

## PRESENCE OF ILLICIT DRUG METABOLITES IN THE INFLUENT OF A WASTEWATER TREATMENT PLANT IN ELDORET, KENYA

<sup>1</sup>John Kevine Ochieng\*, <sup>1</sup>Francis Ongachi Olal, <sup>1</sup>Edward Anino

\*Corresponding Author: [jkevine85@gmail.com](mailto:jkevine85@gmail.com)

<https://orcid.org/0000-0002-3628-635X>; <https://orcid.org/0000-0003-4928-5310>; <https://orcid.org/0000-0003-1936-1212>

<sup>1</sup>Rongo University, P.O. Box 103-40404 Rongo, Kenya

### Abstract

*The increased occurrence of illicit and medical drug residues in urban wastewater raises new environmental and public health problems. Conventional monitoring methods are frequently expensive, invasive, and have limited population coverage, rendering them unsuitable for routine community surveillance. This study used wastewater-based epidemiology (WBE) to investigate the prevalence and temporal trends of selected drug metabolites in influent wastewater from a treatment facility in Eldoret, Kenya. From February to May 2025, weekly composite influent samples were collected and analysed using solid-phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Six metabolites were consistently identified: amphetamines, methylhexanamine, 19-norandrosterone, 3'-hydroxy-stanozolol, erythropoietin (EPO), and cocaine derivatives. Amphetamine concentrations peaked in February ( $50.0 \pm 0.10$  ng/L) and gradually fell, while anabolic steroid metabolites exhibited a moderate but consistent fall. Methylhexanamine levels remained rather steady throughout the study period, but cocaine metabolites showed up occasionally, indicating sporadic community usage. The discovery of EPO (0.003 ng/L) revealed WBE's great analytical sensitivity in detecting low-level biomarkers in complicated wastewater mixtures. Overall, the findings emphasise WBE as a viable, non-invasive, and cost-effective method for monitoring community-level drug exposure and producing real-time local information on emergent substance-use trends. This study presents the first baseline dataset of drug-related metabolites in Kenyan wastewater, paving the way for WBE's future integration into national environmental and public health surveillance regimes.*

**Keywords:** *Liquid chromatography-Mass spectrometry, epidemiology, wastewater, treatment plants, metabolite profiling, illicit drug*

### Introduction

The growing use of illegal and performance-enhancing drugs around the world poses serious risks to environmental and public health. Modern drug use, which was once limited to conventional anabolic steroids, now includes a variety of intricate and challenging-to-detect substances, such as methylhexanamine, amphetamines, micro-dosed erythropoietin (EPO), opioids, and sophisticated masking agents (Petróczi & Boardley, 2022).

According to recent statistics, drug usage is common across a variety of demographics; in 2022, over 1,500 drug-related infractions were reported worldwide (WADA, 2023). This pattern illustrates the enduring nature of substance abuse as well as the shortcomings of current detection and prevention techniques.

Concerns about the abuse of illegal and prescription drugs are becoming more prevalent in Kenya, which is in line with a worldwide trend.

Regional reports and national statistics indicate that substance use is widespread in the general community and is not limited to particular professional groups, frequently going unnoticed by traditional monitoring methods (Jaguga et al., 2023; Owiti & Ogola, 2024; NACADA, 2022). The prevalence of uncontrolled steroid and stimulant use, as well as the distribution of recreational drugs and mislabeled supplements, suggests systemic difficulties in prevention and monitoring. These difficulties show how urgently trustworthy population-level surveillance methods that go beyond case-based detection are needed.

Although scientifically sound, traditional biological testing methods like blood and urine collection are frequently difficult for extensive community surveillance. They only offer a restricted temporal perspective, demand rigorous logistics, and are resource-intensive (Kwaw, 2025). As a result, there is a dearth of information on actual usage patterns and significant segments of the population are not tracked. Effective legislation and intervention are hampered by the substantial gaps in knowledge regarding the scope and dynamics of substance use.

To fill these deficiencies, wastewater-based epidemiology (WBE) has become a viable supplementary strategy. WBE allows for anonymous, real-time monitoring of drug use at the community level, based on the idea that drugs and their metabolites are eliminated in urine or faeces and then end up in wastewater systems (Daughton, 2012; Zuccato et al., 2005). WBE enables highly sensitive identification of trace-level metabolites across several classes of compounds when combined with sophisticated analytical methods as solid-phase extraction and liquid chromatography–tandem mass spectrometry (LC-MS/MS) (González-Mariño et al., 2020; Bade et al., 2024).

In countries like South Africa, Australia, and Spain, WBE has been effectively used to monitor trends in both prescription and illegal drug use. The results of these research demonstrate that WBE can offer important insights into temporal and spatial patterns of substance use that are frequently overlooked by conventional monitoring techniques

(Madikizela et al., 2020; Carnevale Miino et al., 2023). Despite these developments, WBE is still not widely used in Kenya, and baseline data on the presence of restricted chemicals in wastewater is scarce. Because of this disparity, environmental organisations, health authorities, and policymakers lack the data necessary to create well-informed solutions.

By using WBE to detect and measure the metabolites of illegal and regulated compounds in wastewater from Eldoret, Kenya, this work helps close that gap. Over the course of a four-month sample period, these metabolites were examined using LC-MS/MS techniques. The identification of these substances offers crucial information about community-level trends in the usage of performance-enhancing and recreational drugs. Based on these considerations, this study hypothesized that the concentrations of selected illicit and performance-enhancing drug metabolites in wastewater would show temporal variation consistent with shifts in community substance-use patterns. This study shows the promise of WBE as a scalable, affordable, and non-invasive surveillance technology that can support current regulatory and health frameworks by producing localised baseline data.

