Journal of Environmental Chemical Engineering 14 (2026) 121294

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

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

Differences in drug use across three neighbourhoods with varying socioeconomic levels: A wastewater-based surveillance study

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ARTICLE INFO

Keywords:
Wastewater-based epidemiology
Substance use
Socioeconomic disparities
Urban health
Drug monitoring
Public health surveillance

ABSTRACT

This study measured 39 biomarkers of licit (e.g., alcohol, nicotine, benzodiazepines, antidepressants) and illicit drug consumption (e.g., cocaine, amphetamine, cannabis, new psychoactive substances) in wastewater from three neighbourhoods (NGBs) in Barcelona, each representing different socioeconomic and lifestyle profiles. Four weeklong sampling campaigns were conducted between March 2021 and March 2022. The results revealed significant differences in drug use across neighbourhoods that are not apparent in city-level analyses. These differences were particularly notable for illicit drugs—such as higher morphine and cannabis loads in socioeconomically disadvantaged areas—as well as for specific benzodiazepines and antidepressants, which varied in prevalence between neighbourhoods. Alcohol and nicotine patterns were more strongly associated with the local density of bars and restaurants than with socioeconomic status. A clear weekend peak was observed for substances like alcohol. Temporal analysis over the study period showed increased consumption of certain drugs at the end of 2021, likely linked to the reopening of nightlife venues and elevated anxiety related to the COVID-19 pandemic. This study highlights the value of applying wastewater-based epidemiology (WBE) at the intra-city level. By moving beyond aggregated citywide data, intra-urban WBE can reveal spatial and temporal variations in drug use that may otherwise go unnoticed. This approach also enables the identification of communities or socioeconomic groups at higher risk of substance use or mental health challenges, offering critical insights for public health authorities to develop targeted interventions and preventive strategies.

1. Introduction

Wastewater-based epidemiology (WBE) is a cost-effective method to gather information on the consumption of drugs through wastewater (WW) sampling and biomarkers analysis. Most WBE studies have compared drug consumption over time [1–3], between different cities/regions [4–8], or the combination of the two [9–14]. Analysis of WW biomarkers temporal trends can help understand changes in the drug market, caused by changes in their production and distribution or in response to new government interventions. Analysis of spatial trends

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https://doi.org/10.1016/j.jece.2026.121294
Received 3 November 2025; Received in revised form 17 December 2025; Accepted 16 January 2026
Available online 17 January 2026

can provide insights regarding the prevalence, typology and amounts of substances consumed in different geographical areas [11,15].

Socioeconomic level or lifestyle habits influence drug consumption patterns, shaping behaviours and preferences [16,17]. This relationship has been previously explored by comparing WW samples collected from cities with varying socioeconomic characteristics. For instance, previous studies have shown that areas characterized by socioeconomic disadvantage tend to exhibit higher opioid use [18], while higher alcohol consumption is often observed in socioeconomically advantaged groups [9,18]. Additionally, Price et al. [13] found that ecstasy and cocaine consumption correlated negatively with socioeconomic disadvantage, while methamphetamine (MA) use correlated positively. These studies have typically operated by collecting samples at the city level, which can pose limitations due to the coexistence of diverse communities with varying socioeconomic levels and lifestyle habits (dietary practices, physical activity, health behaviours, environmental interaction, housing and living conditions, social and cultural practices), potentially averaging out WW biomarker signals. In large urban areas, where socioeconomic differences are often more pronounced across neighbourhoods (NGBs), analysing WW at finer spatial scales can offer deeper insights into these disparities.

The recent study by Duvallet et al. [19] is the only WBE analysis conducted at the intra-urban scale on illicit drug consumption revealing distinct opioid consumption preferences across urban areas within a municipality in North Carolina (US). The study highlighted areas with particularly high or low consumption to different opioids and indicated which opioids were used in all the studied areas and which had restricted geographical use. Given that the approach has proven effective for opioids, it is of interest to apply it to other compounds and examine whether differences in consumption emerge over space and time. In this study we aimed to investigate whether there are differences in licit and illicit drug consumption preferences across three NGBs *i.e.,* urban areas from the metropolitan city of Barcelona, Spain. The three NGBs have variable socioeconomic levels: NGB-L refers to the urban area with the lowest (L) income level; NGB-M to the one with a medium (M) socioeconomic level; and NGB-H to the one with the highest (H) income level. To achieve this, we applied a WBE approach to sample and analyse 24 lifestyle biomarkers for both licit substances (such as alcohol, nicotine, antidepressants and benzodiazepines) and illicit drugs (including new psychoactive substances (NPS)). In parallel, a computer-assisted telephonic interview (CATI) survey was conducted to assess the consumption of alcohol, nicotine, and sedatives within the same three NGBs. This enabled a comparison between self-reported survey data and findings derived from WW analysis. To the best of our knowledge, this study is the first to further increase the spatial resolution by studying differences at the NGB level (intra-city level). Such a novel approach may provide a more nuanced understanding of drug use preferences and help develop targeted prevention and intervention strategies.

2. Materials and methods

2.1. Neighbourhoods selection and sampling stations

Three NGBs subareas of Barcelona were carefully selected, taking into account socioeconomic disparities as previously detailed in [20]. Each NGB corresponds to a subarea comprising a few representative and homogeneous census tracts. The NGBs subareas differed in terms of household yearly income (between €25,849 and €78,476) and number of bars and restaurants (between 3 and 10 per 1000 inhabitants). The age distribution across the three areas was broadly similar for younger populations (15.3 %–18.4 % under 18 years), with some variation observed in older populations (17.4 %–23.6 % aged 65 and above). No hospital wastewater reached the sampling points. A summary of the main socioeconomic and other indicators of sub catchments, as well as on the sewer infrastructure is provided in Table 1. Detailed sample descriptives on socioeconomic characteristics are found in Table SI-1.

Table 1
Characteristics of the sampled areas.

	NGB-L	NGB-M	NGB-H
Number of Inhabitants*	18,042	8914	5090
Population under 18, year 2019 (%)*	15.3	17.1	18.4
People aged 65 and over, year 2019 (%)*	22,3	17,4	23,6
Household yearly income (€)**	25,849	38,430	78,476
Number of bars and restaurants***	53	89	25
Catchment area (m^2)	719,334	252,195	183,617
Pipes length (m)	11,126	6921	3103
Number of origin sewer holes	500	409	128
Residence time origin sewer holes to sampling point (h) (Q1/**Q2**/Q3)	0.9/**1.4**/2.3	0.8/**1.8**/2.9	0.7/**1.6**/4.3

* National Institute of Statistics (INE), based on the Estadística del Padrón Continuo a 1 de enero de 2019 (INE, 2019) https://www.ine.es/jaxi/Tabla.htm?path=/t20/e245/p07/a2019/l0/&file=0801.px&L=0

** National Institute of Statistics (INE), based on the 2019 edition of the Atlas de Distribución de Renta de los Hogares (INE, 2019) https://www.ine.es/ADRH/?config=config_ADRH_2019.json&showLayers=Renta_neta_media_por_hogar_2019_9410&level=5

*** using commercial census from [21]. Q refers to quartiles (Q1 marks the point below which 25 % of the data falls, Q2 is the median, and Q3 marks the point below which 75 % of the data falls). Data obtained from [20]. NGB-L: low-income neighbourhood; NGB-M: middle-income neighbourhood; NGB-H: high-income neighbourhood.

