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Consumption of illicit drugs and benzodiazepines in six Spanish cities during different periods of the COVID-19 pandemic

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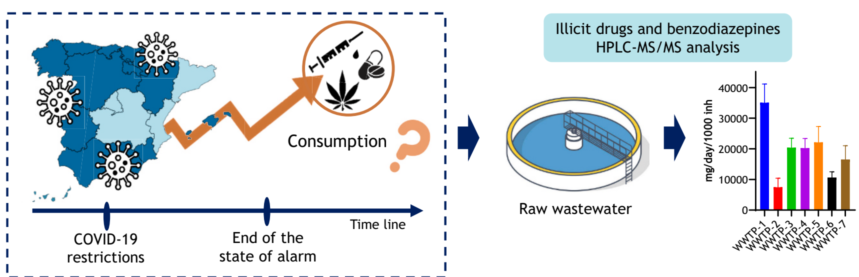
HIGHLIGHTS

- 18 illicit drugs and benzodiazepines were analyzed in urban wastewater.
- Non-homogenous effect of the COVID-19 restrictions on the use of the substances
- Cannabis and cocaine use was higher when the restrictions were more severe.
- Methamphetamine, MDMA and mephedrone use was higher after the end of the state of alarm.
- ANOVA and PCA were used to explore spatial and temporal profiles of use.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater-based epidemiology (WBE) can provide objective and real time information about the use of addictive substances. A national study was conducted by measuring the most consumed illicit drugs, other drugs whose consumption is not so widespread but has increased significantly in recent years, and benzodiazepines in untreated wastewater from seven wastewater treatment plants (WWTPs) in six Spanish cities. Raw composite wastewater samples were collected from December 2020 to December 2021, a period in which the Spanish and regional governments adopted different restriction measures to contain the spread of the COVID-19 pandemic. Samples were analyzed using a validated analytical methodology for the simultaneous determination of 18 substances, based on solid-phase extraction and liquid-chromatography tandem mass spectrometry. Except for heroin, fentanyl, 6-acetylmorphine and alprazolam, all the compounds were found in at least one city and 9 out of 18 compounds were found in all the samples. In general, the consumption of illicit drugs was particularly high in one of the cities monitored in December 2020, when the restrictions were more severe, especially for cannabis and cocaine with values up to 46 and 6.9 g/day/1000 inhabitants (g/day/1000 inh), respectively. The consumption of MDMA, methamphetamine and mephedrone was notably higher in June 2021, after the end of the state of alarm, in the biggest population investigated in this study. Regarding the use of benzodiazepines, the highest mass loads corresponded to lorazepam. This study demonstrates that WBE is suitable for complementing epidemiological studies about the prevalence of illicit drugs and benzodiazepines during the COVID-19 pandemic restrictions.

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1. Introduction

Wastewater-based epidemiology (WBE) is used as a surveillance tool to provide data related to public health, drug use, exposure to chemicals and lifestyle habits (Rousis et al., 2023). It is based on the analysis of urban wastewater that reflects the lifestyle of a population as it represents anonymous urine samples from thousands of people (Castiglioni et al., 2021). Common illicit drugs were the initial target compounds of the WBE approach but in a short period of time it was also applied worldwide to assess the consumption of other classes of compounds, such as pesticides (Rousis et al., 2021), mycotoxins (Gracia-Lor et al., 2020), phthalate plasticizers (Estévez-Danta et al., 2021), psychotropic substances such as caffeine, nicotine and alcohol (Rousis et al., 2023), new psychoactive substances (NPS) (Bade et al., 2023) or pharmaceuticals (Fontanals et al., 2023), among others.

WBE has the potential to offer objective, up-to-date information and real-time evidence of the patterns of use and changes in an entire community, but also to identify changes during special events (Castiglioni et al., 2013). In December 2019, Chinese health authorities reported to the World Health Organization an outbreak of pneumonia caused by the virus SAR-CoV-2, rapidly spreading globally and officially declared a pandemic on March 11, 2020. This disease was named coronavirus or COVID-19. In Spain, to reduce the spread of the virus, the nationwide lockdown was from 14 March 2020 until May 2020. As restrictions were lifted, a new increase of infections forced to reimpose restrictive measures and consequently, in November 2020 the state of alarm was again declared until May 2021. During these months different types of measures were applied depending on the severity of the related symptoms and the number of positive cases. These measures affected the mobility, remote work from home, socialization (opening of bars, restaurants and pubs) and everyday activities of citizens, thus they affected both social and economic aspects of life (Psichoudaki et al., 2023). It was expected that physical and social restrictions, such as closure of night-time economy venues and music festivals, would disproportionally impact people who regularly use illicit drugs and cause substantial shifts in illicit drugs markets, drug use patterns and related harms (Dietze and Peacock, 2020). Social isolation and lifestyle changes (there was a significant decline in physical and other meaningful activities of daily living, working, leisure, social activity, and education) during the COVID-19 pandemic (Caroppo et al., 2021) had also a profound impact on the mental health of the global population (Abdalla et al., 2023). A report published by the World Health Organization highlighted that during the first year of the pandemic there was a significant increase in depression and anxiety problems ("Mental Health and COVID-19 : Early evidence of the pandemic's impact (World Health Organization),", 2022). Benzodiazepines are among the most prescribed pharmaceuticals to treat these symptoms. These substances are potentially addictive drugs (Schmitz, 2016; Tan et al., 2010).

The aim of this study was to apply the WBE approach to assess the consumption of 18 potential substances of abuse, including drugs and benzodiazepines, in six different Spanish cities and to investigate the possible impact of the COVID-19 restrictions on their consumption patterns.

