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Wastewater Analysis and Correlation with Demographic Indicators in the Province of Valencia

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Abstract

While wastewater-based epidemiology (WBE) is well-established in metropolitan areas, its application in rural settings remains largely unexplored. This study addresses this gap by evaluating the consumption patterns of 47 psychoactive biomarkers across 17 municipalities in Valencia, Spain. We demonstrate that monthly grab sampling over 24 months effectively captures long-term trends, overcoming technical constraints in rural infrastructures.

Using SPE-LC-MS/MS, we detected high concentrations of cocaine, cannabis, and benzodiazepines, with significant correlations between parent drugs and metabolites confirming their human origin. A key novelty is the analysis of chemical data alongside socioeconomic indicators. Results show that while cocaine consumption is ubiquitous, cannabis and ketamine loads are driven by urban density and younger demographics. Conversely, therapeutic pharmaceuticals predominate in rural areas with higher aging and dependency indexes. These findings validate WBE as a sensitive tool for monitoring public health across the rural-urban divide, providing essential data for targeted social and health interventions in underserved populations.

Keywords: Wastewater-based epidemiology (WBE), illicit drugs, pharmaceuticals, sociodemographic factors, correlation.

1. Introduction

Drug consumption, encompassing both illicit substances and misused pharmaceuticals, presents a significant and evolving global challenge with profound implications for public health, safety, and socioeconomic stability. According to the United Nations Office on Drugs and Crime (UNODC), an estimated 316 million people used drugs globally in 2023, a 28% increase over the previous decade, highlighting a growing worldwide prevalence [1]. The global drug market is dynamic, characterized by the emergence of new psychoactive substances (NPS), increasing potency of traditional drugs, and shifts in consumption patterns influenced by geopolitical, social, and economic factors. Beyond illicit drugs, the global consumption of pharmaceuticals, particularly psychotropic medications such as anxiolytics, hypnotics, and sedatives, has also surged, leading to concerns about misuse and dependence in many regions [2].

Traditionally, estimations of drug use have relied on population surveys, pharmacy sales data, medical records, and police seizures. However, these methods often suffer from inherent limitations including subjectivity, low spatial resolution, self-reporting bias, and significant delays in data acquisition, which can hinder timely responses to emerging trends [3]. In this context, wastewater-based epidemiology (WBE) has emerged as a powerful, objective, and real-time tool for estimating drug consumption at the population level [4]. By analyzing the concentrations of chemical biomarkers in raw wastewater, it is possible to estimate the per capita consumption of various substances within a defined population catchment area. This methodology provides a unique advantage in its ability to offer near real-time, objective data, allowing for the evaluation of consumption patterns across different regions, the detection of temporal changes, and the identification of new trends, including the appearance of novel psychoactive substances [5]. The utility of WBE has been widely recognized and applied in numerous cities and regions across Europe, North America, Asia, Africa, and Australia, providing invaluable insights into global drug use dynamics [6]. Significant advancements in retrospective calculations and the standardization of correction factors have further improved the reliability of WBE estimates, solidifying its role as a key monitoring tool endorsed by international bodies such as the European Monitoring

Centre for Drugs and Drug Addiction (EMCDDA) and the United Nations Office on Drugs and Crime (UNODC) [7].

Drug use patterns are not isolated phenomena but are intricately linked to a myriad of social and demographic factors that influence prevalence and treatment needs [8]. WBE serves as an invaluable tool for studying these relationships by providing objective, real-time data on consumption patterns across various community settings. Beyond monitoring general trends in large communities, this technique has been adapted to analyze more specific populations, such as within prisons [9,10,11,12] or educational institutions [13,14,15,16], where unique factors influence substance use. International collaborative projects like the European School Survey Project on Alcohol and Other Drugs (ESPAD) highlight the uneven distribution of drug use and its variability with demographic factors, social conditions, behavior, and mental health [17]. Furthermore, studies have explored the correlation between WBE data and various socioeconomic indicators, including income levels, education, economic inequality, and access to healthcare, offering a more holistic view of community well-being and its interaction with substance use [18,19,20]. While genetic factors can play a role in drug-related disorders, the broader social environment remains a critical determinant. Previous research has demonstrated that illicit drug use and criminal behavior are complexly intertwined [21,22].

This study aims to further explore these complex relationships within diverse municipalities in low-density, depopulated rural areas of the Province of Valencia. While WBE has been widely used in urban settings, its novel application in sparsely populated rural municipalities provides an unparalleled, near real-time snapshot of psychoactive substance consumption patterns that complements traditional survey data. The true significance of this research lies in correlating these WBE-derived consumption levels with specific socio-economic characteristics (e.g., income, unemployment rates, education levels). Such a correlation can enable the design of targeted, effective public health and social support interventions that address the unique challenges of rural municipalities. The Province of Valencia, located on the eastern coast of Spain, presents a diverse geographical and demographic landscape, ranging from densely populated urban centers, mainly in coastal areas, to small rural municipalities