The three stations were built on site after identifying these three NGBs and were set up nearly identically. All equipment was housed in a stainless cabin measuring 2 m x 1.4 m x 2 m and installed at street level near a sewer hole. The sampling procedure involved the use of a large peristaltic pump to continuously pump WW from the underground sewer pipe and transfer it to a ground-level reservoir equipped with a top drain. Within this reservoir, online sensors were positioned to monitor wastewater parameters, and an inlet silicone tube connected to a secondary sampling pump (Watson Marlow Qdos 30) used for sample collection. The sampling pump was linked to a Scan Concube unit (s::can GmbH) via a 4–20 mA analogue connection to regulate the flow rate. Each composite WW sample spanned a 24-hour period, was obtained in a continuous flow-proportional manner, and was stored in a fridge at 4 °C. Proportional sampling was divided into three stages (peak, day, and night), with each stage corresponding to different flow rates of the sampling pump. The flow rates of the secondary sampling pump were determined based on field observations conducted at the same stations, utilizing the installed flowmeters within the sewer pipe. Those flowmeters were installed into the sewer system at each of the stations to measure the flow rate. A Nivus PCM4 (Eppingen GmbH, Germany) with a submerged ultrasonic combi sensor was installed at station NGB-L. The FLO-DAR AV (Hach; Colorado, USA), equipped with a contactless radar-based sensor for velocity and a contactless ultrasonic sensor for level, was used at NGB-M. Initially, station NGB-H utilized the SIGMA 950AV (Hach; Colorado, USA) with a submerged doppler sensor (from March 2021 until October 2021). On the 20th of October 2021, it was replaced with a Nivus NFM-550 (Eppingen GmbH, Germany) which uses contactless velocity and level (Nivus OFR) sensors to reduce maintenance workload.

2.2. Sampling

Four intensive sampling campaigns were executed in the three NGBs (March 2021, June 2021, November 2021, March 2022); in each campaign, samples were collected daily over 1 week (in total 84, equivalent to 3 sites, 7 days, 4 campaigns). In terms of volume, each composite sample consisted of 32 % collected in the morning (07:00 till 12:00 - peak flow rate), 54 % collected in the afternoon/evening (12:01 till 22:00 - day flow rate), and 14 % collected at night (22:01 till 06:59 of the following day - night flow rate). After 24 h sampling, the pump was stopped, and the resulting composited sample was manually collected within few hours and stored at −20 °C until analysis. To establish a baseline for comparison with the NGBs, we sampled the largest wastewater treatment plant (WWTP) in Barcelona, located downstream of the three monitored NGBs. This WWTP serves a population of 2.1 million within the Barcelona Metropolitan Area. The 24-hour composite influent WW samples were collected over the same full week as the NGB sampling campaigns in June 2021 and March 2022, using a flow-proportional autosampler.

2.3. Analytical methods

The study included 22 biomarkers of consumption of both licit drugs (alcohol, nicotine, benzodiazepines, and antidepressants) and illicit drugs (including cocaine, amphetamine, and cannabis) in wastewater samples together with 17 NPS (e.g., methylone, dipentylone, ethcathinone, Table 2). Fully validated methods were applied for the analysis of these 39 biomarkers. All compounds except ethyl sulfate (EtS), cotinine (COT), hydroxy-cotinine (OH-COT), and NPS were analysed following the method described by López-García et al. [22], based on isotope dilution on-line solid phase extraction-liquid chromatography tandem mass spectrometry (SPE-LC-MS/MS). The system configuration comprised a Prospekt-2 sample preparation system (Spark Holland, Emmen, The Netherlands) coupled on-line with a 1525 binary LC pump and a Xevo TQ-S mass spectrometer (Waters, Milford, MA). Prior to analysis, samples were diluted 1:10 with high performance liquid chromatography water, spiked with a mixture of surrogate internal standards, and centrifuged. On-line SPE of these diluted samples (5 mL) was performed with a polymeric cartridge PLRPs (cross-linked styrene-divinylbenzene polymer, 10 × 2 mm, 15–25 µm particle size) (Spark Holland, Emmen, The Netherlands). MS/MS acquisition was carried out with an electrospray interface operated in positive ionization (ESI+) and in selected reaction monitoring (SRM) mode.

The ethanol metabolite EtS was determined following the method described by Mastroianni et al. [23] on an Aria Mx LC system coupled with a triple quadrupole TSQ Quantiva mass spectrometer, both from Thermo Fisher Scientific Inc. (Waltham, MA). The samples were spiked with deuterated EtS (EtS-d5) at a concentration of 25 µg/L, centrifuged and quantified via ion-pair LC–MS/MS using an electrospray interface operated in negative ionization (ESI-) and acquisition in the SRM mode.

The two nicotine metabolites (COT and OH-COT) were determined as described in [24] after enzymatic deconjugation by LC-MS/MS on an Acquity UPLC Liquid system combined with a Xevo TQD triple quadrupole mass spectrometer, both from Waters (Milford, Massachusetts, USA). COT and OH-COT concentrations were found to be highly correlated (Pearson's correlation coefficient, $r = 0.954–0.999$, mean=0.986), as already described in Rodríguez-Álvarez et al. [25] Therefore, only COT data was used for discussion and statistical analysis as otherwise they would provide redundant data. Details on the analysis of NPS, including internal standards used, sample treatment, instrument operating conditions, and method validation can be found in Celma et al. [26].

All standards of the target compounds and isotopically labelled analogues were purchased from Cerilliant (Round Rock, TX, USA) or Cayman Chemical (Ann Arbor, MI, USA) as high purity (>98 %) solutions in methanol (MeOH) or acetonitrile (ACN) at a concentration of 0.1 or 1 mg/mL. Information on the manufacturer and mass-spectrometry analysis conditions of all target and deuterated standards is provided in Table SI-2.

WW concentrations were multiplied by wastewater daily flow rates to gain daily biomarker mass loads (mg/day or g/day). Finally, loads were normalized to the population of each NGB (PNML—population normalised level, in mg/day/1000 inhabitants).

2.4. Interview-based survey

2.4.1. Design

The interview-based survey unit represents the individuals aged ≥ 16 years and living in the three NGBs covered in the WW sampling. Individuals live in 40 census tracts touching completely or partially the WW catchment areas. A census track is the smallest administrative unit, each with approximately 1500 inhabitants. The number of participants surveyed in each area was predetermined at 350 for NGB-L (the catchment with larger number of inhabitants), 325 for both NGB-M and NGB-H by design, ensuring sufficient responses to analyze the results for each area separately and provide representative findings. Theoretically such number of participants lead to a sample-to-population ratio of 1.84 % and a sample error of 3.07 % on average. The strata were defined by combining 40 census tracts with the sex and age of individuals aged ≥ 16 years, based on the Municipal Population Register as of January 1, 2020. Age groups were categorized as 16–44 years, 45–64 years, and ≥ 65 years, aligning with the age groups used in the Health Survey of Catalonia (Enquesta de Salut de Catalunya, ESCA) (https://salutweb.gencat.cat/ca/departament/estadistiques-sanitaries/enquestes/esca/index.html). This alignment allows the results to be compared with other secondary data sources. The interviewing methodology included computer-assisted telephonic interviews (CATI) conducted via landline and mobile phones, which accounted for approximately 77.3 % of interviews. To complete the sex and age quotas, computer-assisted personal interviews (CAPI) were conducted in the remaining 22.7 % of cases, particularly when CATI was not feasible. The interview-based survey design stratified by sex and age quotas, is presented in Table SI1. The survey procedure was approved by the ethics committee of the Jordi Gol i Gurina Institute for Research in Primary Care (CEIm Code: 21/066-P). Privacy and data protection were ensured by collecting responses anonymously, without any personally identifiable information other than age and sex. No sensitive health data were requested; questions focused on general habits (smoking, alcohol consumption, and sedative use) and perceptions rather than clinical diagnoses. All data were stored securely and processed in compliance with GDPR regulations.

Data analysis included descriptive statistics and tests of statistically significant differences in the indicators by sampling zone, gender, age group, and personal-level socio-economic status, using Bonferroni corrections to explore the data structure and identify key patterns. Results were reported at the 0.05 level of significance.

2.4.2. Harmful use of alcohol

Harmful use of alcohol was assessed using questions 1–3 of the AUDIT (*Alcohol Use Disorders Identification Test*) developed by the WHO [27] (see Table SI-2). According to AUDIT criteria, consumption at risky level was defined as either drinking 3–4 alcoholic drinks (or more) on a typical day, or having six or more drinks on a single occasion, even if this occurs less than once a month.

