The embodiment of wastewater data for the estimation of illicit drug consumption in Spain

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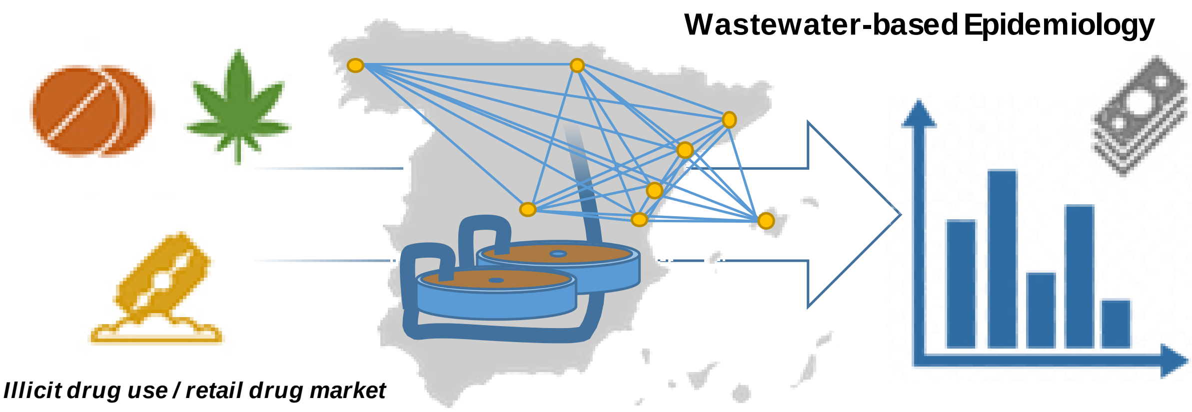
Highlights

- First national wastewater campaign to estimate illicit drug consumption in Spain
- Methamphetamine and MDMA positively correlated to population size
- NPS were only detected sporadically at low concentrations in wastewater
- Agreement between WBE consumption estimates and other indicators for several drugs
- Size of the retail drug market and contribution to gross domestic product estimated

39 **Graphical abstract**



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Abstract

Data obtained from wastewater analysis can provide rapid and complementary insights in illicit drug consumption at community level. Within Europe, Spain is an important country of transit of both cocaine and cannabis. The quantity of seized drugs and prevalence of their use rank Spain at the top of Europe. Hence, the implementation of a wastewater monitoring program at national level would help to get better understanding of spatial differences and trends in use of illicit drugs. In this study, a national wastewater campaign was performed for the first time to get more insight on the consumption of illicit drugs within Spain. The 13 Spanish cities monitored cover approximately 6 million inhabitants (12.8% of the Spanish population). Untreated wastewater samples were analyzed for urinary biomarkers of amphetamine, methamphetamine, MDMA, cocaine, and cannabis. In addition, weekend samples were monitored for 17 new psychoactive substances. Cannabis and cocaine are the most consumed drugs in Spain, but geographical variations showed, for instance, comparatively higher levels of methamphetamine in Barcelona and amphetamine in Bilbao , with about 1-fold higher consumption of these two substances in such metropolitan areas. For amphetamine, an enantiomeric profiling was performed in order to assure the results were due to consumption and not to illegal dumping of production residues. Furthermore, different correction factors for the excretion of cannabis were used to compare consumption estimations. All wastewater results were compared with previously reported data, national seizure data and general population survey data, were a reasonable agreement was found. Daily and yearly drug consumption were extrapolated to the entire Spanish population with due precautions because of the uncertainty associated. These data was further used to estimate the retail drug market, where for instance cocaine illicit consumption alone was calculated to contribute to 0.2-0.5% of the Spanish gross domestic product (ca. 3000-6000 million Euro/year).