## Materials and Methods

In order to detect and measure the metabolites of illegal and regulated substances in a community context, this study used wastewater-based epidemiology, or WBE. A four-month observational, non-invasive, cross-sectional sampling strategy was used to record temporal fluctuations in metabolite concentrations. A systematic routine was put in place to track changes over time.

This study was carried out in the Eldoret Municipal Council Wastewater Treatment Plant (WWTP) in Uasin Gishu County, Kenya (coordinates: 0.55186° N, 35.2655° E). With an average elevation of 2,100 meters above sea level. A rapidly expanding urban centre, Eldoret is distinguished by a growing population density, a wide range of socioeconomic

activity, and an increase in the demand for municipal services. The municipality has steadily seen an increase in institutional, commercial, and residential buildings, all of which add to the wastewater stream. A wastewater treatment facility (WWTP) that receives mixed influent from local companies, residences, and public facilities served as the site of the sampling. Because of its strategic accessibility for routine monitoring and its representativeness of the community's effluent, the plant's inlet was chosen as the sampling location. Wastewater at this location is appropriate for wastewater-based epidemiological studies because it offers a composite matrix that reflects the combined consumption habits of the local population. In order to capture possible temporal fluctuations in community substance use related to seasonal, social, or economic causes, the study period was extended from February to May 2025. An accurate evaluation of drug metabolite trends over the study period was made possible by the consistent intake to the WWTP, which guaranteed dependable sample collection over time. This environment offered a useful and reliable framework for assessing wastewater-based epidemiology's (WBE) suitability for tracking drug use in Kenyan communities.

Field sampling equipment and materials included high-density polyethylene (HDPE) sample containers (1 L capacity) that had been pre-cleaned with laboratory-grade detergents and ultrapure water, insulated ice boxes with ice packs to maintain the cold chain during transport, and personal protective equipment (PPE) such as laboratory coats, nitrile gloves and safety goggles. Filtration equipment, SPE cartridges, solvent-safe amber vials, nitrogen gas cylinders for evaporation, and precision glassware were all employed during sample preparation and extraction. All apparatus were chosen based on their chemical inertness, appropriateness for handling trace pharmaceutical residues, and ability to reduce contamination risk throughout the sampling and analytical workflow.

The sampling location for this investigation was chosen to be the entrance of a wastewater

treatment facility (WWTP) in Eldoret, Kenya. This facility is indicative of wastewater streams throughout the community since it receives influent from a wide catchment area that includes institutional, commercial, and residential sources. Because the WWTP influent collects aggregated effluent that represents the general consumption patterns of the local population, sampling there was deemed suitable for wastewater-based epidemiology (WBE). Over the course of the four-month investigation, a methodical methodology was used to guarantee the representativeness, consistency, and integrity of the samples that were gathered. Samples were taken once weekly, on the first day of each week, to capture temporal change. This allowed for the identification of larger community trends in metabolite occurrence while minimising random daily fluctuations. Because early-morning wastewater often contains more concentrated discharged metabolites from overnight physiological activity, sampling was done over a set two-hour morning window (07:00–09:00). This timetable reduced variability that can result from variations in flow or human activity during the day and increased comparability among sample occasions. High-density polyethylene (HDPE) containers that had been previously cleaned were used to gather composite grab samples, each weighing around 1 L. Because of their proven usefulness for environmental water sampling and chemical inertness, HDPE containers were selected. To remove any chance of contamination, all containers were cleaned with lab-grade detergent, completely rinsed with ultrapure water, and allowed to air dry in a clean laboratory setting prior to deployment. To clear the channel and reduce the possibility of accumulating non-representative stagnant material, the first influent flow was left running at the WWTP intake for a few minutes. After that, containers were carefully submerged to gather samples without coming into contact with the channel's walls or any surface debris. To stop analytes from degrading in transit, samples were put in ice-cooled transport boxes as soon as they were collected. Samples were immediately kept at -20 °C upon arrival at the lab in

order to prevent chemical breakdown and microbiological activity. Each sample was frozen overnight at 4 °C and then gently homogenised by inversion to guarantee homogeneity prior to analysis. A 100 mL subsample was extracted from each homogenised sample for additional processing, such as liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis and solid-phase extraction (SPE). These techniques made it possible to identify target metabolites at the trace level with great sensitivity and selectivity. Date, time, ambient temperature, flow parameters, and any odd observations, including smells or changes in influent appearance, were all noted in the field notes for every sampling event. Interpreting analytical results and determining potential confounding factors connected to wastewater system operation required this kind of contextual knowledge. Strict adherence to documented WBE methods was maintained throughout the whole process, guaranteeing comparability with earlier research (Zuccato et al., 2005; González-Mariño et al., 2020). Furthermore, all fieldwork participants adhered to the proper biosafety protocols. Gloves, lab coats, and safety glasses were among the personal protective equipment (PPE) worn to safeguard the researchers and the sample integrity. Training was given to field workers on proper sampling techniques, biosafety measures, and how to handle potentially dangerous effluent. By taking these steps and implementing strict quality assurance procedures, the gathered samples were guaranteed to be appropriate for instrumental analysis later on. The careful methodology used in this study gave WBE a solid basis on which to evaluate community-level exposure to illegal and controlled substances in Eldoret, Kenya.