2.4.3. Smoking behaviour

The question about tobacco smoking is formulated to distinguish between "non-smokers", "ex-smokers", "daily smokers" and "occasional smokers", as follows from [28] (see Table SI-3).

2.4.4. Sedatives consumption

A questionnaire scale was developed to analyze the self-reported intake of sedatives, with a specific focus on adherence to therapy (taking them "exactly as prescribed"), versus over-the-counter (OTC) usage (without prescription). To minimize the risk of overestimating adherence behaviour, validated scales were used, and the composition and order of questions were carefully designed to reduce social desirability bias [29] (see Table SI-4). In the study, sedatives were defined by their therapeutic indications, and examples were provided to respondents in a clarification note, including medications such as Valium, Orfidal,

Table 2
Biomarkers measured in wastewater samples.

Substance	InChIKey	Abbreviation	Chemical family
Amphetamine	KWTSXDURSIMDCE-UHFFFAOYSA-N	AM	Illicit drug, amphetamine-like compound
Methamphetamine	MYWUZJCMWCOHBA-VIFPVBQESA-N	MA	Illicit drug, amphetamine-like compound
Ecstasy	SHXWCVYOXRDMCX-UHFFFAOYSA-N	MDMA	Illicit drug, amphetamine-like compound
Benzoylecgonine	GVGYEFKIHJTNQZ-RFQIPJPRSA-N	BE	Metabolic marker of cocaine consumption
Cocaethylene	NMPOSNRHZIWLLL-XUWVNRHRSA-N	CE	Metabolite formed after combined consumption of cocaine and alcohol
Ketamine	YQEZLKZALYSWHR-UHFFFAOYSA-N	KET	Illicit drug, hallucinogen
Lysergic acid diethylamide	VAYOSLLFUXYJDT-RDTXWAMCSA-N	LSD	Illicit drug, hallucinogen
Morphine	BQJCRHHNABKAKU-KBQPJGBKSA-N	MOR	Illicit + licit drug, opioid
Heroine	GVGLGOZIDCSQPN-PVHGPHFFSA-N	HER	Illicit drug, opioid
6-Monoacetylmorphine	JJGYGPZNTOPXGV-SSTWWWIQSA-N	6-MAM	Metabolic marker of heroine consumption
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	AJRJPORIQGYFMT-RMOCHZDMSA-N	EDDP	Metabolic marker of methadone (opioid) consumption
Tetrahydrocannabinol-9-carboxylic acid	YOVRGSHRZRJTLZ-UHFFFAOYSA-N	THC-COOH	Metabolic marker of cannabis consumption
Cotinine	UIKROCXWUNQSPJ-VIFPVBQESA-N	COT	Metabolic marker of nicotine consumption
Ethyl sulfate	KIWBPDUYBMNFTB-UHFFFAOYSA-N	EtS	Metabolic marker of alcohol consumption
Alprazolam	VREFGVBLTWBCJP-UHFFFAOYSA-N	ALP	Pharmaceutical, benzodiazepine
Lorazepam	DIWRORZWFLOCLC-UHFFFAOYSA-N	LOR	Pharmaceutical, benzodiazepine
Oxazepam	ADIMAYPTOBDMTL-UHFFFAOYSA-N	OXZ	Pharmaceutical, benzodiazepine
Temazepam	SEQDDYPDSLOBDC-UHFFFAOYSA-	TEM	Pharmaceutical, benzodiazepine
Venlafaxine	PNVNVHUZROJLTJ-UHFFFAOYSA-N	VEN	Pharmaceutical, antidepressant
Fluoxetine	RTHCYVBBDHJXIQ-UHFFFAOYSA-N	FLX	Pharmaceutical, antidepressant
Citalopram	WSEQXVZVJXJVFP-UHFFFAOYSA-N	CIT	Pharmaceutical, antidepressant
Ephedrine	KWGRBVOPPLSCSI-WPRPVWTQSA-N	EPH	Pharmaceutical, stimulant, misuse outside medical guidelines or use in the illegal production of MA
Mephedrone	YELGFTGWJGBAQU-UHFFFAOYSA-N	MEPH	NPS, cathinone
p-Methoxymethamphetamine	UGFMBZYKVQSQFX-UHFFFAOYSA-N	PMMA	NPS, analogue of methamphetamine
4-Fluoromethcathinone	MWKQPIROPJSFRI-UHFFFAOYSA-N	4-FMC	NPS, cathinone
Methoxetamine	LPKTWLVEGBNOOX-UHFFFAOYSA-N	-	NPS, arylcyclohexylamine
Methedrone	MQUIHBQDYYAEMH-UHFFFAOYSA-N	MEPH	NPS, cathinone
Methylone	VKEQBMCRQDSRET-UHFFFAOYSA-N	-	NPS, cathinone
Dimethylone	OSNIIMCBVLBNGS-UHFFFAOYSA-N	-	NPS, cathinone
Butylone	CGKQZIULZRXRRJ-UHFFFAOYSA-N	-	NPS, cathinone
4-Methylethcathinone	ZOXZWYWOECCBSH-UHFFFAOYSA-N	4-MEC	NPS, cathinone
4-Methyl-pyrrolidinopropiophenone	XSVBRRBDIKZEQD-UHFFFAOYSA-N	4-MePPP	NPS, cathinone
α-Pyrrolidinopentiophenone	YDIIDRWHPFMLGR-UHFFFAOYSA-N	α-PVP	NPS, cathinone
4-Chloro-α-pyrrolidinopropiophenone	HIDBRDWDFDSEOP-UHFFFAOYSA-N	4-chloro-α-PPP	NPS, cathinone
Dipentylone	PQTJKFUXRBKONZ-UHFFFAOYSA-N	-	NPS, cathinone
Methylendioxypyrovalerone	SYHGEUNFJIGTRX-UHFFFAOYSA-N	MDPV	NPS, cathinone
3,4-Dimethoxy-α-pyrrolidinopentiophenone	AGRVUDWDZKZCAA-UHFFFAOYSA-N	3,4-DiMeO-α-PVP	NPS, cathinone

(continued on next page)

Table 2 (*continued*)

Substance	InChIKey	Abbreviation	Chemical family
Naphyrone	DTNUPBSOODGRKW-UHFFFAOYSA-N	-	NPS, cathinone
Ethcathinone	LYMHIBZGTAPASQ-UHFFFAOYSA-N	-	NPS, cathinone