Keywords: wastewater-based epidemiology; chiral analysis; drugs of abuse; national drug monitoring; addiction; Spain;

1 Introduction

Illicit drugs are a widespread problem, which does not only affect public health, but also threats security of regions and economic and social development (EMCDDA, 2019a; UNODC, 2018). Information on emerging drug production, distribution and consumption trends is pivotal for policy makers to design strategies and elaborate appropriate responses, both nationally and internationally. The compilation of comprehensive illicit drug consumption datasets requires the consultation of multiple sources of information (EMCDDA, 2019a; UNODC, 2018). One of them is the analysis of wastewater, which provides rapidly information on drug consumption patterns at community level. This methodology, also known as wastewater-based epidemiology (WBE), has been endorsed by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) since 2008, and by the United Nations Office on Drugs and Crime (UNODC) since 2016, incorporating the WBE results provided by the Sewage Analysis Core Group Europe network (SCORE, 2020) in their annual drug reports.

The SCORE monitoring performed in municipal wastewater has provided annually a one-week snapshot of drug volumes consumed in some European cities since 2011 (EMCDDA, 2020). Because results are reported in the same year that the samples are collected, they can also potentially provide an early warning signal of changes in drug consumption patterns (EMCDDA, 2019a). Furthermore, yearly monitoring has allowed evaluating spatial differences and temporal changes in illicit drugs use at international level (González-Mariño et al., 2020; Ort et al., 2014; Thomas et al., 2012). These international studies are logistically challenging, involve voluntary and financial goodwill of participants, and must comply with the high quality criteria standards set by the SCORE network to ensure that different results are reliable and comparable (van Nuijs et al., 2018). Hence, the studies are mostly limited to only a few cities of each participant country. Although the comparisons of wastewater data with other epidemiological indicators are generally in good agreement, uncertainty related to national consumption estimates tends to diminish when increasing the number of cities monitored. However, trends are rather defined by regional geography than by national boundaries (Been et al., 2016a). In any case, illicit drugs use has been assessed through wastewater analysis at regional and national level in Australia (Lai et al., 2013), Belgium (Van Nuijs et al., 2009), China (Du et al., 2015), France (Nefau et al., 2013), Finland (Kankaanpää et al., 2016), Germany and Switzerland (Been et al., 2016a), Italy (Zuccato et al., 2016), the Netherlands (Bijlsma et al., 2012), Poland (Klupczynska et al., 2016), Scandinavia (Löve et al., 2018), Slovakia (Mackulak et al., 2014), South Korea (Kim et al., 2015) and Sweden (Östman et al., 2014). Moreover, the national wastewater monitoring programs of Australia and New Zealand stand out for covering around 60% and 80% of their total populations, respectively ("Australian Criminal Intelligence Commision: National Wastewater Drug Monitoring Program reports," 2020, "New Zealand Police: National Wastewater Testing Programme Quarter 1 2019," 2020; O'Brien et al., 2019).

Although a wastewater monitoring program has not been launched in Spain yet, leading experts and Spanish SCORE members have created the ESAR-net network (ESAR-net, 2020) to promote WBE at national level (Bijlsma et al., 2018) and communicate their findings to authorities and policymakers. Within Europe, Spain is an important country of transit of both cocaine and cannabis due to its cultural, linguistic and colonial ties to Latin America and its proximity to Morocco (EMCDDA, 2019a; UNODC, 2010). In addition, Moroccan organized crime groups are becoming a more important player in cocaine trade making use of their established cannabis trafficking routes (EMCDDA, 2018). Hence, Spain ranks at the top of European countries in terms of quantity of seized cocaine and cannabis, and consumption prevalence (EMCDDA, 2019a). All these matters, as well as the increasing availability, purity and potency of cocaine and cannabis and of other stimulant drugs in general are of national and international concern.

In this work, a national wastewater campaign was performed for the first time in Spain to get more insight into the prevalence of drug use within its territory. Wastewater samples were collected from 13 Spanish cities, covering approximately 6 million inhabitants (12.8 % of the Spanish population). This study also contributes for the first time to shed light on the illicit drug consumption patterns in the Spanish capital