Ultrapure water, methanol, ethanol, and acetonitrile were employed as laboratory grade reagents. Analytical-grade solvents containing 0.1% formic acid were used for chromatographic separation during LC-MS/MS analysis. Internal standards, certified reference standards, and isotope-labeled standards were employed to aid in quantitative calibration and compound

identification. Because of its structural and physicochemical similarities, d5-amphetamine (amphetamine-D5) was utilized as the internal standard for amphetamines, whereas certified d-amphetamine was used as the reference standard. Certified 19-Norandrosterone was utilized as the analytical standard in the instance of 19-Norandrosterone, a major metabolite of nandrolone. To account for matrix effects and extraction variability, 19-Norandrosterone-D4 or D3 was utilized as the internal standard. The recognized analytical standard of 3'-Hydroxy-Stanozolol was utilized for 3'-Hydroxy-Stanozolol, a metabolite of stanozolol, and, when it was available, 3'-Hydroxy-Stanozolol-D3 or Stanozolol-D3 was used as the internal standard. Methylhexanamine-D9 was used as the deuterated internal standard, and methylhexanamine (DMAA), a prohibited stimulant frequently present in sports supplements, was quantified using its certified reference form. Benzoylecgonine-D3 or D8 and norcocaine-D3, respectively, were utilized as internal standards for the quantification of cocaine metabolites, including benzoylecgonine and norcocaine, using certified standards of each component. Traditional deuterated standards were not applicable for erythropoietin (EPO) because of its complex protein structure; instead, recombinant human erythropoietin (rHuEPO) was used as the reference, and internal standardization was carried out using Erythropoietin a specific marker fragments or stable isotope-labeled peptides in accordance with proteomic LC-MS/MS techniques (Mariño and et al, 2020; Bade and et al, 2024). Filtration units, glassware, nitrogen gas cylinders for evaporation, and solvent-safe reconstitution vials were among the other supplies needed for the facility. Because of their great purity and suitability for detecting pharmaceutical residues at the trace level in intricate wastewater samples, all of the materials employed were chosen to preserve the integrity of sensitive target chemicals during the sampling, extraction, and analysis procedures, to avoid contamination, and guarantee analytical accuracy.

Sample extraction and analyte enrichment were performed using Solid Phase Extraction (SPE) using standard WBE-based trace level techniques with slight adjustments to suit local laboratory conditions (González-Mariño et al., 2020; Bade et al., 2024). Following freezing and homogenisation, each 100 mL filtered wastewater sample underwent SPE with Oasis HLB cartridges. Cartridges were pre-conditioned with 5 mL methanol, followed by 5 mL ultrapure water, before being loaded with samples under vacuum at a controlled flow rate. Following sample loading, cartridges were washed with 5 mL ultrapure water to remove matrix interferences before analytes were eluted with 5 mL acetonitrile. Eluates were then evaporated under a gentle nitrogen stream at room temperature and reconstituted in 1 mL mobile phase (water:acetonitrile with 0.1% formic acid) prior to LC-MS/MS analysis. LC-MS/MS analysis was performed using an Agilent 6460 Triple Quadrupole system operating in positive ESI mode with chromatographic separation achieved using a reverse-phase C18 column. Gradient elution employed ultrapure water and acetonitrile, each containing 0.1% formic acid, with a total run time of approximately 12 minutes per sample. MRM transitions optimized for each analyte were used based on certified standards (Lin et al., 2021; McCall et al., 2016). Key LC-MS/MS parameters included a capillary voltage of 4,000 V, drying gas temperature of 300 °C, drying gas flow of 10 L/min, nebulizer pressure of 35 psi, injection volume of 10  $\mu$ L, and flow rate of 0.3 mL/min.

The research focused on detecting and quantifying specific illegal and performance-enhancing drug metabolites in wastewater. Target analytes included amphetamine, 19-norandrosterone, 3'-hydroxystanozolol, methylhexanamine (DMAA), benzoylecgonine, norcocaine, and erythropoietin (EPO). (González-Mariño et al. 2020) chose these substances for their importance in doping control, recreational abuse, anti-doping surveillance, and detectability in wastewater using LC-MS/MS analysis. Based on these considerations, this study hypothesized that the concentrations of selected illicit and performance-enhancing drug metabolites

in wastewater would show temporal variation consistent with shifts in community substance-use patterns. The concentration (ng/L) of each target metabolite was the major dependent variable used to assess community-level substance use behaviour and temporal fluctuation throughout a four-month monitoring period.

The major variable of interest in this investigation was the concentration of illicit drugs and performance-enhancing compounds discovered in municipal influent wastewater, which was expressed in ng/L. These metabolites served as proxy indicators of community-level substance use behaviour among the Eldoret watershed population. To enable compound-specific temporal monitoring, the study treated each metabolite as a single variable rather than as a group of chemical classes. As a result, amphetamine, 19-norandrosterone, 3'-hydroxystanozolol, methylhexanamine (DMAA), benzoylecgonine, norcocaine, and erythropoietin (EPO) were all distinct dependent variables whose variations over time reflected changing drug-use patterns in the population that used the wastewater treatment system during the study period.

Quantification was aided by certified reference standards and deuterated internal standards for each analyte class as described by (González-Mariño et al., 2020; Bade et al. Analytical batches had procedural blanks, matrix-matched spikes, and quality control samples to evaluate instrument drift and contamination. The mean recoveries varied from 78-108%, with matrix effects within  $\pm 15\%$ , showing minor ion suppression or enhancement during SPE clean-up. Calibration curves using six-point standard plots (0.01-100 ng/L) showed excellent linearity ( $R^2 > 0.99$ ) across all analytes (McCall et al., 2016; Bade et al., 2024). LOD and LOQ varied from 0.003 to 0.30 ng/L, which is consistent with trace-level detection capabilities in wastewater reported in previous WBE studies (Bade et al., 2024). The final values were determined from triple analytical measurements per sampling point and are provided as mean  $\pm$  standard error.