2. Methods

2.1. Chemicals and reagents

All the reference standards of illicit drugs (benzoylecgonine, ecgonine methyl ester, heroin, codeine, morphine, 6-acetylmorphine, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), mephedrone, ketamine, fentanyl, cocaethylene) and of benzodiazepines (lorazepam, alprazolam, lormetazepam, diazepam) were purchased from Merck (Steinheim, Germany) in liquid form as 1 mg/mL solutions in methanol (MeOH) or acetonitrile (AcN). Stock solutions were prepared in MeOH at 100 mg/L, except 6-acetylmorphine, ecgonine methyl ester, heroin, cocaethylene, fentanyl and lorazepam which were prepared in AcN. THC-COOH was prepared at 10 mg/L in methanol. A working standard solution containing all target analytes at 1 mg/L was prepared in methanol and subsequently diluted, when required, with HPLC-grade water.

Isotopically labeled compounds for illicit drugs were morphine-d_6, THC-COOH-d_9, methamphetamine-d_9, cocaethylene-d_9, benzoylecgonine-d_3, amphetamine-d_5, MDMA-d_5, ecgonine methyl ester-d_3, and for benzodiazepines, lorazepam-d_4, diazepam-d_5 and alprazolam-d_5 were used. All of them were supplied by Merck as 0.1 mg/mL solutions in MeOH or AcN. Labeled internal stock solutions were prepared separately at 10 mg/L. Two different internal standard (IS) mixtures at 1 mg/L were prepared (a mixture for illicit drugs and another one for benzodiazepines) and were used as surrogates.

Ultrapure water was obtained by using a Milli-Q Millipore system (Bedford, MA, USA). HPLC-grade MeOH and HPLC-grade AcN were purchased from Scharlau (Barcelona, Spain). Formic acid (HCOOH, content >98 %) was supplied by Scharlau (Barcelona, Spain). SPE cartridges used were Oasis HLB (3 mL, 60 mg) from Waters Corp., Milford, MA, USA; Extrabond EB2 (6 mL, 200 mg) from Scharlab and Isolute SLE+ (1 mL) from Biotage. HPLC capillary Avantor ACE Excel C18-PFP column (100 mm × 2.1 mm, 2 μm) was acquired from Symta S.L.L. (Madrid, Spain).

2.2. Sample collection

24-h composite influent samples were collected at the entrance of seven WWTPs in different regions of Spain (Fig. S1): WWTP-1 in the Valencian Community, WWTP-2 in Catalonia, WWTP-3 and WWTP-7 in Castilla-La Mancha and WWTP-4, WWTP-5 and WWTP-6 in the Community of Madrid (WWTP-5 and 6 were in two different districts of one of the cities investigated). These WWTPs were selected because they are in different areas of Spain, with very diverse population sizes and socio-demographic and socio-economic characteristics. Samples were collected daily for seven consecutive days during different periods of the COVID-19 pandemic. Samples from WWTP-1 and WWTP-2 were taken in December 2020; in WWTP-3 and WWTP-4 the sampling was performed in May 2021; in WWTP-5, WWTP-6 and WWTP-7, during June 2021. In addition, samples from WWTP-3 were also collected in December 2021. Each sample was collected as a daily composite sample, using flow-proportional or volume sampling mode. 1 L aliquots collected in polyethylene high-density bottles were received in our laboratory and stored at −20 °C until extraction. Table S1 summarizes the sampling dates, flow rates and the population size served by each WWTP. In addition, the restrictions applied during the different sampling periods have been summarized, as they might have an impact on the consumption.

2.3. Extraction and extract work-up

Prior to the treatment of the samples, an optimization of the extraction procedure was carried out, comparing three different SPE cartridges: Oasis HLB, Extrabond EB2 and Isolute SLE+. Details on the optimization can be found in Section 3.1 and in the Supplementary information.

According to the optimized method, samples were thawed at room temperature before solid-phase extraction. An aliquot of 60 mL was taken and spiked with the internal standards mixture to give a final concentration of 0.5 μg/L. Then, it was filtered to remove the suspended particulate matter through 0.45 μm mixed cellulose membrane filters from Whatman (Kent, UK) and 50 mL of the filtered sample were loaded on Oasis HLB cartridges (3 mL, 60 mg), previously conditioned with 6 mL of MeOH and 6 mL of ultrapure water. Cartridges were vacuum-dried for 15 min, wrapped in aluminium foil and immediately stored at −20 °C. Analytes were eluted with 6 mL of MeOH and the extract was evaporated to dryness under a nitrogen stream (40 °C). Dry residues were redissolved in 0.5 mL of MeOH-water (5–95, v/v) and transferred into glass vials for instrumental analysis. The concentration factor was 100 times.

2.4. Chromatographic and tandem mass spectrometry conditions

A liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) method was used for the determination of the selected compounds. Analysis was done using a EVOQ Qube triple quadrupole (Bruker, Darmstadt, Germany). The compounds were separated on an ACE Excel C18-PFP (100 mm × 2.1 mm, 2 μm) column using the Elute HPG 1300 pumps and Elute Autosampler (Bruker). The column was maintained at 40 °C and the mobile phase consisted of ultrapure water (eluent A) and MeOH (eluent B), both 0.1 % HCOOH. The percentage of eluent B changed as follows: 0 min, 5 %; 0.5 min, 5 %; 10 min, 100 %; 13 min, 100 %; 13.1 min, 5 %; 16 min, 5 %. The flow rate was maintained at 0.4 mL/min and the injection volume was 15 μL.