(Madrid), and other relevant cities in terms of population and geographical location (Palma de Mallorca and Bilbao). Untreated wastewater samples were analyzed for urinary biomarkers of amphetamine, methamphetamine, MDMA, cocaine, and cannabis. Population-normalized mass loads were back-calculated (*i.e.* converted) into the amount of drugs consumed by applying correction factors (CFs) for the excretion of each drug. Cannabis estimates were evaluated by using two different CFs, which are frequently proposed in the scientific literature. In addition to these traditional drugs, weekend samples were monitored for 17 new psychoactive substances (NPS). The selected NPS (*i.e.* phenethylamines and cathinones) have been previously reported and known to be used as adulterants or potential replacement of traditional drugs (Celma et al., 2019; Fontanals et al., 2017; Vidal Giné et al., 2014). Furthermore, enantiomeric profiling of amphetamine was performed in one of the cities, in which high concentrations of the drug were found in wastewater, in order to further check if the results were actually due to consumption or illegal dumping of unused drug or production waste. Finally, wastewater results were critically compared against previously reported data, seizure data and general population survey (GPS) data, and used to estimate daily and yearly consumption of the entire Spanish population and the contribution of the corresponding retail drug market.

2 Materials and methods

2.1 Sample collection

Untreated wastewater samples were collected from 13 Spanish cities (in total 17 wastewater treatment plants (WWTPs)) over 7 consecutive days in spring 2018 (March-June), avoiding any local or national festivity. In the specific case of Palma de Mallorca, two WWTPs were considered together, because a given percentage of the wastewater flow that entered the first WWTP was continuously diverted to the second WWTP. Daily 24-hour composite samples were taken using automatic sampler devices installed by the partners or operational at each WWTP. The flow rate (m^3/day) entering the WWTP each sampling day was recorded and used to calculate daily loads. In addition, pooled weekend samples of every WWTP were obtained by mixing at equal proportions the Friday, Saturday, and Sunday samples. All samples were collected, immediately transported to the laboratory, and stored in the dark at -20 °C until analysis. Important catchment characteristics and details on sampling procedures, such as the estimated population served by the WWTP and sampling mode, were gathered using a simplified Spanish version of the standardized questionnaire reported by Ort et al. (Ort et al., 2014). Where possible, water quality parameters *i.e.* concentrations of biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (N) and total phosphorus (P) as well as the pH were measured. In total, 136 wastewater samples were collected and analysed. **Table 1** gives an overview of the locations and key characteristics of the WWTPs included in this study. More details can be found in **Table S1** of the supporting information (SI).

2.2 Analysis

2.2.1 Target analytes

The parent illicit drugs - amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA) and cocaine (COC) - as well as the specific urinary metabolites of COC and cannabis - benzoylecgonine (BE) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), respectively - were determined in all wastewater samples collected. In addition, the following NPS were searched in all pooled weekend samples: butylone, dimethylone (bk-MDDMA), dimethylpentylone (bk-DMBDP), ketamine, methylenedioxypyrovalerone (MDPV), mephedrone, methedrone, methoxetamine, methylone, N-ethylcathinone, ρ-methoxymethamphetamine (PMMA), α-pyrrolidinopentiophenone (α-PVP), 3,4-dimethoxy-α-pyrrolidinopentiophenone (3,4-DiMeO-α-PVP), 4-chloro-α-pyrrolydinopropiophenone (4-chloro-α-PPP), 4-fluoromethcathinone (4-FMC), 4-methylethcathinone (4-MEC), and 4-methyl-α-pyrrolydinopropiophenone (4-MePPP). All illicit drugs and most NPS were quantified using their corresponding isotope-labelled internal standards applied as surrogate internal standard.

2.2.2 Analytical methodology

Wastewater samples were analyzed for the aforementioned target analytes using fully validated analytical methods. In general, sample treatment consisted of: (i) spike of the sample with internal standards, (ii) centrifugation or filtration (0.45 µm GFC), and (iii) on-line or off-line solid-phase extraction (SPE) using Oasis HLB, PLRP-s or MCX cartridges. Previous recommendations to improve the determination of the cannabis biomarker THC-COOH (i.e. avoiding acidification of the samples and considering the order of sample treatment steps) were taken into account for the analytical procedure (Causanilles et al., 2017).