This study followed the institutional and national ethical criteria for environmental and public health research. WBE was declared non-invasive because no direct human subject sampling or personal identifiers were used. Before beginning data collecting, the institution obtained ethical approval. To safeguard privacy and confidentiality, all sampling was limited to the WWTP influent intake, with no wastewater being isolated based on specific identifiable groups. To avoid indirect identification, the identities and locations of athletic training facilities in the WWTP catchment were anonymised. González-Mariño et al. (2020) followed standard laboratory biosafety procedures, PPE use, secure data storage, and restricted access guidelines throughout their work.

## Results and Discussions

The presence and temporal fluctuations of several drug-related metabolites were determined by analysing wastewater samples that were collected in Eldoret, Kenya. All presented results are the average of triple experiments, and high-sensitivity liquid chromatography–tandem mass spectrometry (LC-MS/MS) was used to verify accuracy and reproducibility.

Strong analytical performance was shown by the LC-MS/MS system, which achieved low detection limits and distinct chromatographic separation of the target analytes. Retention durations, precursor and product ions, and technique sensitivity, represented as limits of detection (LOD) and limits of quantification (LOQ), are among the important performance metrics for the chosen chemicals that are compiled in Table 1.

**Table 1**

*LC-MS/MS Performance Parameters for Selected Analytes.*

| Compound           | RT (min) | Precursor (m/z) | Product (m/z) | LOD (ng/L) | LOQ (ng/L) |
|--------------------|----------|-----------------|---------------|------------|------------|
| Amphetamine        | 2.5      | 136.1           | 119.1         | 0.20       | 0.60       |
| 19-Norandrosterone | 5.6      | 271.2           | 253.2         | 0.10       | 0.25       |
| Benzoylcegonine    | 4.2      | 290.1           | 168.9         | 0.05       | 0.15       |
| Norcocaine         | 3.8      | 303.1           | 181.9         | 0.10       | 0.30       |

Throughout the sampling period, six target metabolites were detected using quantitative analysis. Table 2 shows the monthly mean

concentrations of each metabolite ( $\pm$  standard error), while Figure 1 shows temporal concentration changes.

**Table2**

*Results of Metabolites Concentration from Eldoret WWTP .*

| Metabolite            | Feb (ng/L)         | Mar               | Apr                | May                |
|-----------------------|--------------------|-------------------|--------------------|--------------------|
| 3'-Hydroxy-Stanozolol | 5.00 $\pm$ 0.06    | 3.80 $\pm$ 0.12   | 3.10 $\pm$ 0.09    | 2.50 $\pm$ 0.04    |
| 19-Norandrosterone    | 10.00 $\pm$ 0.34   | 7.50 $\pm$ 0.15   | 6.00 $\pm$ 0.23    | 5.00 $\pm$ 0.11    |
| Amphetamines          | 50.00 $\pm$ 0.10   | 35.00 $\pm$ 0.50  | 22.00 $\pm$ 0.80   | 15.00 $\pm$ 0.37   |
| Cocaine Metabolites   | 1.20 $\pm$ 0.04    | 0.80 $\pm$ 0.01   | 0.00 $\pm$ 0.00    | 0.30 $\pm$ 0.01    |
| Methylhexanamine      | 4.50 $\pm$ 0.16    | 4.10 $\pm$ 0.08   | 3.80 $\pm$ 0.12    | 3.50 $\pm$ 0.06    |
| EPO                   | 0.003 $\pm$ 0.0001 | 0.003 $\pm$ 0.001 | 0.003 $\pm$ 0.0001 | 0.003 $\pm$ 0.0001 |