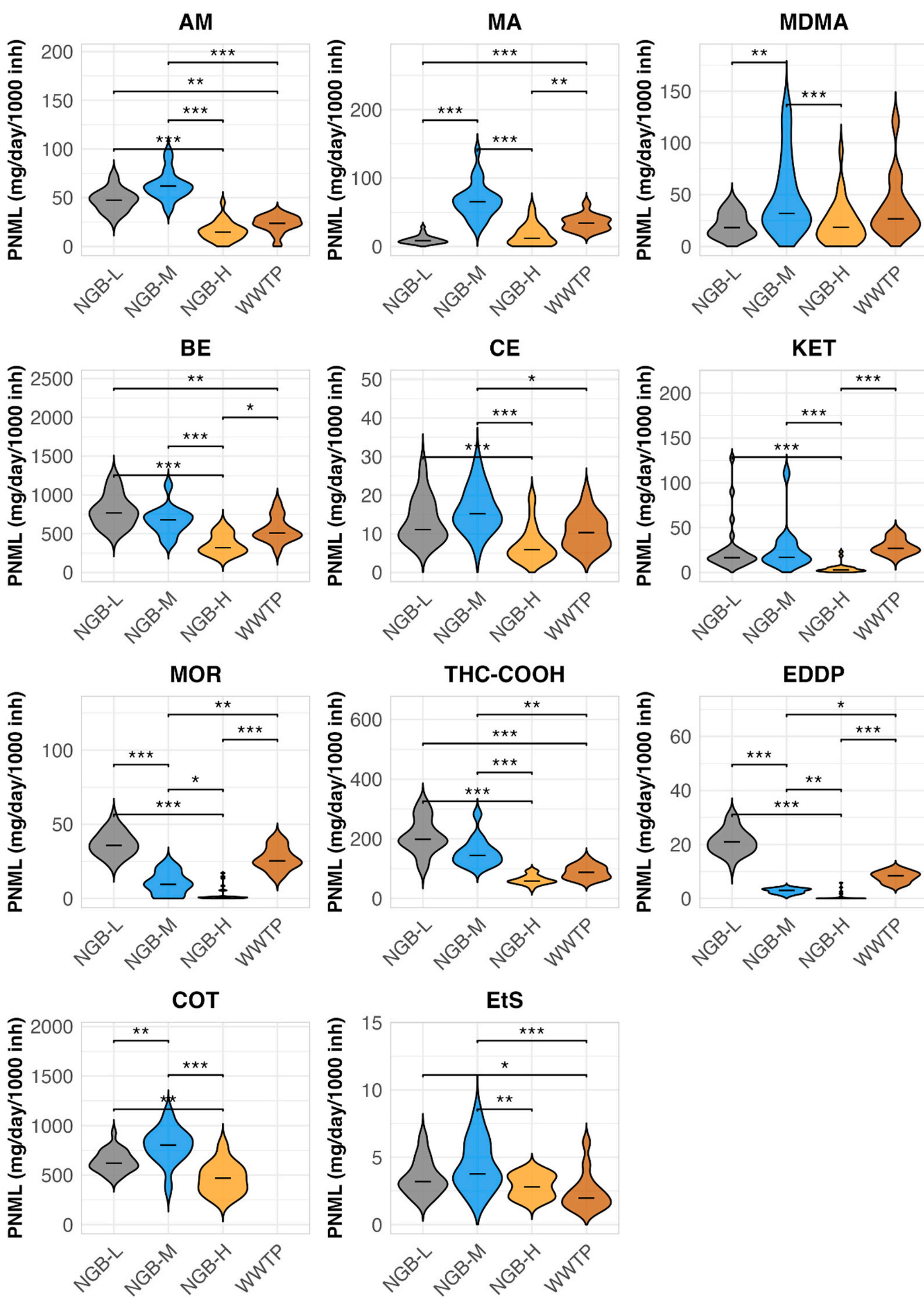

Fig. 1. Licit and illicit drugs PNMLs (mg/day/1000inh) across stations. Statistical differences are shown for pairs with p-values < 0.05.

Lexatín, Cefalexin, Venlafaxine, Zolpidem, Alprazolam, Lorazepam, Diazepam, Clonazepam, Bromazepam, Lormetazepam, or Estazolam.

3. Results

3.1. WW differences in loads across NGBs

Figs. 1 and 2 show PNMLs of all four sampling campaigns. The plotting and statistical analysis include 19 compounds out of the 39 analysed. Lysergic acid diethylamide (LSD), heroine (HER), 6-monoacetylmorphine (6-MAM), and NPS (*e.g.,* Mephedrone (MEPH)) are not included as they were only detected sporadically. Average loads per NGB can be found in Table SI-5. A Kruskal-Wallis test followed by a Bonferroni post-hoc test revealed statistically significant differences in biomarker loads between NGBs. NGB-M exhibited notably higher levels of various substances, including tobacco (COT), alcohol (EtS and cocaethylene (CE)), and amphetamine derivatives (MA, amphetamine (AM) and 3,4-methylenedioxymethamphetamine or ecstasy (MDMA)), compared to other NGBs, with some drugs reaching levels several times greater; up to 7 times greater for MA as compared to NGB-L, up to 4 times greater for AM as compared to NGB-H, up to 2,3 times greater for MDMA as compared to NGB-L, and up to 1,5 times greater for EtS as compared to NGB-H. In contrast, NGB-L showed significantly higher levels of methadone (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)), cannabis (tetrahydrocannabinol-9-carboxylic acid (THC-COOH)), and morphine (MOR) of up to ten times higher than the other NGBs in some cases. Meanwhile, NGB-H consistently exhibited the lowest levels for many substances, particularly the cocaine metabolite (benzoylecgonine (BE)) and ketamine (KET), where concentrations were approximately 20–50 % lower than in the other NGBs. These findings reveal pronounced spatial disparities in drug consumption patterns, with certain NGBs showing substantially higher loads of specific drug classes.

Distinct spatial patterns in licit drug PNMLs were observed across the three NGBs. Both NGB-M and NGB-H exhibited higher levels of several medications compared to NGB-L, particularly antidepressants such as citalopram (CIT), anxiolytics including alprazolam (ALP) and lorazepam (LOR), as well as ephedrine (EPH). Notably, NGB-H showed the highest concentrations of EPH and the anxiolytics ALP and LOR, with EPH levels reaching up to three times those detected in NGB-L. In contrast, higher loads of oxazepam (OXZ), temazepam (TEM), venlafaxine (VEN), and fluoxetine (FLX) were observed in NGB-L and NGB-M compared to NGB-H. The most pronounced difference was found for OXZ and TEM, with concentrations in NGB-M nearly double those in NGB-H. These findings underscore significant spatial variability in pharmaceutical consumption, reflecting diverse healthcare needs, prescription practices, and medication use patterns within the urban area.

The ratios of the PNMLs at each NGB to the PNMLs at the WWTP were calculated for each substance. The average of these ratios was then computed separately for pharmaceuticals and the remaining substances.