Samples were ionized using electrospray ionization in positive polarity. Source setting parameters were: capillary voltage 4000 V; cone temperature 350 °C; cone gas flow 20 psi; source temperature 500 °C; source gas flow 20 psi and nebulizer gas flow 60 psi. Analysis was done using the Selected Reaction Monitoring (SRM) under time-scheduled conditions. The $[M + H]^+$ ion was selected as the precursor except for lorazepam-d_4. In this case, the transition 327.0 > 280.9 was chosen in order to avoid the mass overlap between the natural analyte (isotope peak due to the presence of two chlorine atoms; $2Cl^{37}$) and the internal standard signal, which would occur if the transition 325.0 > 278.9 was chosen. For all the compounds, the two most abundant precursor/product ion transitions were selected. Analytical details and instrumental parameters are provided in Table S2.

LC/MS/MS Bruker Compass EDM 1.2 and TQ control software was used to process quantitative data.

2.5. Validation of the analytical method

Method accuracy (reported as percentage recovery) and precision (reported as relative standard deviation (RSD %)) of the analytical method were tested by triplicate analysis of 50 mL wastewater aliquots spiked with 0.5 μg/L of each analyte and of each IS. An additional aliquot, spiked only with all the IS, was also processed in the same batch to subtract the responses of the target compounds that were present in the wastewater sample used in the validation, in order to correct recovery values. Analytes were quantified using surrogate-deuterated standards. Eleven deuterated substances were evaluated as potential IS during recovery experiments. For each analyte, the deuterated compound providing the best value (closer to 100 %) was finally selected as its IS. Recovery values between 70 % and 120 %, with RSD lower than 20 % were considered as satisfactory.

External calibration curves were prepared freshly before each analytical run. A seven-point calibration curve was constructed by injecting standard solutions in the range 2–500 μg/L in MeOH-water (5:95, v/v) and a fixed amount of the internal standards (50 μg/L). Satisfactory linearity using weighed (1/X) least squares regression was assumed when the correlation coefficient (r) was higher than 0.99, based on analyte peak areas measurement, and when residuals were lower than 20 % without significant trend.

The limits of detection (LODs) and quantification (LOQs) were estimated in real SPE-extracted wastewater samples considering the concentrations of the analytes giving a signal to-noise (S/N) ratio of 3 and 10, respectively. For analytes that were not found in the wastewater, the limits were calculated from spiked samples at the validation level tested.

2.6. Calculations

Concentrations were calculated using calibration standards prepared in solvent, based on relative responses analyte/IS. For confirmation, the ion ratio between the two transitions (within 30 %) as well as retention time (<0.1 min) had to compare with the standard, according to the European Guidelines (European Comission, 2021).

Quality control samples (QCs) were included in every sample sequence to ensure the reliability of results. They consisted of a wastewater sample spiked at 0.5 μg/L of illicit drugs, benzodiazepines and internal standards. They were prepared for each WWTP, randomly selecting one of the samples and were analyzed following the same analytical procedure than the samples. QCs recoveries in the range 70–120 % were considered as satisfactory.

Normalized mass loads (mg/day/1000 inhabitants) were estimated using the measured concentrations (μg/L), the daily wastewater flow rates (m^3/day) and the number of inhabitants served by each WWTP (see Supplementary information). Back-calculation of the consumption to the parent substance was performed for the compounds where a correction factor (CF) has been established (Castiglioni et al., 2013; Gracia-Lor et al., 2016) and widely used; cocaine consumption was calculated from benzoylecgonine (CF = 3.59), MDMA consumption from MDMA (CF = 4.4), amphetamine consumption from amphetamine (CF = 3.30), methamphetamine consumption from methamphetamine (CF = 2.44) and cannabis consumption from THC-COOH (CF = 182). Consumption of the rest of the compounds was not back-calculated and only normalized-mass loads of parent drugs or metabolites were considered.

2.7. Statistical analysis

Analysis of variance (ANOVA) and principal component analysis (PCA) were used to explore spatial and temporal profiles of use. The software package used was Statgraphics 19 program (Statgraphics Technologies. Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Optimization of the solid-phase extraction

Three different extraction cartridges were compared. Oasis HLB and Extrabond EB2 cartridges have a similar mechanism of retention, based on a balance between hydrophilic and hydrophobic properties. Oasis HLB is made from a co-polymer of divinylbenzene and vinyl pyrrolidone and is suitable for the retention of acidic, neutral and basic compounds. Extrabond EB2 is made from divinylbenzene polystyrene modified with pyrrolidone and is recommended for most analytes, but specially for acidic compounds. Isolute SLE+ cartridges are packed with a modified form of diatomaceous earth. All of them were tested at neutral pH, passing 25, 50 and 100 mL of wastewater, and using the same conditioning (ultrapure water and MeOH) and elution conditions (MeOH).

As expected, the absolute recoveries (i.e. without correction with internal standards) using Oasis HLB and EB2 were similar for all the compounds, with the exception of heroin. This analyte was not recovered with EB2 cartridges while low recoveries were obtained with Oasis HLB. In general, the compounds were poorly retained (<10 %) on Isolute SLE+ or were not recovered (mephedrone, heroin and THC-COOH). In order to improve the extraction efficiency of Isolute SLE+, this cartridge was tested using specific elution and reconstituted conditions recommended by the manufacturer. Thus, elution was performed with dichloromethane and MeOH (both solvents were collected in the same tube) and 50 mM HCl in MeOH was added to each collection tube before evaporation because the presence of HCl is supposed to stabilize amphetamines and ketamine and minimize analyte losses during evaporation, according to the supplier. Isolute cartridges were also tested using dichloromethane and ethyl acetate in the elution step because using these conditions THC-COOH was efficiently recovered in oral fluid in a method previously developed (unpublished data). However, although recoveries improved for most compounds using these conditions, it did not work for THC-COOH.