**Figure 1**

*Monthly Concentrations of Key PEDs and Stimulants in Eldoret WWTP*

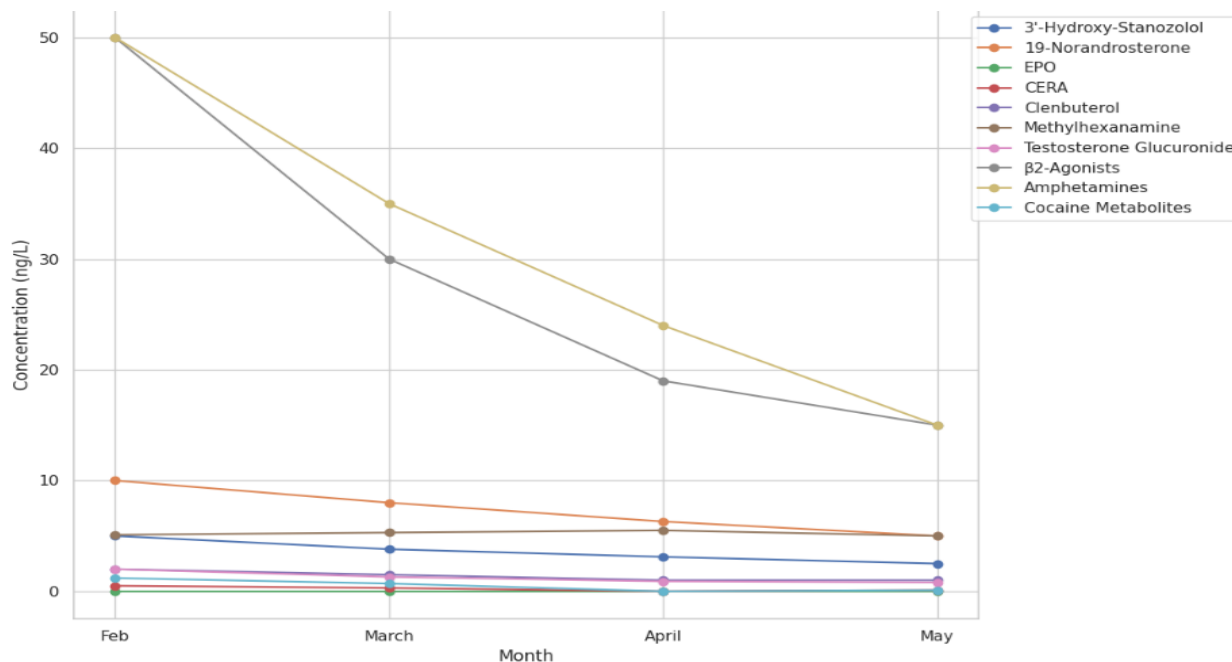


Figure 1 depicts the temporal fluctuation in the concentrations of selected illicit and performance-enhancing drug metabolites in wastewater between February and May 2025. Amphetamines had the highest amounts throughout all months and dropped sharply from 50 ng/L in February to around 15 ng/L by May, indicating a significant decrease in community use over time. A comparable decrease trend was detected for steroid metabolites, such as 19-norandrosterone and 3'-hydroxy-stanozolol, indicating a proportional drop in anabolic agent exposure during the same time period. Methylhexanamine concentrations were relatively steady, with just minor monthly changes, indicating continued but mild use, most likely due to dietary stimulant supplements. Cocaine metabolites were intermittently present, indicating occasional, non-continuous population consumption. EPO trace levels were stable throughout the study period, suggesting that wastewater-based epidemiology can detect biomarkers at extremely low concentrations inside complicated wastewater matrices. Overall, the data shows a decrease in temporal intensity across most substance classes, indicating behavioural, seasonal,

or availability-related changes in drug consumption patterns in the Eldoret community.

In order to identify the existence and temporal fluctuations of specific drug-related metabolites, sixteen wastewater samples were taken from a treatment facility in Eldoret, Kenya, during the four-month investigation. Amphetamines, 19-Norandrosterone (a primary metabolite of nandrolone), 3'-Hydroxy-Stanozolol (a stanozolol metabolite), methylhexanamine, cocaine derivatives (benzoylecgonine and norcocaine), and erythropoietin (EPO) were the six main metabolites that were identified using liquid chromatography–tandem mass spectrometry (LC-MS/MS). These results show how wastewater-based epidemiology (WBE) can be used to identify a variety of compounds in the general population, from recreational drugs to stimulants and anabolic agents.

The most commonly found metabolites were amphetamines, which had an average concentration of  $50.0 \pm 0.10$  ng/L in February before steadily declining over the following months. This implies a high starting intake that

declined in May, which can be a reflection of changes in social behaviour, substance availability, or access over time. Amphetamine levels spiked during times of increased social activity, such as festivals, exams, or economic cycles, and then tapered off, according to similar patterns shown in European WBE research (Boogaerts et al., 2021; Huizer et al., 2021). This volatility may be related to seasonal economic fluctuations that impact recreational drug usage and purchasing power, agricultural labour cycles, or school or university calendars in Kenya. The sustained presence of amphetamines underscores their widespread use at the community level, possibly for both recreational purposes and cognitive or physical stimulation in occupational settings.

Attention should also be paid to the identification of anabolic steroid metabolites, particularly 3'-hydroxy-stanozolol and 19-norandrosterone. By May, 19-norandrosterone levels had dropped from  $10.0 \pm 0.34$  ng/L in February to  $5.0 \pm 0.11$  ng/L. In the same time frame, 3'-Hydroxy-Stanozolol dropped from  $5.0 \pm 0.06$  ng/L to  $2.5 \pm 0.04$  ng/L. These substances may be used by the general public for weight control, body image enhancement, or perceived therapeutic effects, despite their historical association with performance enhancement. Similar steroid metabolites have been found in urban wastewater in studies conducted in Europe and Asia, indicating widespread social use outside of elite contexts (Carnevale Miino et al., 2023; Celma et al., 2022). The decline observed across the sampling period may indicate intentional cycling or tapering practices, in which individuals modulate their use of such substances to minimize health effects, manage costs, or respond to local supply fluctuations.

The levels of methylhexanamine, a stimulant frequently found in dietary supplements, were found to be rather steady, ranging from  $3.5 \pm 0.06$  ng/L in May to  $4.5 \pm 0.16$  ng/L in February. Its persistent presence might be the result of continuous, frequent use of supplements promoted for energy boost, weight loss, or mental

clarity. According to earlier research, methylhexanamine is commonly found in supplements that are mislabeled or tainted (Lemas et al., 2021; Oleksak et al., 2024). Its presence in municipal wastewater indicates either accidental exposure from poorly regulated supplements or deliberate use as a stimulant. Because consumers could unintentionally consume banned chemicals, this presents serious public health issues and emphasises the need for regulatory supervision and public education initiatives regarding supplement safety.

The quantities of cocaine metabolites were found sporadically, peaking in February at  $1.2 \pm 0.04$  ng/L, disappearing in April, and then resurfacing in May at lower concentrations ( $0.30 \pm 0.01$  ng/L). Instead of regular consumption, this inconsistency suggests intermittent community use. WBE studies conducted in Asia and Australia have documented similar intermittent detection patterns, with cocaine metabolites peaking during social gatherings or vacations (Lin et al., 2021; Yi et al., 2023). These swings in Eldoret might be related to times when social gatherings are more frequent, regional celebrations, or shifts in illegal supply chains. Despite being found in comparatively small amounts, the existence of cocaine metabolites suggests that recreational drug use is widespread in the community and should not be disregarded when discussing law enforcement and public health.

Throughout the experiment, erythropoietin (EPO) was regularly found at trace amounts of around 0.003 ng/L, which is comparable to the method's lower quantitative capabilities. These findings were validated against recombinant EPO reference standards to verify they were above instrumental background; nonetheless, values should be reported to two significant figures to reflect true analytical precision under wastewater conditions.