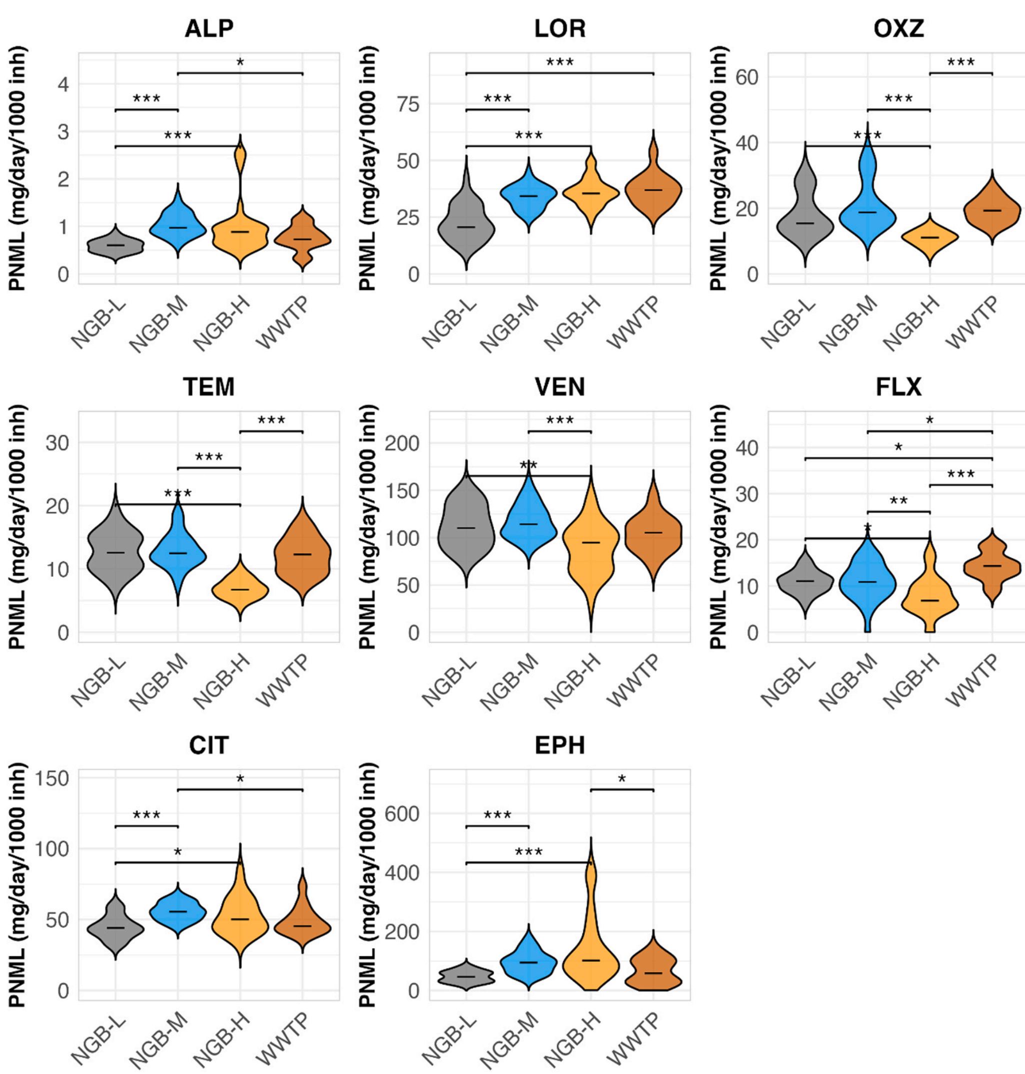

Fig. 2. Pharmaceutical PNMLs (mg/day/1000inh) across stations. Statistical differences are shown for pairs with p-values < 0.05.

For pharmaceuticals, the average ratios were consistent across NGBs, with values of 0.9, 1.1, and 1.0 for NGB-L, NGB-M, and NGB-H, respectively. In contrast, for the remaining substances, the average ratios exhibited greater variability, with values of 1.4 for both NGB-L and NGB-M, and 0.5 for NGB-H.

3.2. *Consumption preferences per NGB*

We employed principal component analysis (PCA) to investigate drug consumption preferences in WW samples collected at the NGB level (Fig. 3). Loadings indicate how strongly each variable (drug) influences a particular principal component (PC) (Figure SI-1). Higher loadings (longer arrows) mean that the variable has a greater impact on that PC. All variables (drugs) except LOR and EPH are positively correlated with PC1 (Fig. 3). Here, PC1 represents the average behaviour of all variables and is associated with the intensity of drug consumption. The scores are the observations projected onto the new axis provided by the PCs. Scores on the right side of PC1 indicate excretions higher than the average, while scores on the left indicate excretions lower than the average. LOR, MOR, EDDP, MA, ALP, EPH, and CIT contribute the most to PC2, and MDMA to PC3 (Figure SI-2). We color-coded the scores by NGB income and observed that they naturally formed distinct clusters, providing valuable insights into the underlying drug consumption preferences within each NGB. Our analysis unveiled distinct drug consumption preferences across the NGBs: NGB-L exhibited a preference for drugs such as MOR, EDDP, THC-COOH, and BE, while NGB-M showed a preference for more recreational drugs like MDMA and MA. Scores associated with NGB-L are clustered in the region that negatively correlates with PC2. In contrast, NGB-H displayed overall lower drug consumption, with a preference for LOR and EPH. The PCA plots also shows compounds which are correlated, such as LOR, EPH and CIT (pharmaceuticals); MOR and EDDP (opioids); TEM, BE, THC-COOH and AM; EtS and COT (recreational licit drugs); MA and MDMA (recreational illicit drugs).

3.3. *Weekend patterns*

The average PNMLs per NGB station, calculated using the data from all campaigns, are shown in Fig. 4. To compare biomarker loads between weekends and weekdays, four Mann-Whitney/Wilcoxon rank-sum tests were conducted using (i) the combined data from all three stations, and (ii) the data separated by NGB. For EtS, only Saturday and Sunday were considered weekend days due to the rapid metabolization of alcohol and its excretion as ethyl sulfate within few hours [30]. For the remaining, slower metabolized substances, Saturday, Sunday, and Monday loads (accounting mainly for Friday to Sunday consumption) were considered as weekend loads.

Only alcohol and MDMA showed a clear pattern of weekend consumption both when analyzing the combined data from all stations and when examining each station separately. For BE, statistically significant higher loads were found during the weekend in NGB-M, but not in NGB-L and NGB-H. When the three NGBs were analyzed together, higher BE loads were also observed during the weekend, a pattern likely influenced by the measurements in NGB-M. Among the other substances, differences between weekend and weekday loads were only statistically significant in some specific NGBs: AM in NGB-L, CE and KET in NGB-M (increased use during the weekend in both cases).

3.4. *Temporal trends along the four campaigns*

To evaluate temporal consumption trends across the four sampling campaigns, PNMLs of the biomarkers were analyzed by combining data from the three NGBs. Separate evaluations were performed for each NGB to identify and highlight patterns in homogeneous intra-urban communities. A Kruskal-Wallis test, followed by a Bonferroni correction, was used to assess statistically significant differences between the campaigns. When considering the three NGBs collectively none of the illicit drug biomarkers exhibited statistically significant differences across the four sampled periods (Figure SI-3). Regarding licit drug biomarkers, EtS loads increased significantly from March 2021 until November 2021 (Fig. 5), while COT levels showed a notable decrease during the full studied period. For pharmaceuticals, some differences were observed. For instance, EPH levels were significantly lower after March 2021 (Fig. 5), while LOR and OXZ levels increased after March 2021 (Figure SI-3). MDMA, CE, and KET remained stable over the four sampling campaigns in all NGBs, with considerable variability only in some campaigns (Figure SI-3).

When biomarker loads were evaluated for each NGB separately, distinct differences emerged. Elevated PNMLs for certain biomarkers were only observed at the NGB level (Fig. 5, and Figure SI-3). In NGB-L, increases were seen for BE, CIT, EDDP, FLX, MA (with a sharp rise in March 2022), and THC-COOH (between March 2021 and November 2021); EPH and EtS also showed increases but were already noted in the collective analysis. In NGB-M, increased excretion of EPH was observed between June 2021 and November 2021, and elevated excretion of THC-COOH between June 2021 and March 2022. A market increase in EtS

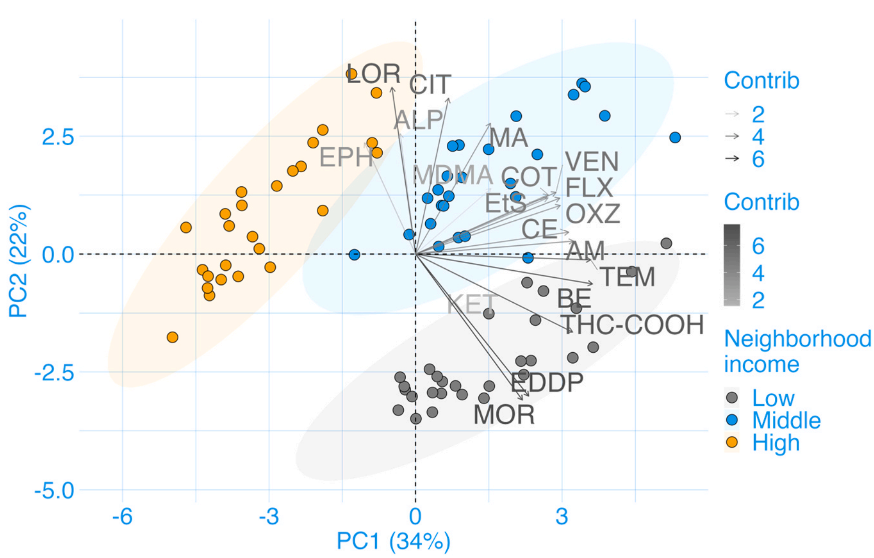

Fig. 3. Drug consumption preferences through principal component analysis.