In order to simultaneously extract all the compounds, and supported by many similar studies found in literature (Adhikari et al., 2023; Fontanals et al., 2023; Massano et al., 2023), Oasis HLB cartridges were finally selected. The of use internal standards improved recoveries, leading to satisfactory values for most analytes (16 out of 18 showed recoveries higher than 70 %) (see Table 1 and Fig. S2). Regarding the volume of wastewater, 100 mL was discarded because passing this volume fentanyl was not recovered. This might be consequence of exceeding the breakthrough volume of the cartridge or because of a higher matrix effect (ion suppression). Similar recoveries were obtained for all the compounds when comparing 25 and 50 mL. Therefore, 50 mL was selected.

3.2. Validation of the analytical method

Method validation was performed in terms of accuracy, precision, linear range and sensitivity (Table 1). Calibration curves were linear in the range 2–500 μg/L (corresponding to 0.02–5 μg/L in the raw wastewater considering the pre-concentration factor applied along the sample procedure), except for amphetamine, 6-acetylmorphine, codeine and fentanyl (linear response from 2 to 250 μg/L) and ketamine (2–100 μg/L). In all cases, calibration curves showed satisfactory correlation coefficients (>0.9910) and residuals lower than 20 % for all compounds.

Accuracy and precision were estimated from recovery experiments ($n = 3$) of the target compounds by spiking 50 mL aliquots with 0.5 μg/L of each analyte as well as with the IS mixtures (11 labeled compounds). Satisfactory recoveries were obtained for the compounds that were quantified using their own labeled analyte (Table 1). This indicates that losses during the filtration and/or SPE step and matrix effects were corrected. For the remaining seven compounds, an IS with a similar structure than analyte under study or a close retention time was selected. The deuterated compound providing the best value (closer to 100 %) was finally selected as its IS (see Table 1). In most cases, this correction yielded recoveries between 74 and 117 %. However, three compounds (mephedrone, heroin and fentanyl), still presented recoveries ≤50 % (mephedrone 42 %, heroin 51 %, fentanyl 44 %). Thus, quantitative data presented for these analytes (if present in the samples) should be taken as estimated levels.

For most compounds, the method presented satisfactory precision with RSD values below 10 %. Regarding the LOQs (calculated in real wastewater samples), they were < 6 ng/L for 16 out of 18 compounds. For the remaining compounds, the LOQs ranged from 14 to 88 ng/L (Table 1).

3.3. Presence of illicit drugs and benzodiazepines in wastewater

The validated method was applied to the analysis of 49 raw wastewater samples collected from seven WWTPs in six Spanish cities. Detailed values are set out in Table 2. Regarding the illicit drugs, all were detected except 6-acetylmorphine and heroin. In the case of 6-acetylmorphine it might be due to its poor stability in untreated wastewater, which might be reduced by acidifying the samples (Lin et al., 2021). Benzoylecgonine, cocaethylene, codeine, ecgonine methyl ester, morphine and THC-COOH were detected in all the samples. The highest concentrations corresponded to benzoylecgonine, ecgonine methyl ester (both are metabolites of cocaine) and THC-COOH (metabolite of cannabis). The highest values for the three compounds were measured in WWTP-1 in December 2020 (weekly mean values 4530, 690 and 680 ng/L, respectively).

The remaining illicit drugs were measured at low concentrations (mean concentrations from 30 to 140 ng/L) in all the WWTPs (Table 2), except amphetamine and methamphetamine, which were found in 4 and 6 of the treatment plants, respectively, and mephedrone and ketamine,

Table 2
Mean, median and range concentrations of the compounds found in wastewater and frequency of detection.

Analyte	Mean (ng/L)	Median (ng/L)	Min (ng/L)	Max (ng/L)	Number of cities (frequency %)
Amphetamine	56	46	25	139	4 (57)
6-Acetylmorphine	n.d.	n.d.	n.d.	n.d.	n.d.
Benzoylecgonine	1795	1475	577	5972	7 (100)
Cocaethylene	30	25	3	103	7 (100)
Codeine	143	101	23	567	7 (100)
Ecgonine methyl ester	466	427	161	1296	7 (100)
Heroin	n.d.	n.d.	n.d.	n.d.	n.d.
MDMA	41	25	6	227	7 (92)
Methamphetamine	44	14	2	191	6 (86)
Morphine	75	65	22	202	7 (100)
THC-COOH	400	360	100	753	7 (100)
Fentanyl	n.d.	n.d.	n.d.	n.d.	n.d.
Ketamine	31	31	14	57	2 (24)
Mephedrone	17	17	12	21	1 (4)
Alprazolam	n.d.	n.d.	n.d.	n.d.	n.d.
Diazepam	13	13	3	40	7 (100)
Lormetazepam	50	50	22	86	7 (100)
Lorazepam	111	102	55	248	7 (100)

Abbreviations: Min (minimum level found); max (maximum level found).

Table 1
Method validation. Recovery (%) and relative standard deviation (RSD%) for three replicates.

Analyte	Linear range (μg/L)[a]	Recovery (%)	RSD (%)	LOD (ng/L)	LOQ (ng/L)	IS used
Amphetamine	2–250	87	5	0.9	3	Amphetamine-d_5
6-Acetylmorphine	2–250	109	11	1.3	4.33	Amphetamine-d_5
Benzoylecgonine	2–500	96	1	0.1	0.35	Benzoylecgonine-d_3
Cocaethylene	2–500	90	4	0.32	1.07	Cocaethylene-d_3
Codeine	2–250	92	19	0.28	0.96	Morphine-d_6
Ecgonine methyl ester	2–500	103	3	0.39	1.31	Ecgonine methyl ester-d_3
Heroin	2–500	51	11	1.08	3.6	Benzoylecgonine -d_3
MDMA	2–500	98	6	0.36	1.21	MDMA-d_5
Methamphetamine	2–500	98	7	0.55	1.83	Methamphetamine -d_9
Morphine	2–500	97	4	0.12	0.41	Morphine-d_6
THC-COOH	2–500	97	5	26.4	88.1	THC-COOH-d_9
Fentanyl	2–250	44	2	0.3	1	Cocaethylene-d_3
Ketamine	2–100	74	11	0.53	1.76	Benzoylecgonine-d_3
Mephedrone	2–500	42	6	0.21	0.69	Benzoylecgonine-d_3
Alprazolam	2–500	102	5	1.54	5.14	Alprazolam-d_5
Diazepam	2–500	101	5	0.42	1.41	Diazepam-d_5
Lormetazepam	2–500	117	7	0.31	1.05	Lorazepam-d_4
Lorazepam	2–500	99	4	4.32	14.4	Lorazepam-d_4