The continuous detection demonstrates the sensitivity of WBE for recognising physiologically active chemicals, even while its concentration stayed incredibly low. The prevalence of EPO in wastewater may partially reflect its legal medicinal

use in the general population. EPO is used medically to treat anaemia, especially in patients with cancer or chronic renal disease. Nevertheless, degrading processes within the wastewater system or micro-dosing procedures may potentially lead to trace detection (Gerber et al., 2023; Metushi et al., 2024b). The efficacy of WBE as a monitoring tool for both medicinal and non-therapeutic substance use is demonstrated by its capacity to detect such low quantities, regardless of the source.

All things considered, the identification of these six metabolites demonstrates how effective WBE is in offering up-to-date, neighborhood-wide insights into drug usage patterns. WBE provides an anonymous, non-invasive, and economical tool that captures collective consumption trends across whole populations, in contrast to conventional biological testing procedures. The study's temporal patterns—such as the decrease in anabolic steroids and amphetamines, the consistent presence of methylhexanamine, and the intermittent emergence of cocaine metabolites—reflect a variety of consumption dynamics that can guide focused public health initiatives.

There are a number of theories that could account for these trends. First, temporal variations are probably influenced by socioeconomic factors, such as changes in disposable income and the availability of drugs. Second, as has been shown in other areas, seasonal increases in recreational drug use may be influenced by social and cultural events. Third, the consistent existence of stimulants linked to supplements, such as methylhexanamine, highlights how the nutrition and supplement industries influence consumption patterns, frequently without adequate consumer awareness of the hazards. Lastly, the regular identification of medically significant substances like EPO highlights how crucial it is to differentiate between therapeutic and non-therapeutic sources in community surveillance.

These findings have important policy and public health ramifications. WBE can offer data for developing community education and outreach initiatives about the dangers of using steroids and

stimulants, as well as for bolstering laws against mislabeled supplements at the local level. The creation of baseline data on substance use using WBE can direct the distribution of resources for harm-reduction programs, law enforcement, and healthcare services on a national level. These findings contribute to a growing body of evidence showing that WBE is a globally applicable scalable framework for drug surveillance, especially in environments with low resources.

WBE makes it possible to comprehend substance use trends more thoroughly by exposing patterns that traditional testing frequently overlooks. Policymakers and public health professionals may be better equipped to address new issues in drug use, regulation, and community health if WBE is incorporated into national monitoring systems. Although this study found detectable temporal changes in metabolite levels, the relatively small number of weekly samples collected ( $n = 16$ ) over a four-month period is a limitation; future studies should include higher sampling frequency and longer monitoring durations to improve temporal trend interpretation.

### Conclusion and Recommendations

This study found that wastewater-based epidemiology can detect and quantify illicit drugs and performance-enhancing compounds in Eldoret's municipal wastewater system. Amphetamines had the greatest quantities of any analyte and showed a distinct decline trend from February to May 2025, although anabolic steroid metabolites also declined with time, indicating reduced exposure or usage intensity in the examined group. Cocaine metabolites were intermittent and low, indicating occasional consumption patterns, but methylhexanamine levels were rather stable across the sample period. Trace identification of EPO verified the method's potential to detect very low-abundance biomarkers in complicated wastewater matrices. These findings jointly indicate WBE's applicability for tracking community-level substance use patterns in Kenya, as well as providing initial baseline data for future national monitoring frameworks. However, the

limited sampling frequency ( $n = 16$ ) should be acknowledged as a constraint on long-term temporal interpretation, and future studies should incorporate higher-frequency sampling and expanded multi-site monitoring to strengthen trend analysis reliability and enhance applicability for public health, anti-doping intelligence, and policy decision-making.

It is advised that wastewater-based epidemiology (WBE) be incorporated into Kenya's larger national drug surveillance framework in view of these findings. WBE might boost early-warning systems for new trends by offering ongoing, anonymous insights on substance use at the community level, supplementing clinical monitoring systems, hospital records, and law enforcement data. Adding more urban and peri-urban areas to monitoring activities will facilitate the creation of a comprehensive national database and enable cross-regional comparisons, ultimately providing a more accurate image of the drug landscape in the nation. Campaigns for public education and awareness should also be given top priority in order to address the dangers of uncontrolled stimulants and supplements, especially those like methylhexanamine that may be inadvertently ingested. To reduce contamination and mislabeling that lead to unintentional exposure, regulatory bodies must simultaneously strengthen laws controlling pharmaceuticals and nutritional supplements in regional marketplaces. In order for policymakers to foresee issues and carry out prompt, evidence-based responses, future research should prioritise long-term surveillance and the creation of predictive models that account for the seasonal, cultural, and socioeconomic factors that influence drug use.

#### **Declaration of funding sources**

No specific grant from a public, private, or nonprofit funding organisation was obtained for this study. The authors paid for the entire study themselves. All expenses related to data processing, laboratory analysis, and field sampling were paid for out of pocket.

#### **CRedit authorship contribution statement**

**John Kevine Ochieng:** Conceptualization; Methodology; Investigation; Data Curation; Formal Analysis; Visualization; Writing – Original Draft.

**Dr. Olal:** Supervision; Validation; Writing – Review & Editing; Methodological Oversight.

**Dr. Anino:** Supervision; Project Administration; Writing – Review & Editing; Critical Revisions

#### **Declaration of conflict of interest**

The authors state that there are no competing interests or personal relationships that could have influenced the work presented in this study.

#### **Data Availability Statement**

The datasets created and analysed during the current study are not publically available due to institutional data protection policies and ethical concerns over the training facility's location and identity. However, anonymised data supporting the study's conclusions are available from the corresponding author, **John Kevine Ochieng**, upon reasonable request and institutional approval.