76 located in the mountainous inland region. This diversity makes it an ideal microcosm for studying
77 substance consumption patterns in varying socio-demographic contexts. The study area includes a
78 selection of municipalities across this province, allowing for a comprehensive analysis of consumption
79 trends.
80
81 ## 2. Experimental
82 ### 2.1. Standards, reagents and solvents
83 The analytes used in this study include: 3-methoxy-phencyclidine (3MeO-PCP), 6-monoacetylmorphine
84 (6-MAM), 11-nor-9-carboxy-Δ9-THC (11-COOH-THC), alprazolam, amiloride, amphetamine (AMP),
85 benzoylecgonine (BEC), benzocaine, bromazepam, buprenorphine, butylone, caffeine, cannabidiol
86 (CBD), cathinone, clonazepam, clorazepate, chlordiazepoxide, cocaine, codeine, cotinine, diazepam,
87 doxylamine succinate, ecgonine methyl ester (ECME), escitalopram, scopolamine, fentanyl, heroin,
88 ketamine, lorazepam, lysergic acid diethylamide (LSD), 3,4-methylenedioxymethamphetamine
89 (MDMA), methylenedioxypyrovalerone (MDPV), medazepam, methadone, methamphetamine
90 (MAMP), methylone, naphazoline, nicotine, norketamine, o-desmethylvenlafaxine, oxazepam,
91 oxycodone, oxymethazolyne, tramadol, venlafaxine, α-pyrrolidinopentiophenone (α-PVP) and Δ-9-
92 tetrahydrocannabinol (Δ9-THC); all of which were purchased from Merck (St. Louis, Missouri, United
93 States).
94 Isotopically labeled internal standard solutions of 100 µg mL^{-1} (in methanol) of 6-MAM-D3, 11-COOH-
95 THC-D3, alprazolam-D5, AMP-D5, BEC-D3, benzocaine-D4, bromazepam-D4, butylone-D3, caffeine-D9,
96 cathinone-D5, clonazepam-D4, cocaine-D3, diazepam-D5, fentanyl-D5, heroin-D9, ketamine-D4,
97 lorazepam-D4, LSD-D3, MDMA-D5, MAMP-D5, methadone-D3, methylone-D3, nicotine-D4,
98 norketamine-D4, O-desmethylvenlafaxine-D6, oxazepam-D5, oxycodone-D3, scopolamine-D3,
99 venlafaxine-D6, tramadol-^{13}C-D3, α-PVP-D8 and Δ9-THC-D3, were obtained from Merck.
100 Methanol, acetonitrile, and buffer constituents were provided by Scharlab (Barcelona, Spain). Milli-Q
101 water was purified in a Crystal B30 EDI Adrona deionizer (Riga, Latvia) with a resistivity lower than 18

MΩ cm^{-1}. Visiprep™SPE vacuum manifold and Supel™ Swift HLB SPE Tubes (200 mg 6 mL) were purchased from Merck.

A multicomponent stock solution was prepared at 10 mg L^{-1} concentration level in methanol and stored at -20 ºC in an amber glass vial. Using this solution, calibration curves ranging from 1 to 100 µg L^{-1} were prepared by dilution in water. A solution containing deuterated internal standards was prepared at a concentration of 1 mg L^{-1} in methanol. Suitable volumes of this solution were added to both samples and standards to achieve a final standard concentration of 20 µg L^{-1} for deuterated internal standards.

2.2. Sample location and sampling procedure

Wastewater samples analyzed in this study were collected from wastewater treatment plants (WWTPs) of the different low-density municipalities of the Province of Valencia (see Figure 1). Samples were collected monthly, on working days (Monday to Friday) from the influent of each WWTP. Grab samples were collected from the respective sewer trunk lines prior to their entry into WWTPs due to most of the WWTPs of the small municipalities evaluated in this study are not equipped with autosamplers capable of collecting composite samples. Additionally, several more-densely populated municipalities located in the Province of Valencia were also analyzed for comparative purposes. Each wastewater sample was stored in glass bottles and frozen until analysis.

This study was carried out over a period of two years (2024-2025) and involved the analysis of drugs and psychotropic substances in wastewater, including anxiolytics, antidepressants, and sedatives in WWTPs of 17 different municipalities.