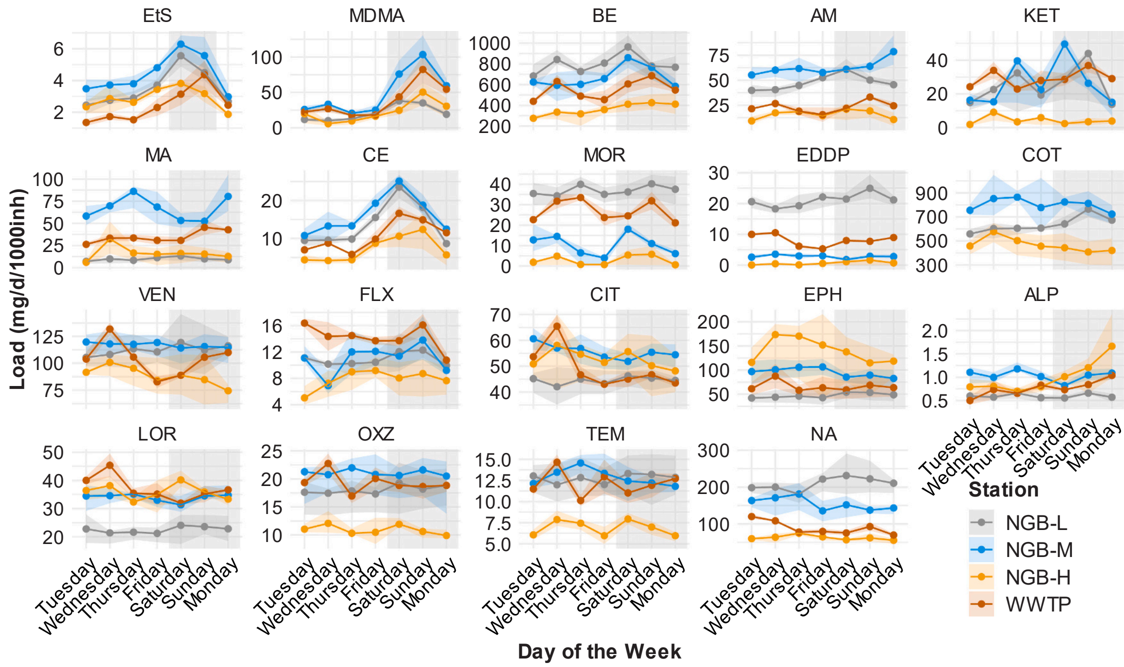

Fig. 4. Average excretion loads per day and station using the values generated in all campaigns. The first row shows the compounds for which statistically significant differences are observed between weekdays and weekends (EtS and MDMA all stations, BE_NGB-M, AM_NGH-L, KET_NBG-M). Weekends are Saturday, Sunday and Monday for all compounds except for EtS which are Saturday and Sunday only. COT was not measured at the WWTP. Shadows reflect Q1 and Q3 values.

excretion was also observed in NGB-M, which was visible in the collective analysis. In NGB-H, MOR excretion significantly increased from March 2021 to March 2022. Conversely, decreases in PNMLs were observed as follows: in NGB-M, CIT decreased, along with COT, which was detected in the collective analysis. In NGB-H, reductions were observed for AM, EPH (large reduction after the march 21 campaign), and FLX (COT also decreased but was detected in the collective analysis). In NGB-L, a significant decrease in KET excretion was observed from March 2021 through the subsequent two campaigns.

3.5. Interview-based survey

A total of 1004 interviews were conducted, resulting in a sample-to-population ratio of 3 %, which is lower than the widely accepted 10 %. The final survey included 354 individuals in NGB-L, 325 in NGB-M, and 325 in NGB-H, with a total sampling error of 3.1 %. The distribution of interviews across NGBs was balanced in terms of gender, and the average age of respondents was approximately 50 years, as shown in Table SI-1. Notable differences in personal income were observed across NGBs, reflected in the percentage of individuals with personal income levels below the mean index: 74.6 % in NGB-L, 50.1 % in NGB-M, and 37.0 % in NGB-H. Harmful alcohol use was relatively higher in NGB-M (27.1 %) and NGB-H (27.8 %) compared to NGB-L (21.1 %), although these differences were not statistically significant (Table 3). Risky drinking behaviour was more prevalent among men than women in NGB-L and NGB-M (Table SI-6); however, females from NGB-H exhibited similar risky alcohol use to males in NGB-H and males in the other NGBs. Risky alcohol use was higher among females in NGB-H compared to females in NGB-L. The strongest indications of harmful alcohol use were found among young adults (ages 16–44) in both NGB-M and NGB-H, with statistically significant higher consumption compared to the elderly (65 + years) (Table SI-6). In NGB-M and NGB-H, young adults also had significantly higher alcohol consumption than those aged 45–64.

The proportion of everyday smokers was similar across NGBs, at around 21 % (Table 3). In terms of smoking, gender differences were observed, with a larger proportion of women, compared to men, having never smoked (Table SI-6). The highest proportion of former smokers was noted in NGB-M (32.1 %), while the largest number of occasional smokers was found in NGB-H (11.6 %). No significant differences were observed in the consumption of sedatives with prescription, with the lowest consumption for long-term therapy (15 + days) recorded in NGB-M with 12 % compared to the 17 % from both the NGB-L and NGB-H. In contrast, significant differences were observed in the daily use of sedatives taken without a prescription in both NGB-L and NGB-H, which were higher compared to NGB-M. Gender differences were more notable in terms of long-term treatments (15 + days) with sedatives; significantly higher proportion of women in NGB-L compared to other categories.

4. Discussion

4.1. Licit and illicit drugs preferences in communities with differences in socioeconomic level and lifestyle habits

Our main finding is that applying WBE at the NGB level helps reveal substance use preferences in homogeneous communities. Analysing NGBs, which are more homogeneous in terms of socioeconomic level relative to an entire city, enables a deeper understanding of health status (through pharmaceutical loads) and lifestyle habits (through alcohol, nicotine, and illicit drug loads). Our findings align with previous studies by Choi et al. [18] and Price et al. [13]; which examined the relationships between sociodemographic factors and loads of MOR and THC-COOH in wastewater collected at the WWTP level. Both studies reported moderate to strong correlations between opioid use and socioeconomic disadvantage, consistent with our observation of higher EDDP and THC-COOH use in NGB-L, a NGB with lower socioeconomic level. Notably, EDDP and THC-COOH use were highest in this lower socioeconomic level area and decreased in NGBs with middle and high-income socioeconomic levels. Both Choi et al. [18] and Price et al. [13] found a positive correlation between MA use and socioeconomic disadvantage, while Price et al. [13] observed that MDMA and cocaine use were positively correlated with socioeconomic advantage. In contrast, our study observed a statistically significant higher use of all