#### **Acknowledgements**

I, **John Kevine Ochieng**, would like to convey my heartfelt gratitude to **Dr. Olal** and **Dr. Anino** of **Rongo University** for their great academic supervision, mentorship, and continuous support during the course of my study. Their valuable insights and assistance helped shape the research strategy, data interpretation, and final publication. I would also want to thank the management and staff of Eldoret's sports training centre for their cooperation and help during the wastewater sample collecting period. Special thanks go to the laboratory staff for their technical assistance with sample analysis utilizing LC-MS/MS techniques. Finally, I want to thank the Anti-Doping Agency of Kenya (ADAK) for giving access to essential data and recommendations that enhanced the context and significance of our study.

## Reference

- Bade, R., Huchthausen, J., Huber, C., Dewapriya, P., Tschärke, B. J., Verhagen, R., Puljevic, C., Escher, B. I., & O'Brien, J. W. (2024). Improving wastewater-based epidemiology for new psychoactive substance surveillance by combining a high-throughput in vitro metabolism assay and LC–HRMS metabolite identification. *Water Research*, 253. <https://doi.org/10.1016/J.WATRES.2024.121297>
- Boogaerts, T., Ahmed, F., Choi, P. M., Tschärke, B., O'Brien, J., De Loof, H., Gao, J., Thai, P., Thomas, K., Mueller, J. F., Hall, W., Covaci, A., & van Nuijs, A. L. N. (2021). Current and future perspectives for wastewater-based epidemiology as a monitoring tool for pharmaceutical use. *Science of The Total Environment*, 789, 148047. <https://doi.org/10.1016/J.SCITOTENV.2021.148047>
- Bowes, D. A. (2024). Towards a Precision Model for Environmental Public Health: Wastewater-based Epidemiology to Assess Population-level Exposures and Related Diseases. *Current Epidemiology Reports* 2024 11:3, 11(3), 131–139. <https://doi.org/10.1007/S40471-024-00350-6>
- Carnevale Miino, M., Macsek, T., Halešová, T., Chorazy, T., & Hlavínek, P. (2023). Pharmaceutical and narcotics monitoring in Brno wastewater system and estimation of seasonal effect on the abuse of illicit drugs by a wastewater-based epidemiology approach. *Science of the Total Environment*, 891. <https://doi.org/10.1016/j.scitotenv.2023.164386>
- Celma, A., Gago-Ferrero, P., Golovko, O., Hernández, F., Lai, F. Y., Lundqvist, J., Menger, F., Sancho, J. V., Wiberg, K., Ahrens, L., & Bijlsma, L. (2022). Are preserved coastal water bodies in Spanish Mediterranean basin impacted by human activity? Water quality evaluation using chemical and biological analyses. *Environment International*, 165, 107326. <https://doi.org/10.1016/J.ENVINT.2022.107326>
- Criminal Cases - Anti-Doping Agency of Kenya*. (n.d.). Retrieved June 7, 2025, from <https://www.adak.or.ke/criminal-cases/>
- Daughton, C. G. (2012). Real-time estimation of small-area populations with human biomarkers in sewage. *Science of The Total Environment*, 414, 6–21. <https://doi.org/10.1016/J.SCITOTENV.2011.11015>
- Gerber, C., Jaunay, E. L., Simpson, B. S., & White, J. M. (2023). Moving beyond wastewater analysis toward epidemiology. *Wastewater-Based Epidemiology for the Assessment of Human Exposure to Environmental Pollutants*, 33–60. <https://doi.org/10.1016/B978-0-443-19172-5.00006-8>
- González-Mariño, I., Baz-Lomba, J. A., Alygizakis, N. A., Andrés-Costa, M. J., Bade, R., Bannwarth, A., Barron, L. P., Been, F., Benaglia, L., Berset, J. D., Bijlsma, L., Bodík, I., Brenner, A., Brock, A. L., Burgard, D. A., Castrignanò, E., Celma, A., Christophoridis, C. E., Covaci, A., ... Ort, C. (2020). Spatio-temporal assessment of illicit drug use at large scale: evidence from 7 years of international wastewater monitoring. *Addiction*, 115(1), 109–120. <https://doi.org/10.1111/ADD.14767>
- Huizer, M., ter Laak, T. L., de Voogt, P., & van Wezel, A. P. (2021). Wastewater-based epidemiology for illicit drugs: A critical review on global data. In *Water Research* (Vol. 207). Elsevier Ltd. <https://doi.org/10.1016/j.watres.2021.117789>
- Jaguga, F., Mathai, M., Ayuya, C., Francisca, O., Musyoka, C. M., Shah, J., ... et al. (2023). 12-month substance use disorders among first-year university students in Kenya. *PLOS ONE*,