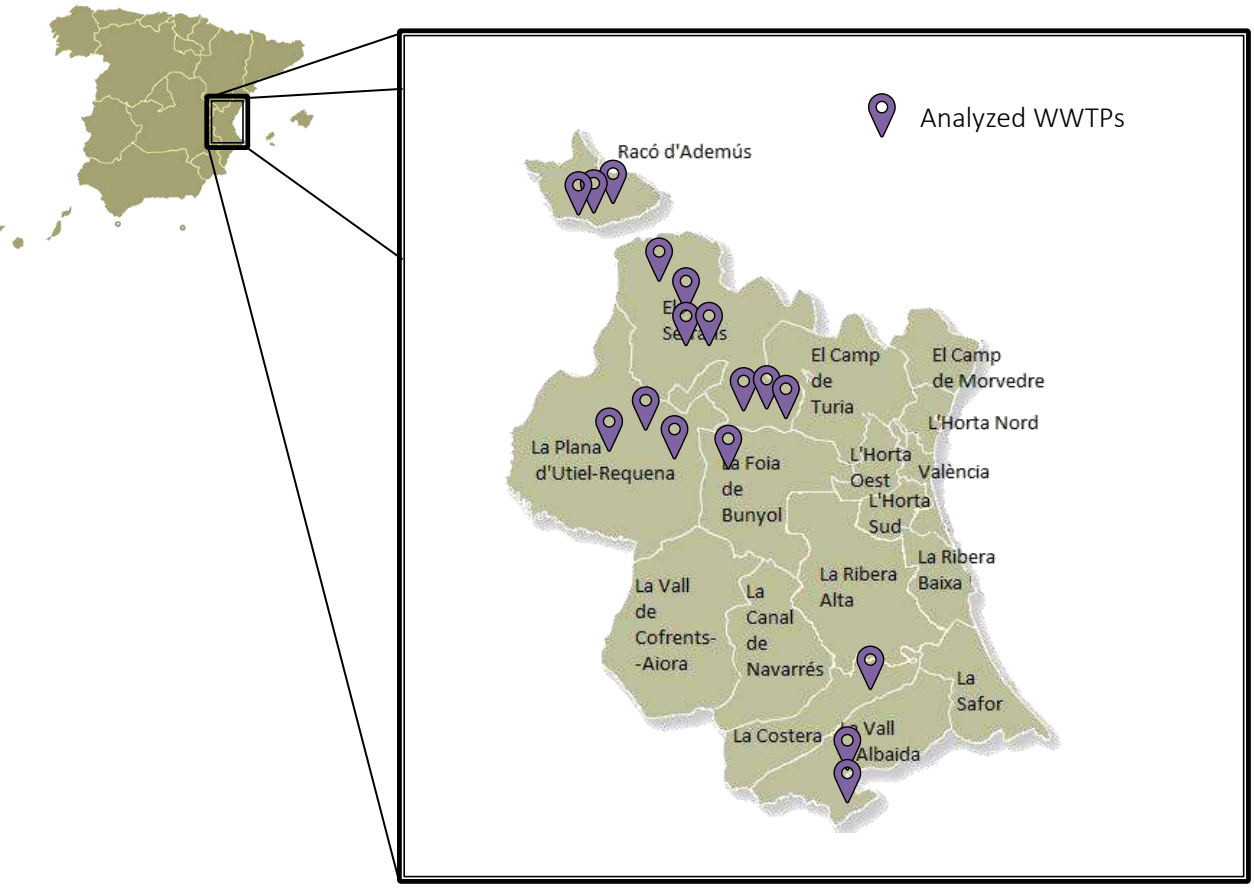


Figure 1. Geographical location of the Wastewater Treatment Plants (WWTPs) sampled in the province of Valencia (Spain).

2.3. Analytical procedure

The following analytical procedure, adapted from a previous publication [23], was used. Each wastewater sample without pH adjustment were filtrated through 1.2 µm glass fiber followed by 0.22 µm nylon filter (Whatman, Maidstone, UK). Then, 250 mL of the filtrated sample were loaded at a flow-rate of 2 mL min^{-1} into Supel™ Swift HLB SPE Tubes (200 mg) cartridges previously conditioned with 5 mL of methanol and 5 mL of ultrapure water. After that, the cartridges were rinsed with 10 mL of ultrapure water, air-dried for 15 min, and the analytes were eluted with 6 mL of methanol. Extracts were evaporated to dryness using a rotary evaporator and reconstituted with 1 mL of ultrapure water. The extracts were filtered through a 0.22 µm nylon syringe filter (Scharlab, Barcelona, Spain) and analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

For LC-MS/MS analysis, an AB SCIEX (Redwood City, CA, USA) ExionLC AD coupled to a Sciex QTRAP 6500+ mass spectrometer system was employed. Separations were performed in a Phenomenex Kinetex C18 column (100 × 2,1 mm, 1,7 µm) from Waters (Milford, Massachusetts, USA), and a 0.4 mL min^{-1} mobile phase flow rate. The mobile phase was made up of 2 mM ammonium formate in water (A) and acetonitrile (B), using a gradient from 5 to 30% B for 6 min, from 30 to 50% B for 2 min, and then from 50% to 100% B for 4 min. MS/MS acquisitions were done using electrospray ionization in positive ionization, using an ionization temperature of 550°C and a voltage of 5.5 kV. Data were evaluated using the SCIEX OS software from SCIEX. To perform quantitation of analytes, multiple reaction monitoring (MRM) mode was used. Two MRM transitions were obtained for each compound (see Supplementary Material Table SM1).

2.4. Normalization of psychoactive substance concentration

The population-normalized daily loads (PNDL) of psychoactive substances in wastewater were normalized to milligrams per 24 h per 1000 inhabitants, following equation (1).

$$PNDL\ (mg\ day^{-1}\ 1000\ inh^{-1}) = \frac{concentration\ (mg\ L^{-1}) \times\ wastewater\ daily\ flow\ rate\ (L\ day^{-1})\ \times 1000}{served\ population\ WWTP\ (inhab)}$$

(equation 1)

This was achieved using official data (treated water flow rate in L per day and the served population in equivalent inhabitants) provided by the Public Entity for Wastewater Sanitation of the Valencian Community (EPSAR) [24].

In this study, other aspects to estimate psychoactive substances consumption such as the stability of the molecules in wastewater and the excretion rate of the parent compounds and/or metabolites, were not considered in order to minimize the potential of false correlations caused by data pretreatment processes.

2.5. Descriptive statistics of social indicators

Data regarding social indicators of the different studied municipalities have been obtained from the National and Regional Institutes of Statistics (INE [25] and IVE [26]). The selected descriptors try to summarize the economic and social conditions of people and households in the area by using indicators that reflect relative socioeconomic advantage and disadvantage. In this study, total population, population density, gender, immigration, the dependency index, the aging index, the longevity index, the maternity index, the renewal of the active population, the percentage of social security affiliates, the percentage of pensioners, the unemployed ratio, the per capita income and the education degree have been considered (see Supplementary Material Table SM2).

2.6. Statistical analysis

In terms of the correlation analysis, Pearson linear correlation was used. To verify the normal distribution of data and to clearly identify the presence of outliers, Q-Q and box plots were obtained.

It should be mentioned that correlation analysis can only capture the direct bivariate correlation

between different descriptors (analyte-analyte or analyte-social descriptor).

3. Results and discussion

3.1. Method validation and analyte selection criteria

The present paper focuses on 47 biomarkers measured in wastewater. The analytical methodology, based on SPE-LC-MS/MS, was successfully validated in terms of linear range, linearity, accuracy, precision, and matrix effect, achieving limits of quantification (LOQs) in the low ng/L range (see Supplementary Material Table SM3). The LC-MS/MS method demonstrated robust linearity, with R^2 values exceeding 0.991 across all analytes. In terms of sensitivity, LOQs were dynamically set based on strict performance criteria (RSD<20% and recoveries of 70–120%), resulting in a value of 4 ng L^{-1} (LOD of 1.2 ng L^{-1}) for the bulk of the compounds, and 40 ng L^{-1} (LOD of 12 ng L^{-1}) for doxylamine succinate, caffeine, medazepam, and buprenorphine. Method precision was highly satisfactory, with RSD values consistently below 18%, confirming the reliability of the protocol for the determination of psychoactive drugs in wastewater.