[a] Linear range in the calibration curve injected (corresponding to 100 times less in the raw wastewater considering the pre-concentration factor applied along the sample procedure).

which were measured in a few samples (4 % of frequency of detection for mephedrone and 24 % frequency of detection for ketamine). Mephedrone was only detected in WWTP-5 in only two samples (mean value 17 ng/L), probably due to its low stability in untreated wastewater frozen at −20 °C (Lin et al., 2021). Ketamine was found in WWTP-5 and in WWTP-2 (mean values 27 and 35 ng/L, respectively). Mean, median and range of concentrations (ng/L) of the compounds measured in positive WW samples are shown in Table 2. Fentanyl, a potent opioid which can also be used as an analgesic and anaesthetic, was not detected in any sample. Thus, our monitoring suggests that the use of mephedrone and fentanyl in Spain is very low. This is quite in accordance with the concentrations found in other European countries. For instance, mephedrone was found in 5 out of 20 cities in the range 1.6–23 ng/L (Castiglioni et al., 2021) and fentanyl was recently found in Italy at very low concentrations (< 0.2 ng/L) in 27 % of the cities investigated (Salgueiro-González et al., 2022). These values are quite close to our LOQs and our methodology may not be able to determine them. All these studies were performed during a normal period, i.e. with no special events such as the COVID-19 pandemic.

With respect to benzodiazepines, two of them (lormetazepam and diazepam) were found in all the samples at the very low ng/L levels, while lorazepam, which was also present in all the samples, was found up to 248 ng/L. On the contrary, alprazolam was not detected in any of the cities. Except for alprazolam, our data is in accordance with the information about the most consumed benzodiazepines in Spain with medical prescription in the last years, being lorazepam the most prescribed anxiolytic active principle followed by alprazolam and diazepam ("Utilización de medicamentos ansiolíticos e hipnóticos en España," 2021). The absence of alprazolam might be explained because its excretion was mainly as metabolites (Greenblatt et al., 1993) or due to the parent compound transformation/degradation in the sewer system. The high frequency of detection of lormetazepam (100 % of the samples) might be explained because it is the most sedative hypnotic prescribed active principle in Spain. Some studies show that lorazepam is also a major metabolite of lormetazepam (Hümpel et al., 1979). This might explain the high concentration levels found for lorazepam in our analysis. Our values are very similar to those found in different cities of US (mean results: alprazolam < Method Detection Limit (MDL) (24 ng/L); diazepam < MDL (139 ng/L); lorazepam (248 ± 90 ng/L)) (Adhikari et al., 2023) and North West of Italy (mean results: lorazepam 72 ± 20 ng/L and lormetazepam 63 ± 30 ng/L) (Massano et al., 2023).

3.4. Spatial profiles

Normalized-mass loads (mg/day/1000 inh) of the different WWTPs were compared to assess spatial trends on use. Fig. 1 shows the average mass loads (in mg/day/1000 inh) for the compounds estimated in each treatment plant (for cocaine, cocethylene and lorazepam, average mass loads can be calculated from the daily mass loads shown in Fig. 2).

The highest consumption was, by far, for cannabis (calculated from the loads of THC-COOH) and cocaine (calculated from the loads of benzoylecgonine) in WWTP-1 (up to 46,178 and 6912 mg/day/1000 inh, respectively) followed by WWTP-5 (up to 29,910 and 2159 mg/day/1000 inh).

Heroin was not found in any of the samples whereas morphine, which is the most abundant metabolite of heroin and can be also used to estimate the consumption of codeine (Gracia-Lor et al., 2016), was found in 100 % of the samples, as well as codeine. 6-acetylmorphine, an exclusive metabolite of heroin (1–3 % of a dose, according to (Castiglioni et al., 2008)) was not detected. This might be due the low in-sample and in-sewer stability of this compound (it can revert quickly to morphine in the wastewater matrix, according to (McCall et al., 2016)) or due to the very low consumption of heroin in the populations investigated. Moreover, the consumption of codeine and morphine seems to be correlated (i.e. in the cites where more codeine was found, higher loads of morphine were obtained), so morphine mass loads probably reflect the therapeutic use of codeine and/or the therapeutic use of morphine itself, but not the contribution from heroin.

The consumption of amphetamine-type stimulants was quite different among the cities. MDMA was found in 92 % of the samples, but its consumption was especially higher in WWTP-5 (average mass loads 134.0 ± 80.0 mg/day/1000 inh). For the rest of the WWTPs, the average mass loads ranged from 17.0 ± 7.0 mg/day/1000 inh (WWTP-3) to 35.0 ± 14.0 mg/day/1000 inh (WWTP-4). Methamphetamine was quantified in up to 42 samples. The highest use was in WWTP-5 (85.5 ± 18.7 mg/day/1000 inh) and in WWTP-6 (37.8 ± 25.8), and the lowest in WWTP-

