to 110.42 mg/day/1000 people, with a yearly average of 75.37 mg/day/1000 people. It is important to note that the calculated levels are overestimated, as it is assumed all morphine detected in the wastewater originates from heroin use.

Δ9-Tetrahydrocannabinolic acid A (THCA-A), the non-psychoactive precursor to THC, is converted to the psychoactive compound THC through decarboxylation, which then activates cannabinoid receptors in the brain (McPartland and Russo 2001). In Greece, the cultivation and use of industrial hemp containing THC levels not exceeding 0.2% THCA-A is permitted under Law 4139/2013. Consequently, products derived from hemp that comply with this THC limit are generally legal. In Patras, cannabis consumption was estimated at 207.7 mg/day/1000 people, with notable fluctuations ranging from undetectable levels for five months to 844.44 mg/day/1000 people in March 2024. In comparison, cannabis consumption in Athens averaged 36.9 mg/day/1000 people, while Seattle, USA, reported the highest consumption at 598.54 mg/day/1000 people in 2024 (EMCDDA, 2024).

Undetected Compounds

Despite a thorough analysis, several analytes were found to be below the limits of detection (LOD). The absence of certain analytes in wastewater-based analyses can be attributed to several factors. Low consumption rates within the population may result in minimal excretion of these substances, leading to concentrations that fall below detection limits (Gros et al. 2007). Additionally, rapid metabolism may transform these compounds into metabolites that are not targeted in the analysis, further lowering detectable levels (Bijlsma et al. 2009). Environmental processes such as photolysis and biodegradation can also reduce analyte concentrations before sampling occurs (Fatta-Kassinos et al. 2011). Analytical limitations, including insufficient sensitivity of detection methods, may prevent the identification of trace amounts of substances. Furthermore, complex wastewater matrices can interfere with the detection process, making accurate measurement more challenging (Petrović et al. 2005). Dilution effects due to factors like increased water flow, can lower analyte concentrations, making them harder to detect (Ort et al. 2010). Finally, temporal variability in substance uses and excretion patterns can lead to fluctuating concentrations, with some analytes falling below detection thresholds during certain periods (Zuccato et al. 2008).

Environmental Contamination

Many studies are being conducted around the globe, according to which many pharmaceutical substances, illicit drugs and micropollutants from healthcare products are detected in surface waters, resulting from inadequate treatment of urban wastewater in cities (Thomaidi et al. 2015). The contamination of aquifers by pharmaceuticals and other toxic substances represents a new form of pollution, and while the full effects on living organisms remain unknown, they cannot be overlooked.

The results underscore the continuous input of pharmaceuticals and other chemicals into the wastewater system. The presence of persistent compounds, such as gabapentin, venlafaxine, metformin etc. suggests potential risks to aquatic ecosystems and necessitates further investigation into removal efficiencies during wastewater treatment (Gros et al. 2007). Several of the substances identified in this study, such as carbamazepine, diclofenac, and clarithromycin, are well known for their ability to persist in the environment long after their intended use. These compounds are not fully removed by conventional wastewater treatment processes and often end up in rivers and other surface waters, where they can pose risks to aquatic life. They have been recognized internationally as contaminants of emerging concern due to their widespread occurrence and potential ecological impacts (aus der Beek et al. 2016) Specifically, carbamazepine, is highly resistant to breakdown in the environment and has been found in water bodies around the world. Studies show that long-term exposure, even at low concentrations, can affect the development, reproduction, and behavior of aquatic organisms, including fish and invertebrates (Subedi et al., 2013). Diclofenac, a widely used painkiller and anti-inflammatory drug, has also raised concern. It has been linked to kidney and liver damage in fish and is considered toxic to aquatic life with long-lasting effects. Because of its harmful impact, the European Union has proposed stricter environmental controls on its use and disposal (Schwaiger et al. 2004). Similarly, venlafaxine, and its metabolite O-desmethyl-venlafaxine have been shown to interfere with fish behavior, including feeding and mating. Since these substances act on the central nervous system, their presence in water, even in trace amounts, can lead to unintended effects on the nervous systems of aquatic animals, especially with continuous exposure over time (Brooks et al. 2003). Clarithromycin, a macrolide antibiotic, poses a notably high environmental risk. Risk assessments show that its concentration in WWTP effluents results in a risk quotient (MEC/PNEC) greater than 7, indicating significant ecological threat (Baranauskaitė-Fedorova and Dvarionienė, 2022).

These examples highlight why it is important not only to detect these substances but also to understand their long-term consequences for aquatic ecosystems. Addressing their persistence and potential toxicity calls for better removal technologies and ongoing environmental monitoring.

As a result, the scientific community must prioritize the development of methods to more effectively purify urban wastewater from these contaminants, preventing their release into the environment.

Public Health Monitoring

Wastewater-Based Epidemiology provides an efficient and reliable method to monitor illicit and pharmaceutical substance use at the population level. It offers valuable insights into drug trafficking, public health, and community well-being, especially during global health crises like the COVID-19 pandemic. WBE allows for timely, anonymous health management while safeguarding citizen privacy. Comparisons with other Greek and international cities helped clarifying how local consumption patterns align with or diverge from regional public-health indicators. Differences in pharmaceutical and illicit drug levels between Patras and other cities likely stem from demographic, socioeconomic, and healthcare-related factors. As a university and port city with a large transient and young adult population, Patras may show elevated use of certain pharmaceuticals, such as antidepressants and stimulants, as well as distinct recreational drug patterns. Variations in prescribing habits, healthcare access, and disease prevalence further shape these differences. Together, these contextual elements explain intercity variability and underscore the need to interpret WBE data within the local setting.

In the future, WBE is poised to evolve with advances in wastewater analysis, increasing sensitivity and accuracy in detecting low concentrations of pathogens and chemicals. The integration of genomics, metabolomics, proteomics, and automated analysis will enable faster, more comprehensive detection. Additionally, AI and machine learning will enhance big data analysis, enabling accurate predictions and swift responses to potential health threats.

Conclusions

This is the first study to examine pharmaceutical substances in the wastewater influent of Patras, Greece's third-largest city. A broad spectrum of compounds from various pharmacological groups was detected, reflecting the drug-use behaviors and health status of its residents. High concentrations of common pharmaceuticals such as metformin, venlafaxine, and rosuvastatin indicate prevalent chronic diseases, including diabetes, cardiovascular disorders, and mental health conditions within the community. Moreover, the consistent detection of illicit drugs such as cocaine and methamphetamine demonstrates the persistence of recreational drug use, aligning with trends observed in other European cities. These findings confirm that Wastewater-Based Epidemiology (WBE) provides an accurate, non-invasive tool for assessing public health, lifestyle habits, and environmental contamination at the population level. A key next step is to integrate wastewater analysis into national and international monitoring systems. Future research should expand WBE coverage to develop a comprehensive and comparative framework for population health assessment. Establishing wastewater networks across cities and countries would enable real-time data collection, promote global collaboration, and enhance health assessments, ultimately supporting coordinated strategies to address public health and environmental challenges.

Declarations

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