Matrix effects (ME) and relative recoveries were systematically evaluated at two concentration levels (10 and 100 ng L^{-1}) as detailed in Supplementary Material Table SM4. The matrix effect was low to moderate for most target compounds, remaining within the ±15% threshold for the vast majority of the analytes. The most prominent exceptions were 3MeO-PCP and oxymetazoline, which exhibited signal suppressions of −36% and −20%, respectively, while MDPV showed a +15% enhancement. Relative recoveries were highly satisfactory, mostly ranging between 80% and 120% for both concentration levels. Although certain compounds could not be determined at the lowest concentration (10 ng L^{-1}) because they fell below the method's LOQ, their recoveries at 100 ng L^{-1} were optimal (95–106%). These findings demonstrate the robustness and suitability of the method for the trace analysis of psychoactive substances and pharmaceuticals in water samples.

From the initial list, 29 analytes, MDMA, 11-COOH-THC, alprazolam, amiloride, AMP, benzocaine, BEC, buprenorphine, caffeine, clorazepate, cocaine, codeine, cotinine, diazepam, doxylamine succinate,

ECME, escitalopram, ketamine, lorazepam, methadone, MAMP, nicotine, norketamine, O-desmethylvenlafaxine, oxazepam, oxycodone, tramadol, and venlafaxine were quantified in more than 75% of the samples. The remaining compounds, which were either below the LOQs or present in fewer than 75% of the samples, were excluded from the statistical analyses. In some cases, this could be partly due to a low consumption rate and/or the poor stability of those compounds in wastewater samples (e.g., Δ9-THC and heroin). Due to the potential sensibility of the data, the 17 different municipalities were randomly anonymized (Municipalities 1-17) for the following analyses to protect the privacy of the sampled populations.

3.2. Assessment of sampling strategy and temporal variability

Considering the technical constraints of the WWTPs of rural municipalities included in this study, which lack automated equipment for 24-hour composite sampling, a grab sampling strategy was implemented. As it has been aforementioned, samples were collected monthly over a two-year period to evaluate the temporal evolution of the target analytes.

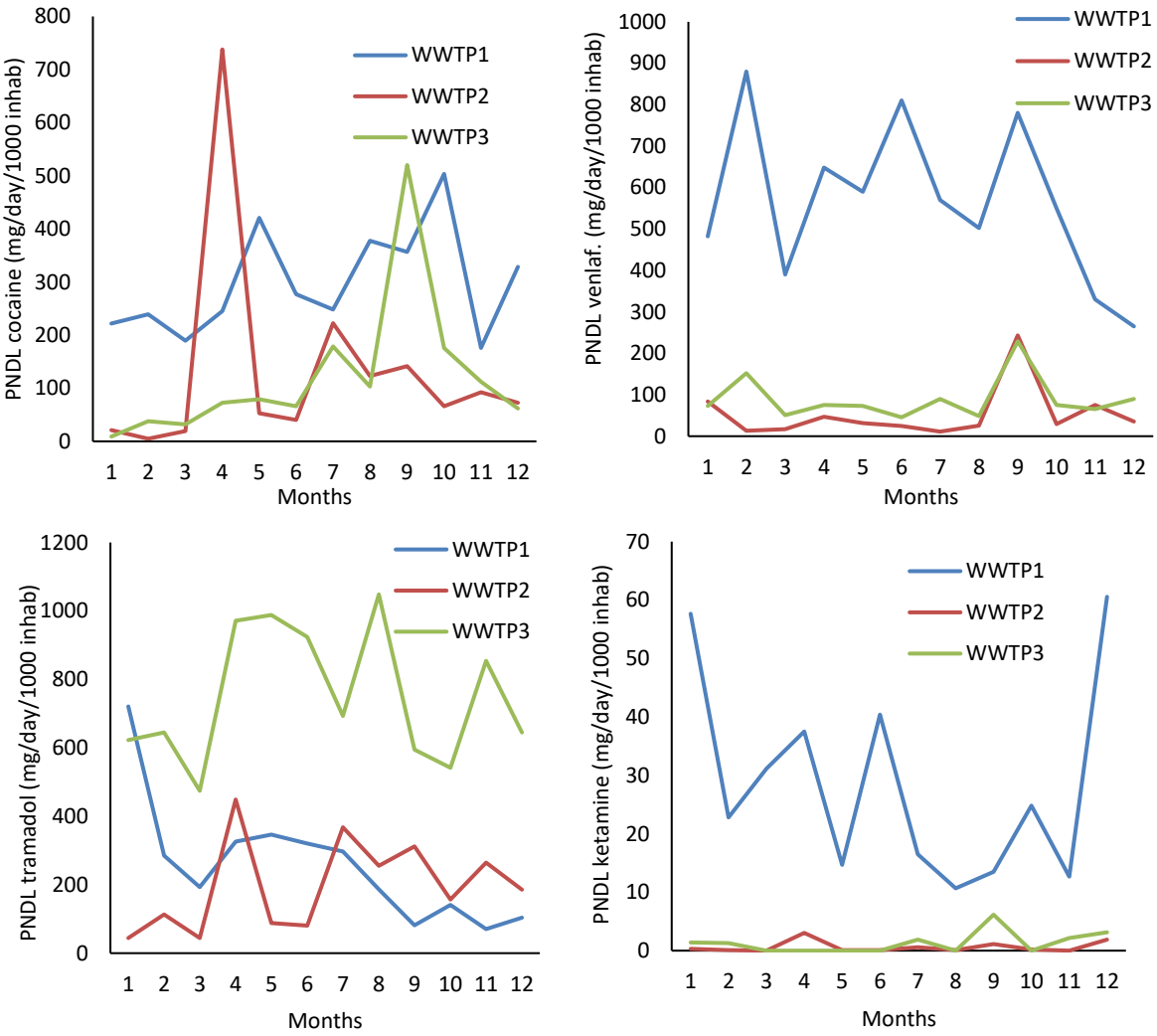
The temporal evolution of the PNDL for cocaine, venlafaxine, tramadol, and ketamine is shown in Figure 2. By expressing the data in mg/day/1000 inhabitants, the specific consumption patterns of each catchment area can be compared regardless of their absolute population size. Figure 2 illustrates the concentration trends for representative substances throughout 12 months monitoring period. This temporal analysis is crucial to assess the representativeness of the obtained mean values.