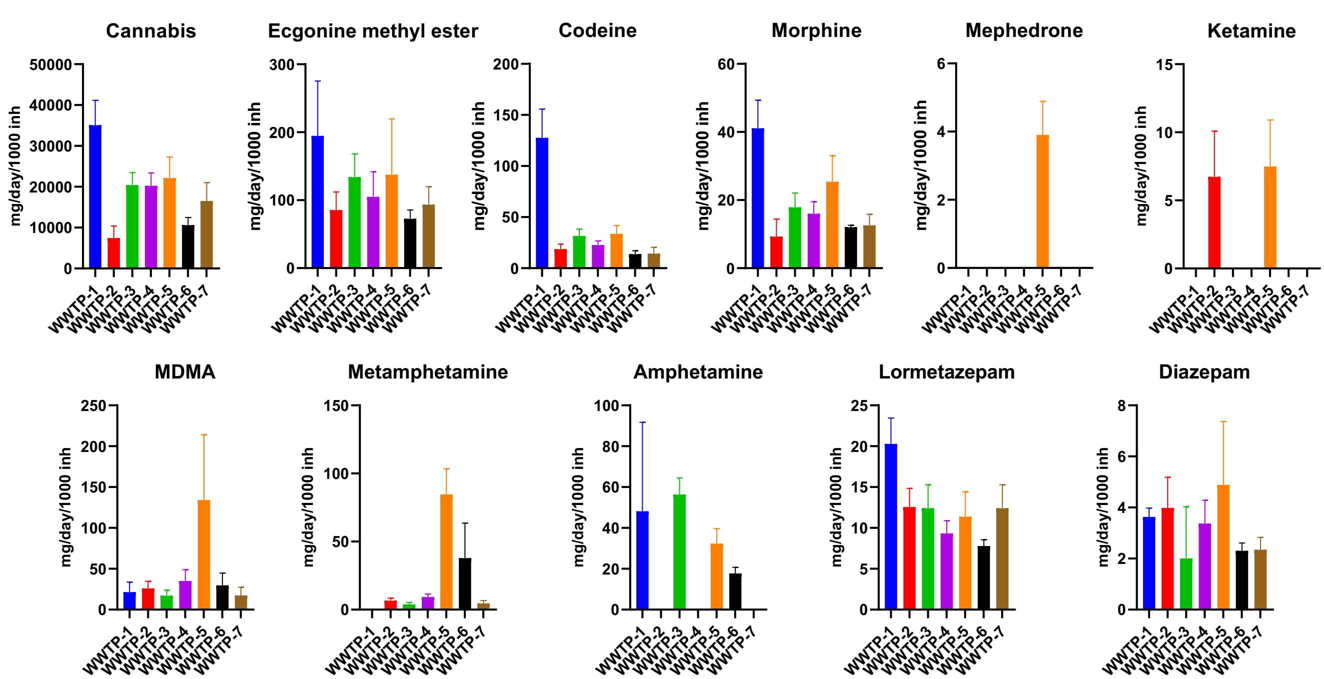

Fig. 1. Average estimates of consumption of the compounds found in seven WWTP in six Spanish cities investigated (WWTP-1 and WWTP-2 in December 2020; WWTP-3 and WWTP-4 in May 2021; WWTP-5, WWTP-6 and WWTP-7 in June 2021).

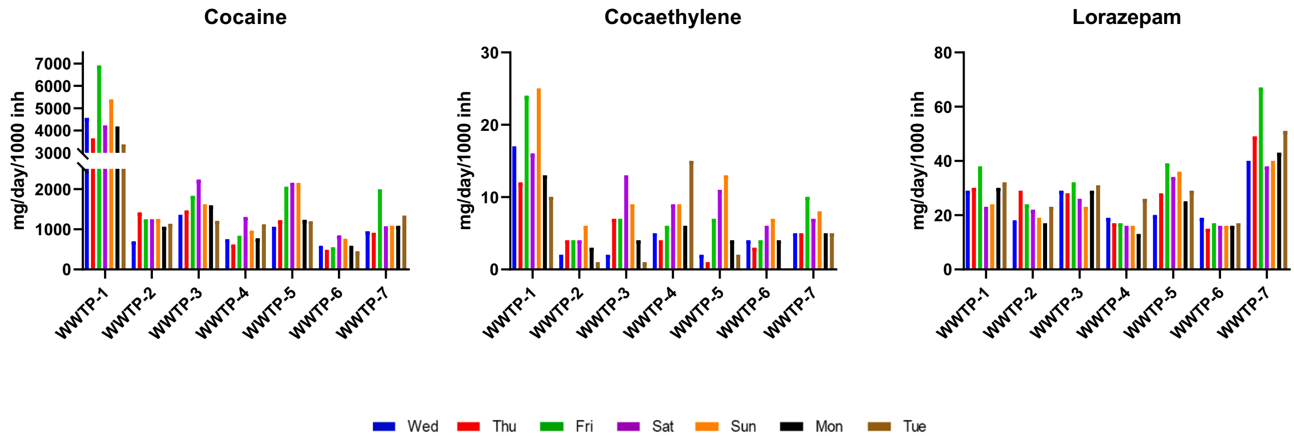

Fig. 2. Weekly profiles of use of cocaine, cocaethylene and lorazepam (WWTP-1 and WWTP-2 in December 2020; WWTP-3 and WWTP-4 in May 2021; WWTP-5, WWTP-6 and WWTP-7 in June 2021).

3 (3.9 ± 1.4 mg/day/1000 inh). Amphetamine was found in 4 of the 7 WWTPs. In this case, mass loads were higher in the small populations (i.e. WWTP-1 and WWTP-3) than in the biggest one (i.e. WWTP-5).

Mephedrone was only found in WWTP-5 in only two samples (corresponding to Sunday and Monday) at very low levels (< 4.6 mg/day/1000 inh). Ketamine tested positive in WWTP-2 in all the samples with loads from 3.2 to 12.9 mg/day/1000 inh and in WWTP-5 in the range 2.8–12.2 mg/day/1000 inh.