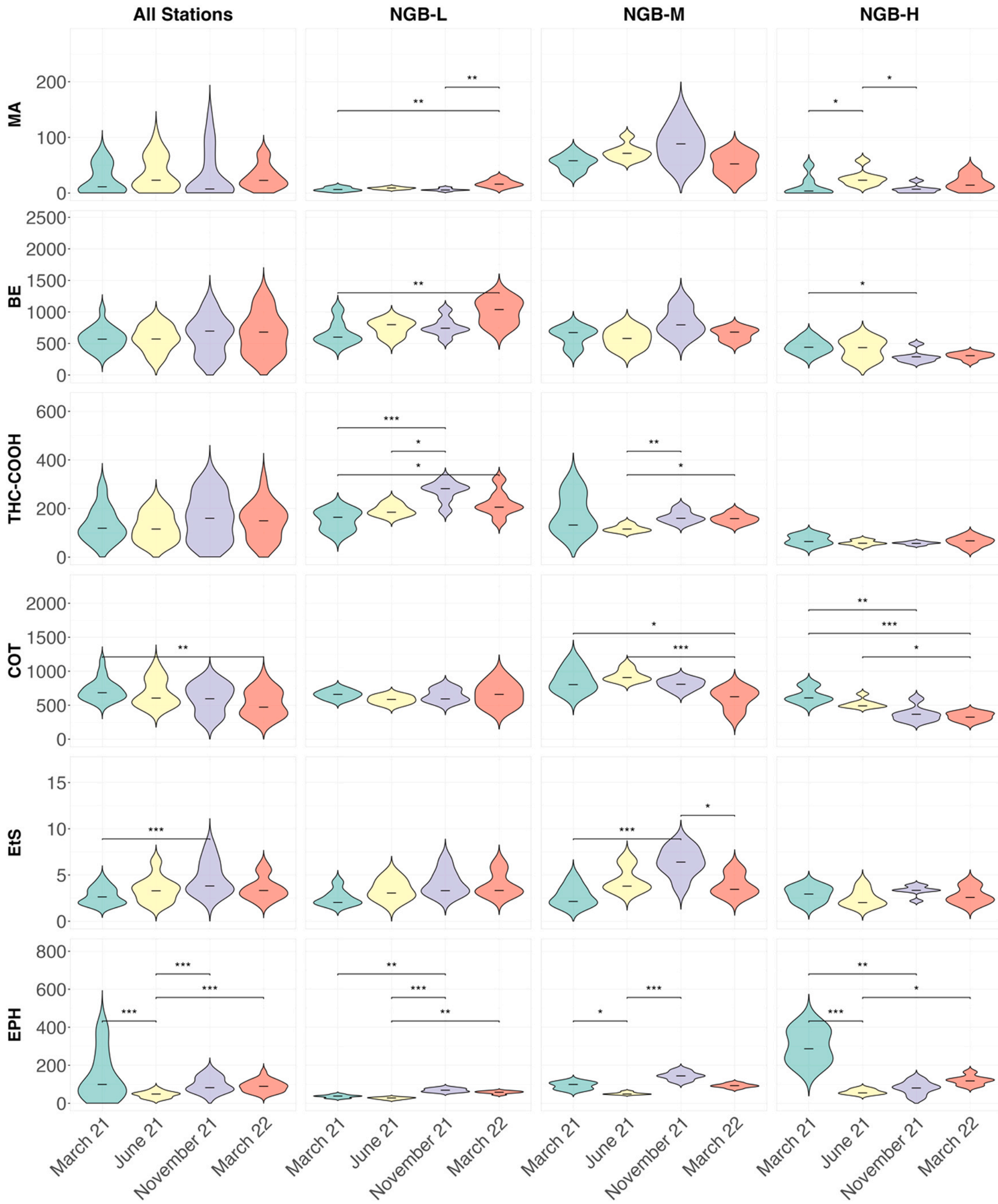

Fig. 5. Representative drugs PNMLs across campaigns. Statistically significant differences are shown for pairs with p-values < 0.05.

amphetamine derivatives, including MA, in NGB-M, whereas NGB-L did not show elevated MA use. Additionally, we found significantly lower cocaine use in NGB-H. Regarding alcohol and nicotine, previous studies such as Choi et al. [18] noted a positive correlation between alcohol consumption and indicators of socioeconomic advantage, while nicotine consumption exhibited a negative correlation. However, in our analysis, NGB-M again stood out with the highest levels of both alcohol and nicotine use. A key factor driving these patterns is the density of bars and restaurants in each NGB. For instance, NGB-L has approximately 3 bars per 1000 inhabitants, NGB-H has around 5, and NGB-M has a significantly higher ratio of 10 bars per 1000 inhabitants. These figures underscore the importance of considering confounding variables such as recreational contexts when selecting sampling points at the intra-urban level, as the higher density of bars in NGB-M likely contributes to increased recreational substance use compared to NGB-L and NGB-H. Interestingly, when we exclude NGB-M from the analysis and focus solely on comparing NGB-L and NGB-H, our results do not align with the findings of Tscharke et al. [9], who observed higher alcohol consumption in high-income settings. This discrepancy may be due to the higher prices of alcohol (x2) and tobacco (x5) in Australia compared to Spain. In our case, no significant differences in alcohol or nicotine consumption were found between these two NGBs, suggesting that factors other than socioeconomic level, such as local recreational infrastructure, may play a more pivotal role in shaping consumption patterns. In terms of pharmaceuticals, the lower loads of EPH, ALP, LOR, and CIT in NGB-L, compared to the other two NGBs, and the lower levels of OXZ, TMZ, VEN, and FLX in NGB-H reflects distinct prescription patterns of benzodiazepines and antidepressants. This may be related to differences in

Table 3
Results from the survey.

Variable	NGB-L (A)	NGB-M (B)	NGB-H (C)	*p*-value	Test
Participants n	354	325	325		
Smoking behaviour % (n)				0.030	13.999
Never smoker	47.7 % (169)	38.9 % (127)	40.0 % (130)		
Former smoker	26.1 % (92)	32.1 % (104)	28.7 % (93) A		
Someday smoker	5.5 % (19)	8.4 % (27)	11.6 % (38)		
Every day smoker	20.7 % (73)	20.6 % (67)	19.6 % (64)		
Harmful use of alcohol % (n)				0.090	4.821
At risk	21.1 % (75)	27.1 % (88)	27.8 % (90)		
Risk free	78.9 % (279)	72.9 % (236)	72.2 % (234)		
Received medical treatment w/ sedatives (last year) % (n)				0.097	13.458
Never	77.0 % (272)	77.5 % (252)	74.9 % (243)		
1 time this year	2.1 % (7)	4.1 % (13)	4.5 % (14)		
2 times this year	1.7 % (6)	1.5 % (5	0.9 % (3)		
3 + times this year	2.1 % (7)	4.8 % (16)	2.5 % (8)		
Long-term therapy (+15 days)	17.1 % (61)	12.1 % (39)	17.3 % (56)		
Took sedatives without prescription (last 7 days) % (n)				0.017	18.609
No	97.0 % (342)	97.0 % (315)	7.0 % (315)		
Once	1.1 % (4)	2.1 % (7)	0.0 % (0)		
Two or three times	0.0 % (0)	0.0 % (0)	1.5 % (5)		
More than four times	0.4 % (1)	0.0 % (0)	0.3 % (1)		
Every day	1.5 % (5)	0.9 % (3)	1.2 % (4)		

disease prevalence, variations in prescriptions depending on whether treatment is provided by private or public health centers, doctor-patient prescription preferences, as well as factors like patient age, sex, and pharmaceutical availability.

4.1.1. *Weekends consumption patterns*

We confirm findings from previous studies regarding the absence of distinct weekend consumption patterns for pharmaceuticals and substances commonly used, such as nicotine [25,31], and antidepressants used to treat chronic conditions [32]. Moreover, EtS and MDMA loads were significantly higher during the weekend, confirming increased alcohol and MDMA use during non-working days [33,34], a pattern observed consistently across all three NGBs. Furthermore, in NGB-M, BE and KET exhibited significant weekend increases. This is likely due to the large number of pubs and bars in this area, and the strong association of these substances with recreational settings. Rodríguez-Álvarez et al. [35] observed a significantly higher consumption of both alcohol and cocaine during the weekends (~23–75 % more than on weekdays) in two European cities *i.e.,* Santiago de Compostela, and Milan [35]. The case of BE is particularly relevant, as the analysis combining the three NGBs did not reveal these weekend trends. Therefore, it is important to include BE in awareness campaigns aimed at reducing its consumption in recreational settings. In contrast, campaigns targeting EtS and MDMA should be more broadly implemented across all NGBs, regardless of income level, with a focus on weekends.