Regarding temporal stability, pharmaceuticals such as venlafaxine and tramadol exhibited relatively steady profiles throughout the year, consistent with their use in chronic medical treatments. In contrast, illicit or recreational substances like cocaine and ketamine showed greater fluctuations, with notable peaks, such as the significant increase in cocaine loads in WWTP2 during the fourth month, likely associated with specific local events or seasonal consumption shifts. Thus, despite the inherent variability of grab sampling, which captures a specific snapshot in time, the longitudinal data provided a consistent baseline for most of the analyzed compounds.

The data further reveal that the per daily load of these substances does not correlate directly with the total population size of the catchments (WWTP1 > WWTP2 > WWTP3). While WWTP1 (the largest population) showed the highest normalized loads for venlafaxine and ketamine, WWTP3 (the smallest catchment) exhibited the highest levels of tramadol for most of the year. These findings suggest that local demographic factors, such as age distribution or socioeconomic characteristics, exert a stronger influence on specific drug consumption patterns than the overall size of the urban center. This lack of proportionality highlights the importance of WBE in identifying unique community-level behaviors that would otherwise be masked by aggregate national data.

In summary, although 24 h composite sampling remains the gold standard in WBE, our findings demonstrate that high-frequency longitudinal analysis over an extended multi-year period, such as 24 months, successfully mitigates short-term temporal variability, thereby validating the suitability of monthly grab sampling for public health surveillance in logistically challenging rural settings.

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279

280 **Figure 2.** Temporal evolution of PNDL (mg/day/1000 inhabitants) for cocaine, venlafaxine, tramadol,

281 and ketamine across 12 months in three different WWTPs of varying population sizes.

3.3 Occurrence and concentration levels of psychoactive substances

Following the assessment of temporal stability, a comprehensive statistical summary of the mass loads recorded from the 408 samples collected over the 24-month monitoring period is presented below. For each facility, the minimum, maximum, mean, and median values, among other descriptive statistics of the PNDL in mg day^{-1} 1000 inh^{-1} for the 28 quantified analytes were calculated (see Table 1).

Table 1. Statistical parameters including minimum, maximum, average, and median for the target analytes monitored in this study.

Analyte	PNDL (mg day^{-1} 1000 inh^{-1})					
	Min	Max	Average	Lower quartile	Median	Upper quartile
ECME	24.3	518.6	171.9	73.9	132.0	179.8
Nicotine	160.2	2845.8	1506.5	907.6	1498.1	1865.8
Cotinine	299.3	4110.9	1803.8	1010.8	1574.9	2004.2
Amiloride	<LOQ	7.6	1.8	0.7	0.9	2.5
AMP	<LOQ	45.8	8.0	0.8	1.4	12.9
Codeine	19.0	846.5	206.6	66.2	97.4	241.6
MAMP	<LOQ	5.4	0.5	0.1	0.2	0.3
Oxycodone	<LOQ	21.2	5.7	1.2	3.3	9.9
Doxylamine Succinate	0.3	78.6	26.7	7.4	22.4	32.6
MDMA	0.7	134.1	18.8	3.8	6.5	12.3
Caffeine	204.8	16543.8	7058.6	4610.9	5337.5	9866.3
Norketamine	<LOQ	21.8	2.3	0.1	0.4	1.6
Ketamine	<LOQ	13.7	3.2	0.7	1.1	4.7
BEC	351.7	3157.0	1272.1	632.9	1402.8	1627.5
O-desmethylvenlafaxine	174.1	1979.8	690.9	270.7	459.4	1166.3
Tramadol	63.3	965.5	310.6	144.8	196.8	375.2
Cocaine	46.3	551.3	250.4	174.0	205.8	344.0
Venlafaxine	18.7	721.4	177.8	51.5	98.4	307.2
Escitalopram	7.5	106.9	48.5	22.0	42.9	74.2
Buprenorphine	<LOQ	28.3	9.4	0.1	3.4	18.3
Methadone	<LOQ	50.9	6.2	0.6	1.8	2.7
Benzocaine	1.4	15.6	5.6	2.7	4.4	5.8
Oxazepam	<LOQ	506.4	94.3	16.4	48.6	82.7
Lorazepam	16.4	365.5	83.1	29.4	48.9	75.4
Alprazolam	<LOQ	16.3	6.3	1.8	5.7	8.7
Clorazepate	<LOQ	47.7	13.0	0.2	13.4	19.0
Diazepam	<LOQ	76.6	7.3	0.1	1.2	3.0
11-COOH-THC	<LOQ	53.6	8.3	0.1	0.3	1.0

Table 1 presents the descriptive statistics for the biomarkers quantified in the influent wastewater of the 17 studied municipalities. A wide range of concentrations was observed, with caffeine and nicotine metabolite showing the highest levels, as expected. The distribution of the data, characterized by the median and interquartile range (Q1–Q3), reveals a significant variability in drug use patterns across the different locations, reflecting diverse consumption habits within the sampled populations.

The spatial distribution and consumption variability of representative biomarkers are illustrated in Figure 3 using box-plot analysis. The box represents the interquartile range (Q1–Q3), the horizontal line indicates the median, and whiskers extend to 1.5 times the interquartile range (IQR). Beyond the higher mass loads for caffeine and nicotine and the marked inter-municipal heterogeneity, the distributions reveal the presence of multiple outliers (isolated points represent outliers). While the median values represent the baseline consumption for the region, the presence of numerous outliers, such those observed AMP or MDMA, suggests that certain locations experience significantly higher exposure levels. These 'hotspots' in mass loads could be attributed to differences in demographic profiles or variations in the availability of these substances. Furthermore, the compact IQR observed for some pharmaceuticals (e.g., oxycodone, buprenorphine or alprazolam) indicates a more uniform consumption pattern across the studied area compared to the more volatile distribution of recreational drugs.