Cocaine and alcohol co-consumption was also investigated through the analysis of cocaethylene. The highest average mass loads corresponded to WWTP-1 (17 mg/day/1000 inh), while average values ranged from 4.4 to 8.1 mg/day/1000 inh in the other cities.

Regarding benzodiazepines consumption, the highest average loads were found for lorazepam, with the maximum values of 47.1 mg/day/1000 inh in WWTP-7 and the lowest in WWTP-6 (17.0 mg/day/1000 inh). Lormetazepam and diazepam were also detected but at lower levels, especially diazepam (2.0–4.9 mg/day/1000 inh). As commented in Section 3.3, the high consumption of lorazepam might be due to two different facts: because it is the most prescribed anxiolytic in Spain, and due to conversion of lormetazepam to lorazepam.

To check whether there were significant differences in the consumption among the populations investigated, we ran a multifactorial ANOVA analysis, which was conducted using the WWTPs and the day of the week as factors, and the amount consumed (mg/day/1000 inh) of each substance as response. *p*-Value < 0.05 indicated statistical significance. For all the compounds detected, there were significant differences among the WWTPs (see Fig. S3).

3.5. Weekly profile

Differences in the use of each substance in each WWTP along the week were evaluated by comparing daily normalized-mass loads. ANOVA analysis, which was conducted for each analyte considering all the mass loads (in mg/day/1000 inh) in the seven treatment plants, gave significant differences for the days of the week for cocaine ($p = 0.0153$), cocaethylene ($p = 0.0012$) and lorazepam ($p = 0.0072$), as reported in Fig. S3. Weekly profiles of cocaine, cocaethylene and lorazepam are shown in Fig. 2. Cocaine (estimated using benzoylecgonine as its biomarker of consumption) was more consumed during the weekends (from Friday to Sunday). This is in agreement with other investigations in Europe (Zuccato et al., 2016). The normalized-mass loads of ecgonine methyl ester also increased during the weekends in most of the populations investigated in this study. In the case of cocaethylene, mass loads notably increased during the weekend in all the WWTPs. Benzodiazepines are compounds which consumption is supposed to be constant over the week, although in the case of lorazepam the consumption was significantly higher on Fridays (except in WWTP-2, WWTP-4 and WWTP-6). For the remaining compounds, although interesting trends were observed, there were not significant differences in daily consumption ($p > 0.05$) (Fig. S3). Cannabis consumption was regularly consumed during the week, although in some cities, such as those corresponding to WWTP-5, WWTP-1 or WWTP-2, increased during the weekend (especially on Friday). In general, MDMA mass loads were significantly higher on Sundays, with a tail on Monday. This is in accordance with the trends observed in other studies (Zuccato et al., 2016) and is probably because of excretion of residues on Monday morning. Mephedrone was only found in two samples taken in WWTP-5 (Sunday and Monday) while ketamine was used along the week, but it increased during the weekends, especially on Friday. Their weekly profiles are depicted in Fig. S4.

Our data was also subjected to a PCA analysis to visually assess the associations between the consumption (in mg/day/1000 inh) and the day of the week. Fig. 3 shows the two-dimensional representation, which explained 70 % of the data set. Thus, cocaine, ecgonine methyl ester and cocaethylene were more consumed on Saturday; MDMA was more consumed on Sunday; the highest mass loads of amphetamine and methamphetamine were found on Monday; on Friday the consumption of THC-COOH, ketamine, morphine, diazepam and lorazepam was higher than during the rest of the week; lormetazepam and codeine consumption increased on Thursday. The higher consumption of illicit drugs during the weekend may be motivated for different reasons. For instance, in the case of MDMA, due to euphoric and socialising effects while cannabis is more consumed due to enhancement and social motives (it is taken for its calming and euphoric effects, specifically to reduce stress or to relax, to get high or for fun and to improve sleep)

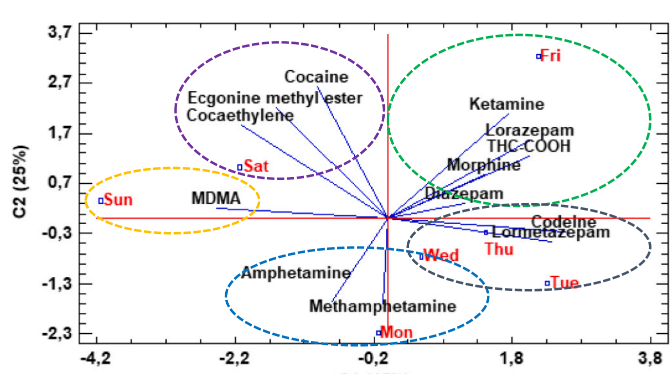

Fig. 3. Two dimensional PCA of the mass loads of each compound based on the day of the week.

(Buckner et al., 2019; "European Web Survey on Drugs 2021: top level findings, 21 EU countries and Switzerland (European Monitoring Centre for Drugs and Drug Addiction)," 2021).

3.6. Impact of COVID-19 on the consumption

From December 2020 to June 2021 the restrictive measures adopted during the COVID-19 pandemic were modified, as reported in Table S1.

As previously mentioned, the highest levels of consumption of cocaine, THC-COOH, ecgonine methyl ester, cocaethylene, morphine and codeine were in WWTP-1. However, on the exact same dates, the mass loads in WWTP-2 were much lower (see Figs. 1 and 2). Thus, it seems that the strict regulations to reduce SARS-CoV-2 infection during December 2020 had a different impact on drug consumption in each population.

For amphetamine-type compounds and for mephedrone and ketamine, in general the highest mass loads were found in WWTP-5, which were significantly higher than in WWTP-6 (also sampled in June 2021), as shown in Fig. 1. As both WWTPs were located at the same city, in two different districts, this suggests that the differences of consumption between people living in both districts were due the different habits of the population connected to each WWTP.