4.1.2. *Night curfew restrictions influenced consumption patterns*

The increases observed for certain illicit drug loads in specific NGBs in November 2021 and/or March 2022 (e.g., MA, BE, EPH, and EDDP in NGB-L, and THC-COOH in NGB-L and NGB-M) can be attributed to the lifting of COVID-19 pandemic night curfew restrictions in Spain, which ended on June 20th, 2021. The resurgence of nightlife activities likely contributed to the elevated EtS loads observed in NGB-L and NGB-M in November 2021. As previously mentioned, the latter district is home to a large number of pubs and bars, which clearly influences alcohol consumption and even affects the overall results when the three NGBs are analyzed collectively. In contrast, the generalized decrease in COT levels in March 2022 may reflect increased awareness of the harmful effects of tobacco on the respiratory system following the pandemic. The OEDA-COVID survey conducted by the Spanish Observatory of drugs and addictions indicated a slight decrease in tobacco consumption during the pandemic compared to pre-pandemic levels [36]. Additionally, the growing consumption of benzodiazepines (LOR, OXZ and TEM) in lower-to-middle-income NGBs (NGB-L and NGB-M) likely reflects the mental health challenges exacerbated by the pandemic, such as increased anxiety, stress, and socioeconomic difficulties. The health and socioeconomic impacts of the global crisis are evident in these trends. It is important to note that generalizing these findings is challenging, as the study focused on only three NGBs. However, we hope this work will inspire health agencies to further expand WBE efforts. Such measures would help design interventions and awareness campaigns aimed at reducing anxiety, as well as curbing the consumption of both licit and illicit drugs.

4.1.3. *WBE findings alignment with survey results*

The WBE findings align with survey results when comparing NGB-L and NGB-H, as no statistically significant differences in alcohol consumption were observed between these two NGBs in both datasets. However, the sharp increase in EtS levels observed in NGB-M, particularly over the weekends, can likely be attributed to the higher concentration of bars and restaurants in this area. This surge in alcohol consumption is not reflected in the survey results, which were conducted among the general residential population rather than visitors frequenting bars and restaurants. These establishments may attract patrons from other NGBs or outside areas, leading to elevated alcohol markers in the wastewater that are not captured by the survey focused solely on local residents. The survey indicated that daily smoking prevalence was similar across the three NGBs, at around 20 %. Wastewater analysis also showed comparable nicotine use, with NGB-M exhibiting slightly higher levels, although the differences were not statistically significant. The sedative loads do not align with the survey results, as respondents indicated the lowest consumption occurred in NGB-M (12 % everyday consumers as compared to 17 % in NGB-L and NGB-H). However, the wastewater data for ALP, LOR, OXZ, and TEM showed that their loads in NGB-M are never the lowest; instead, they consistently ranked either the highest or middle positions. The demographic differences across the three areas are relatively small, but they could still influence the results and should be considered in future studies. Furthermore, the survey captures the consumption of all sedatives, whereas the wastewater analysis is limited to a subset of specific compounds. Finally, because the survey was restricted to the same areas sampled for wastewater, the findings are specific to these neighborhoods and may not be fully generalizable to other areas with similar socioeconomic profiles.

4.1.4. *Findings relevant for health authorities*

The findings of this study hold valuable implications for health authorities. They shed light on areas with notably high or low exposures to various drugs and highlight which ones exhibit widespread community-level exposure (alcohol and nicotine) versus those with specific socioeconomic preferences (cannabis and methadone-associated to low socioeconomic level- or the active principles of sedatives and antidepressants-distinct in distinct NGBs). Our analysis of the temporal trends over the whole sampling period revealed a significant increase in the consumption of methamphetamine, cocaine, and methadone in NGB-L and of cannabis and alcohol in NGB-L and NGB-M at the end of 2021/beginning of 2022, what can be attributed to the end of the night curfew restrictions in Spain after the COVID-19 and which may be subjected to further control and analysis. It is important to acknowledge that this study focused solely on three NGBs within the city of Barcelona, encompassing approximately 1.5 % of the total population serving the WWTP used for comparison purposes. To operationalize this method effectively, a broader network with an expanded set of sampling sites should be established, covering a diverse range of communities. Such an approach could be integrated into a comprehensive health prevention strategy and linked with public awareness campaigns addressing drug consumption. WBE at NGB level in large cities may help identify specific areas and socioeconomic groups that are more affected (and, therefore, need more assistance than others) by public health issues (substance abuse, anxiety and depression disorders).

Applying WBE at an intra-urban scale raises additional ethical considerations compared with city-level monitoring, because smaller catchments increase the risk that results could be interpreted as reflecting specific communities and may lead to indirect harms such as stigmatization, reputational damage, or sensationalised media narratives. International WBE ethics guidance and related scholarship on sewage epidemiology therefore recommend proactive risk mitigation, including protecting anonymity where special sensitivities may apply (e.g., marginalised residential districts), anticipating how findings may be interpreted within their socio-political context, and managing relationships with research partners and media outlets through clear communication protocols and, where appropriate, ethics committee oversight [37–41]

In this study, three neighbourhoods representing low, middle, and high socioeconomic contexts were selected as a proof of concept to demonstrate that intra-city differences can be detected; accordingly, findings should not be over-generalised nor framed as a "competition" between neighbourhoods. Rather, the intended value is to generate actionable evidence for public health authorities (e.g., targeted prevention messaging or early warning for emerging threats such as new psychoactive substances). If implemented as routine practice, neighbourhood-level WBE should be governed by an explicit ethics and governance protocol that defines data and information flows, access control and secure storage, and limits use to clearly specified public health purposes. Structured ethical review tools, such as the "Consolidated Framework for a Structured Ethical Review to Assess Potential Adverse Outcomes of WBT Efforts" proposed by Bowes and colleagues [41], could be adopted to systematically identify, assess and mitigate potential adverse outcomes before scaling up routine WBE at this spatial resolution.

5. Conclusions

This study highlights the value of WBE at the NGB level for uncovering substance use trends and health behaviours that may go unnoticed in city-wide analyses. By examining three NGBs with different socioeconomic and lifestyle characteristics, we identified distinct patterns in the use of licit and illicit substances. Key findings include the association of opioid and cannabis use with lower socioeconomic level and the role of bar density in driving higher alcohol and nicotine consumption in middle-income NGBs. Temporal trends revealed the impact of the COVID-19 pandemic and the lifting of curfews on increased substance use in late 2021 and early 2022. These findings suggest that widening the scope of WBE sampling to include homogenous communities at the intra-urban level can improve our understanding of consumption patterns and NGB-specific trends, providing valuable insights that may support health authorities in designing targeted interventions.

CRediT authorship contribution statement

Iria González-Mariño: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Elena Domene:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Ian Zammit:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Mar Satorras:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Sergi Badia:** Methodology, Investigation, Data curation. **Miren López de Alda:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Sara Rodríguez-Mozaz:** Writing – review & editing, Validation, Investigation, Formal analysis. **Lubertus Bijlsma:** Writing – review & editing, Validation, Conceptualization. **Carlos Pernas-Fraguela:** Writing – review & editing, Methodology. **Rosario Rodil:** Writing – review & editing, Investigation. **Rosa Montes:** Writing – review & editing, Project administration, Investigation. **Andrea Estévez-Danta:** Writing – review & editing, Methodology. **Lluís Corominas:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **José Benito Quintana:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Rocío Inés Bonansea:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Marta García Sierra:** Writing – review & editing, Validation, Resources, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Given her role as Editor, Rosario Rodil had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by Delegación del Gobierno para el Plan Nacional Sobre Drogas (2020I009), Instituto de Salud Carlos III-Next Generation EU/ERDF (RD21/0009/0012, RD24/0003/0001 & RD24/0003/0020 – RIAPAd Network). L. Bijlsma acknowledges grant RYC2020–028936-I funded by MCIN/AEI/10.13039/501100011033 and by "ESF Investing in your future". We acknowledge: the SCOREwater project, which received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no 820751; BCASA, s::can Iberia Sistemas de Medición S.L., Institut Metròpoli and EURECAT for their efforts in setting up and maintaining the SCOREwater stations; the Generalitat de Catalunya through the Consolidated Research Group grants ICRA-Tech 2021-SGR-01283 and 2021 SGR 00753, SA-2021–443, SA-2022–430, and the funding from the CERCA program; the Spanish Agencia Estatal de Investigación, MCIN/AEI/10.13039/501100011033, through grants RED2022–134363-T, PID2022–139446OB-C21, PID2022–139446OB-C22, and PTA2019–017397-I.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2026.121294.

Data availability

Data will be made available on request.

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