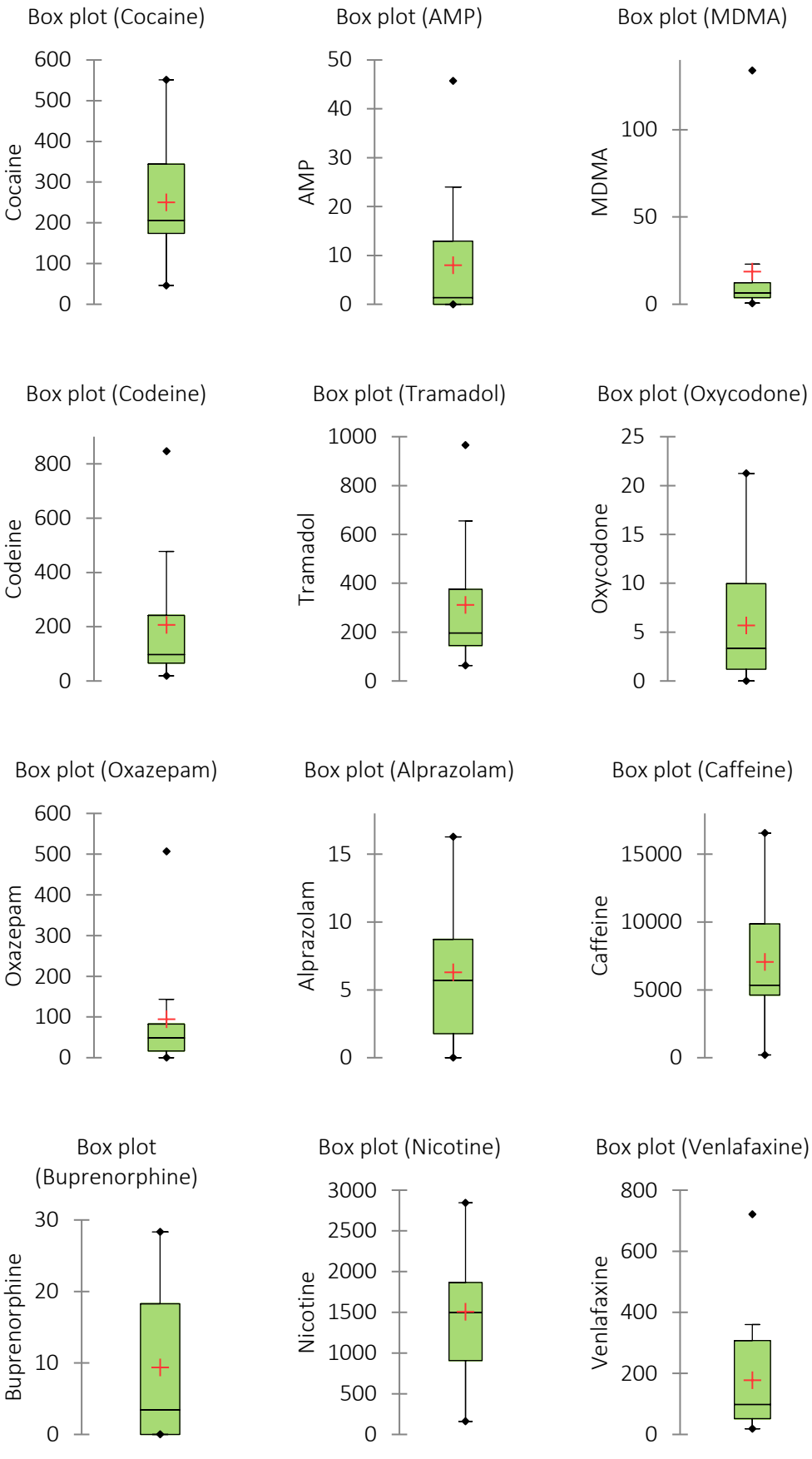


Figure 3. Box-plot distribution of mass loads (mg day^{-1} 1000 inhabitants^{-1}) for representative biomarkers.

3.4. Analysis of Psychoactive Compound Correlations

The relationships between the mass load of the different psychoactive compounds in the evaluated municipalities were also calculated using the Pearson correlation (see Figure 4). The Pearson correlation matrix reveals distinct association patterns between psychoactive substances, providing insights into co-consumption habits and market-related factors. As expected, the strongest correlations correspond to primary analytes and their respective metabolites, validating the consistency of the analytical data. Robust associations were found between cocaine and its metabolites, benzoylecgonine (r = 0.91) and ecgonine methyl ester (r = 0.62), as well as between nicotine and cotinine (r = 0.80). Similar metabolic signatures were observed for the antidepressant venlafaxine and its metabolite O-desmethylvenlafaxine (r = 0.68), and to a lesser extent, between ketamine and norketamine (r = 0.44), probably due to the lower concentrations found in this later case. A significant finding is the strong correlation between cocaine and benzocaine (r = 0.60). This relationship is not probably due to use of benzocaine as a topical anesthetic, but rather to its frequent use as a cutting agent in street cocaine, as previously documented in forensic literature [27,28]. Regarding recreational patterns, cocaine loads showed positive correlations with other stimulants, including amphetamine (r = 0.57), MDMA (r = 0.32), and methamphetamine (r = 0.30). These associations suggest a trend toward polydrug use within certain nightlife or recreational environments across the evaluated municipalities.

Beyond metabolic links, several cross-category correlations emerged that reflect common lifestyle or demographic factors. For instance, the significant correlations between tramadol and stimulants such as caffeine (r = 0.64) and nicotine (r = 0.61), as well as with venlafaxine (r = 0.70), may point to a profile of polypharmacy or common consumption drivers in specific population segments. Furthermore, the relationship between norketamine and 11-COOH-THC (r = 0.72) and diazepam (r = 0.69) suggests a combined use of dissociative and sedative substances. Finally, the moderate associations between caffeine, nicotine, and alprazolam ($r \geq 0.56$) likely represent generalized baseline consumption patterns of legal stimulants and common psychotropic medications within the urban exposome.