- 18(11), e0294143.  
<https://doi.org/10.1371/journal.pone.0294143>
- Kwaw, E. (2025). *Understanding the context of anti-doping in Africa: A communication approach to the “dopogenic” environment*.
- Lemas, D. J., Loop, M. S., Duong, M., Schleffer, A., Collins, C., Bowden, J. A., Du, X., Patel, K., Ciesielski, A. L., Ridge, Z., Wagner, J., Subedi, B., & Delcher, C. (2021). Estimating drug consumption during a college sporting event from wastewater using liquid chromatography mass spectrometry. *Science of The Total Environment*, 764, 143963.  
<https://doi.org/10.1016/J.SCITOTENV.2020.143963>
- Lin, X., Choi, P. M., Thompson, J., Reeks, T., Verhagen, R., Tschärke, B. J., O'Malley, E., Shimko, K. M., Guo, X., Thomas, K. V., & O'Brien, J. W. (2021). Systematic Evaluation of the In-Sample Stability of Selected Pharmaceuticals, Illicit Drugs, and Their Metabolites in Wastewater. *Environmental Science and Technology*, 55(11), 7418–7429.
- Lorenzo, M., & Picó, Y. (2019). Wastewater-based epidemiology: current status and future prospects. *Current Opinion in Environmental Science & Health*, 9, 77–84.  
<https://doi.org/10.1016/J.COESH.2019.05.007>
- Madikizela, L. M., Ncube, S., & Chimuka, L. (2020). Analysis, occurrence and removal of pharmaceuticals in African water resources: A current status. *Journal of Environmental Management*, 253, 109741.  
<https://doi.org/10.1016/J.JENVMAN.2019.109741>
- Maida, C. M., Amodio, E., Mazzucco, W., La Rosa, G., Lucentini, L., Suffredini, E., Palermo, M., Andolina, G., Iaia, F. R., Merlo, F., Chiarelli, M. G., Siragusa, A., Vitale, F., Tramuto, F., Segreto, D., Schembri, P., Cuffari, G., Conti, A., Casamassima, G., ... Graziano, G. (2022). Wastewater-based epidemiology for early warning of SARS-COV-2 circulation: A pilot study conducted in Sicily, Italy. *International Journal of Hygiene and Environmental Health*, 242, 113948.  
<https://doi.org/10.1016/J.IJHEH.2022.113948>
- McCall, A. K., Bade, R., Kinyua, J., Lai, F. Y., Thai, P. K., Covaci, A., Bijlsma, L., van Nuijs, A. L. N., & Ort, C. (2016). Critical review on the stability of illicit drugs in sewers and wastewater samples. *Water Research*, 88, 933–947.  
<https://doi.org/10.1016/J.WATRES.2015.10.040>
- Metushi, I. G., Wagner, A., Ahrens, B., Ericsson, M., Rabin, O., Sobolevsky, T., & Tygart, T. T. (2024a). Breaking Boundaries: Exploring Performance Enhancement and Anti-Doping Testing in Sports. *Clinical Chemistry*, 70(7), 897–904.  
<https://doi.org/10.1093/CLINCHEM/HVAE074>
- National Authority for the Campaign Against Alcohol and Drug Abuse. (2022). *National survey on the status of drugs and substance use in Kenya* (abridged version).  
<https://nacada.go.ke/sites/default/files/2023-05/National%20Survey%20on%20the%20Status%20of%20Drugs%20and%20Substance%20Use%20in%20Kenya%202022.pdf>
- Oleksak, P., Nepovimova, E., Valko, M., Alwasel, S., Alomar, S., & Kuca, K. (2024). Comprehensive analysis of prohibited substances and methods in sports: Unveiling trends, pharmacokinetics, and WADA evolution. *Environmental Toxicology and Pharmacology*, 108, 104447.  
<https://doi.org/10.1016/J.ETAP.2024.104447>
- Owiti, J., & Ogola, S. (2024). Substance use among healthcare professionals in Kenya: Prevalence, patterns, and implications for occupational health. *Global Public Health*, 19(4), 587-599.  
<https://doi.org/10.1371/journal.pgph.0003863>
- Petróczi, A., & Boardley, I. D. (2022). The Meaning of “Clean” in Anti-doping Education and Decision Making: Moving Toward Integrity

- and Conceptual Clarity. *Frontiers in Sports and Active Living*, 4, 869704.  
<https://doi.org/10.3389/FSPOR.2022.869704/>  
BIBTEX
- Petróczi, A., Cruyff, M., de Hon, O., Sagoe, D., & Saugy, M. (2022). Hidden figures: Revisiting doping prevalence estimates previously reported for two major international sport events in the context of further empirical evidence and the extant literature. *Frontiers in Sports and Active Living*, 4, 1017329.  
<https://doi.org/10.3389/FSPOR.2022.1017329/>  
BIBTEX
- Rice, J., Kannan, A. M., Castrignanò, E., Jagadeesan, K., & Kasprzyk-Hordern, B. (2020). Wastewater-based epidemiology combined with local prescription analysis as a tool for temporal monitoring of drugs trends - A UK perspective. *Science of The Total Environment*, 735, 139433.  
<https://doi.org/10.1016/J.SCITOTENV.2020.139433>
- Shimko, K. M., O'Brien, J. W., Barron, L., Kayalar, H., Mueller, J. F., Tscharke, B. J., Choi, P. M., Jiang, H., Eaglesham, G., & Thomas, K. V. (2019). A pilot wastewater-based epidemiology assessment of anabolic steroid use in Queensland, Australia. *Drug Testing and Analysis*, 11(7), 937–949.  
<https://doi.org/10.1002/dta.2591>
- Verovšek, T., Šuštarčič, A., Laimou-Geraniou, M., Krizman-Matasic, I., Prosen, H., Eleršek, T., Kramarič Zidar, V., Mislej, V., Mišmaš, B., Stražar, M., Levstek, M., Cimrmančič, B., Lukšič, S., Uranjek, N., Kozlovič-Bobič, T., Kosjek, T., Kocman, D., Heath, D., & Heath, E. (2023). Removal of residues of psychoactive substances during wastewater treatment, their occurrence in receiving river waters and environmental risk assessment. *Science of the Total Environment*, 866.  
<https://doi.org/10.1016/j.scitotenv.2022.161257>
- Yi, R., Zeng, T., Chen, J., Liu, D., Yang, X., Zhao, M., & Zhou, Z. (2023). Wastewater-Based Epidemiology: Assessing Illicit Drug Usage and Impact through an Innovative Approach. *Water*, 15(23), 4192.  
<https://doi.org/10.3390/w15234192>
- Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., & Fanelli, R. (2005). Cocaine in surface waters: A new evidence-based tool to monitor community drug abuse. *Environmental Health: A Global Access Science Source*, 4(1), 1–7. <https://doi.org/10.1186/1476-069X-4-14/TABLES/2>