The weekly pattern of consumption of cocaine, MDMA and methamphetamine in most WWTPs (shown in Figs. 2 and S4) agreed with the local consumption in Italy in a study carried out before the pandemic (Zuccato et al., 2016) (i.e. cocaine and MDMA were more consumed during the weekend while methamphetamine was steadily consumed throughout the week). This is in accordance with studies of other countries during the post-lockdown period where a similar weekly trend has been observed (Oertel et al., 2023; Psichoudaki et al., 2023). So, it seems that the weekly patterns of these substances did not change during the pandemic.

Regarding the use of benzodiazepines, and according to official data, its consumption increased during the pandemic (Gili et al., 2021; Llorens et al., 2021; Mattiuzzi et al., 2022) and due to the "pandemic fatigue". Among the benzodiazepines investigated in this study, the highest loads of lormetazepam were found in WWTP-1, at the beginning of the pandemic. However, in the case of lorazepam, the highest consumption was after the end of the state of alarm (i.e. WWTP-7 and WWTP-5), which could be associated with the effects of the pandemic fatigue.

3.7. Temporal profiles

WWTP-3 was also monitored in December 2021 (from 29 December 2021 to 4 January 2022) as case study to evaluate changes in use patterns. We chose this WWTP because, although the consumption might be influenced by the special events (i.e. New Year's Eve), it is in a small city, with few commuters. Generally, patterns of consumption were similar in both periods, except for a few compounds whose use increased in December 2021 (Fig. 4). A multifactorial ANOVA was done using the day of the week and the month as factors, and the amount consumed (mg/day/1000 inh) of each compound as responses. Significant differences ($p < 0.05$) were observed for the month for codeine ($p = 0.0038$), morphine ($p = 0.0387$), diazepam ($p = 0.0014$), methamphetamine ($p = 0.0000$) and MDMA (p = 0.0038), as shown in Fig. S5.

MDMA consumption rose significantly over New Year's Eve as well as cocaethylene but for the last compound, no significant differences were seen. The higher use of codeine in December might be explained because it can be used to reduce coughing, which is more common during winter. Diazepam consumption was more constant over the week in December than in June. For methamphetamine, our data suggests a change in the pattern of used, as its consumption considerable increased.

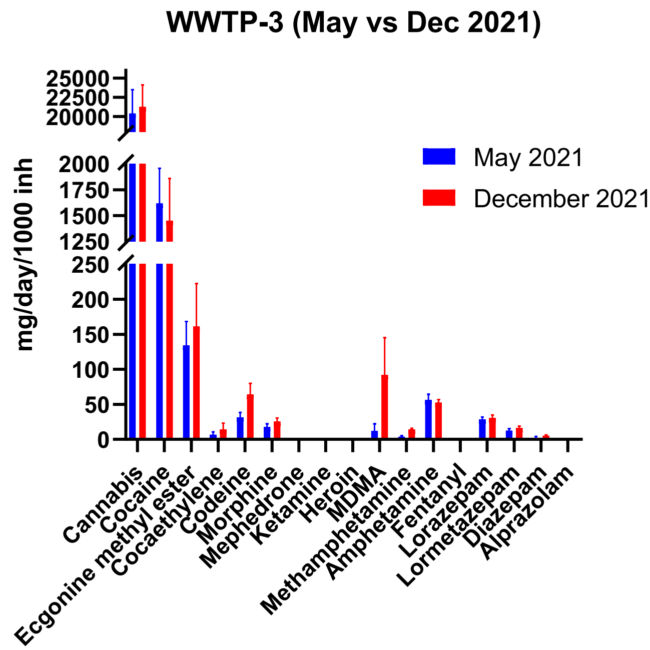

Fig. 4. Mean consumption in WWTP-3 in May and December 2021.

4. Conclusions

In this work, the use of the most consumed illicit drugs and benzodiazepines in six different cities in Spain was investigated during different periods of the COVID-19, from December 2020 when the restrictions were more severe, to June 2021, after the end of the state of alarm when the measures were relaxed. Although sampling campaigns were performed at different times, the results point to a non-homogenous effect of the pandemic restrictions on the use of the substances investigated. That is, when comparing the mass loads of different cities and regions or of two different districts of the same city during the same period, different patterns of consumption were observed. This might be due to a different impact of the pandemic restrictions applied in the populations investigated or to different population consumption habits. The highest loads were for benzoylecgonine, THC-COOH, ecgonine methyl ester, codeine and morphine in December 2020. For amphetamine-type compounds, the consumption was, in general, higher in June 2021 in the treatment plant which serves the biggest population investigated in this study (WWTP-5), as well as for mephedrone and ketamine, which were detected in only a few samples. Temporal consumption trends were also evaluated through the comparison of the normalized-loads mass in one of the cities investigated in two different periods (May and December 2021) and significant differences were observed only for methamphetamine, MDMA, codeine, morphine and diazepam. This study highlights the need for monitoring substance use during high-stress events, such as the COVID-19 pandemic, and complement data about the prevalence of consumption of illicit drugs and benzodiazepines. WBE can give objective information of the real consumption of these substances and could be applied to follow changes under normal circumstances and special events.

CRediT authorship contribution statement

Emma Gracia-Lor: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - Original draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **Azara Pérez-Valenciano:** Validation, Formal analysis, Writing - Review & Editing. **Paloma De Oro-Carretero:** Formal analysis, Writing - Review & Editing. **Lorena García-Ramírez:** Investigation, Validation, Formal Analysis. **Jon Sanz-Landaluze:** Conceptualization, Formal analysis, Resources, Writing - Review & Editing. **Mª. Justina Martín-Gutiérrez:** Conceptualization, Resources, Writing - Review & Editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.173356.

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