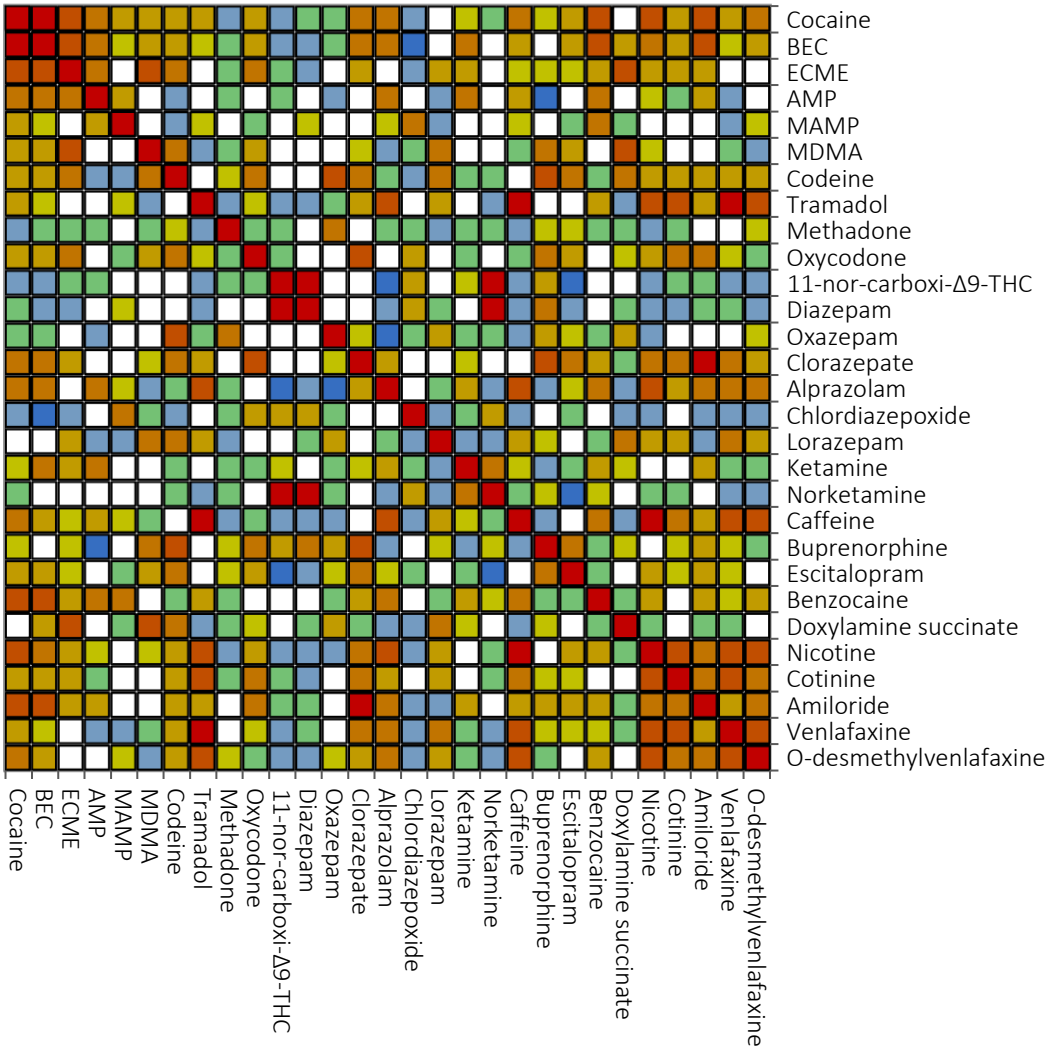


Figure 4. Pearson correlation matrix of the mass loads for the monitored biomarkers across the evaluated municipalities. The color scale represents the strength and direction of the correlation (r), ranging from blue (negative correlation) to red (positive correlation).

3.5. Correlation of psychoactive substances consumption and social indicators

The relationship between the occurrence of psychoactive substances in wastewater and the socioeconomic profile of the monitored populations was evaluated through a Pearson correlation matrix (see Figure 5). Several significant patterns emerged, highlighting the influence of demographic

and economic factors on drug consumption habits.

A key finding of this study is the absence of a significant correlation between cocaine (and its metabolites, BEC and ECME) and population size or density. This lack of proportionality indicates that cocaine consumption is not restricted to large urban centers but is ubiquitously distributed across all municipalities regardless of their scale. This observation is consistent with previous research conducted across various Spanish cities [29], which found no relationship between city size and cocaine use (Spearman's test, p-value: 0.39). Thus, it can be concluded that cocaine consumption in Spain should be considered a deeply rooted social issue that transcends the rural-urban divide.

Additionally, cocaine loads did show distinct relationships with specific economic sectors. A negative correlation was identified between cocaine, BEC, and ECME and service (tertiary) sector (-0.18, -0.30, and -0.69, respectively) and the construction sector (-0.44, -0.43, and -0.38, respectively) and a slight positive correlation was observed with the secondary (industry) sector (0.27, 0.36, and 0.59, respectively). These results agree with the 2019/2020 survey on the use of psychoactive substances in the workplace in Spain, conducted by the OEDA [30], indicated that cocaine use in the working population in 2019-2020 reached 3.1 %, especially in people with a low level of education. Prevalence of cocaine use is higher among manual workers, with higher than average use among skilled manual workers. In contrast, other recreational substances like 11-COOH-THC, ketamine and its metabolite norketamine followed a different pattern, showing a greater affinity for indicators of urban density (correlations of 0.87, 0.29 and 0.94 with population density, and 0.68, 0.63 and 0.85 with total population, respectively). Those compounds exhibited a strong negative correlation with the average age of the population and indicators such as the dependency (-0.40, -0.60 and -0.56), aging (-0.38, -0.63 and -0.60), longevity (-0.37, -0.59 and -0.57) indexes and the pensioners' ratio (-0.34, -0.66 and -0.60). This trend suggests that the consumption of THC and ketamine is predominantly concentrated in municipalities with a younger demographic profile. In this sense, ketamine is likely associated with nightlife and recreational environments, a phenomenon that has been increasingly reported in wastewater-based epidemiological studies across Europe [31]. The fact that this correlation remains

consistent for both the parent compound and its metabolite reinforces the reliability of the data. Conversely, therapeutic compounds such as oxazepam, clorazepate, tramadol, codeine, buprenorphine, and venlafaxine correlated more closely with demographic factors such as aging and the dependency ratio, reflecting their clinical application in specific population segments.

The consumption of several psychoactive compounds such as venlafaxine, nicotine and tramadol demonstrated positive correlations with the educational attainment level of the population. Nevertheless, this trend could be a direct consequence of the socioeconomic characteristics of smaller municipalities, characterized by an older age structure and a lower proportion of individuals with higher education.

Overall, these correlations underscore that WBE not only tracks substance use but also reflects the underlying socio-demographic fabric of the community, providing a nuanced view of how economic stability, age, and occupation shape public health trends. In summary, the correlation results can enable the design of targeted, effective public health and social support interventions that address the unique challenges of rural municipalities.

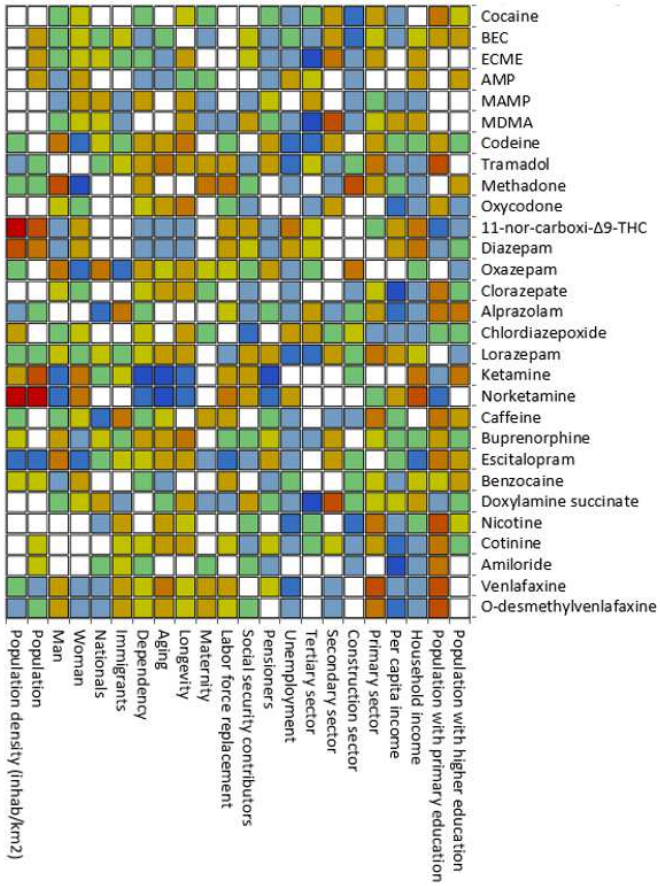


Figure 5. Correlation heatmap between population-normalized daily loads of psychoactive substances in wastewater and socioeconomic indicators. The color scale represents the strength and direction of the correlation (r), ranging from blue (negative correlation) to red (positive correlation).

4. Conclusions

The present study establishes a comprehensive framework for the application of WBE across diverse demographic scales, demonstrating its particular efficacy in characterizing underserved rural environments in the Province of Valencia.

By systematically evaluating 47 psychoactive substances over a biennial period, the research confirms that longitudinal grab sampling, when sustained with high frequency, provides a reliable methodology to overcome the technical and infrastructural limitations inherent to small-scale WWTPs. The robust correlation observed between parent compounds and their metabolites further validates the analytical integrity of the data, ensuring that the mass loads accurately reflect human metabolic excretion.

Beyond the methodological validation, the integration of chemical findings with socioeconomic indicators reveals a complex socio-demographic fabric. The ubiquitous distribution of cocaine, showing no statistical dependence on population density, identifies it as a pervasive social challenge that transcends the rural-urban divide in the Province of Valencia. In contrast, the consumption of other substances follows a clear demographic stratification: while cannabis and ketamine loads are predominantly driven by younger populations in dense urban centers, the prevalence of psychoactive pharmaceuticals is significantly higher in rural municipalities characterized by aging populations and higher dependency ratios. Consequently, direct bivariate socioeconomic correlations must be interpreted with caution and systematically cross-referenced with population age structures, such as through Principal Component Analysis (PCA), to prevent confounding biases or ecological fallacies.

Ultimately, these insights underscore the necessity of moving beyond aggregate national statistics. By identifying local consumption hotspots and specific socioeconomic drivers, WBE emerges as an essential tool for public health authorities to design and implement localized, evidence-based prevention and social support programs. This research not only fills a geographical gap in the current literature but also underscores the potential of wastewater analysis to inform targeted public health policies tailored to the unique challenges of both rural and urban communities.

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435

436 Legend of Figures:

437 **Figure 1.** Geographical location of the Wastewater Treatment Plants (WWTPs) sampled in the province
438 of Valencia (Spain).

439 **Figure 2.** Temporal evolution of PNDL (mg/day/1000 inhabitants) for cocaine, venlafaxine, tramadol,
440 and ketamine across 12 months in three different WWTPs of varying population sizes.

441 **Figure 3.** Box-plot distribution of mass loads (mg day^{-1} 1000 $inhabitants^{-1}$) for representative biomarkers.

442 **Figure 4.** Pearson correlation matrix of the mass loads for the monitored biomarkers across the
443 evaluated municipalities. The color scale represents the strength and direction of the correlation (r),
444 ranging from blue (negative correlation) to red (positive correlation).

445 **Figure 5.** Correlation heatmap between population-normalized daily loads of psychoactive substances
446 in wastewater and socioeconomic indicators. The color scale represents the strength and direction of
447 the correlation (r), ranging from blue (negative correlation) to red (positive correlation).

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