The determination of illicit drugs was performed by the University of Valencia (UV), University Jaume I (UJI), IDAEA-CSIC, and the University of Santiago de Compostela (USC) using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with triple quadrupole or quadrupole-linear ion trap instruments, which is the most widely applied technique in this field (Hernandez et al., 2018). These four laboratories participate since 2011 in the multi-city study published by the EMCDDA (EMCDDA, 2020), where high quality and comparable data is ensured annually by the participation in inter-laboratory comparison exercises (van Nuijs et al., 2018). The determination of NPS, and the enantiomeric profiling of amphetamine, methamphetamine and MDMA were performed by UJI and USC, respectively. Internal quality controls (QCs) were prepared and analyzed in each sample batch to support the quality of analysis. Satisfactory recoveries of QCs were considered between 60 and 140% and reliable identification of positives needed to comply established deviations in ion intensity ratios (≤ 30%) and retention time (≤ 0.1 min) in comparison with the reference standard.

More details on chemicals and materials, sample treatment, target analytes, instrument operating conditions and method validation can be found in publications from the UV (Andrés-Costa et al., 2014), the UJI on illicit drugs (Bijlsma et al., 2014) and NPS (Celma et al., 2019), the IDAEA-CSIC (López-García et al., 2018), and the USC (González-Mariño et al., 2018). Furthermore, limits of detection (LOD) and limits of quantification (LOQ) of each target analyte by each method can be found in **Table S2**. More details on the enantiomeric analysis of AMP performed by USC can be found in the SI (**Text S1**).

2.3 Estimation of drug consumption through wastewater data: normalization and back-calculation

Daily illicit drug consumption by the population was assessed by measuring parent drugs or specific urinary metabolites in (untreated) municipal wastewater. Concentrations (ng/L) measured in 24-h composite samples were multiplied by their corresponding wastewater flow rates (m^3/day) in order to obtain daily mass loads (mg/day). Data were then normalized by dividing daily mass loads by the estimated number of inhabitants who contributed to the sample, within the catchment area, to allow the comparison between locations of different sizes. Finally, population-normalized mass loads (mg/day/1000 inhabitants) were back-calculated into the amount of drugs consumed by applying CFs for the excretion (and in-sewer) degradation of each drug. The latter, however, requires careful interpretation as variable excretion profiles contribute to the uncertainty associated to the back-calculation of drug use through wastewater analysis e.g. 26% for COC use (Castiglioni et al., 2013). The CFs applied in this work were based on extensive review studies performed by the research group of the Mario Negri Institute for Pharmacological Research of Milan (Italy) and were: 3.59 for COC (measured as BE) (Castiglioni et al., 2013), 2.77 for AMP, 2.44 for METH, 4.40 for MDMA, and 182 for cannabis (measured as THC-COOH) (Gracia-Lor et al., 2016). For cannabis, a CF of 36.4 originally proposed by Postigo et al. (Postigo et al., 2011) was also used in order to evaluate and critically discuss both cannabis estimates obtained. The difference between both CFs is that the factor proposed by Postigo et al. (2011) includes also the other major cannabis metabolite, 11-hydroxy-THC. Although this metabolite was not measured, the sum of both excretion percentages (i.e. 2% of 11-hydroxy-THC plus 0.5% of THC-COOH) was considered when applying this CF, by assuming that 11-hydroxy-THC completely oxidizes into THC-COOH during in-sewer transport. The use of both CFs, allows to report a range and account for the uncertainty associated with this aspect. No CFs were applied for NPS owing to the limited excretion data available.

Weighted average loads and consumption estimates were performed by weighting the values obtained for each WWTP according to the population served. Then national extrapolations were obtained from these by considering the total population served by the sampled WWTPs (5.98 Million inh.) to the overall Spanish population (46.66 Million inh. according to the Spanish census as on January 2018).

2.4 Correlation analysis

Correlation of population size and loads of the different biomarkers in wastewater was investigated by a Spearman-rank correlation test, after checking that data was not normally distributed (Saphiro-Wilk test), with the software Statgraphics Centurion 18.1 (Statgraphics Technologies, The Plains, VA, USA).

3 Results and Discussion

In order to perform data analysis, the concentrations below the method LOQ were assigned as the LOQ /2. In addition, a value of LOD/2 was used when a compound was not detected (*i.e.* concentration < LOD) in order to facilitate the statistical analysis and graphical representations. Note, that LODs were sufficiently low in order to not overestimate illicit drug consumption based on samples falling below the LOQ or non-detects. However, when several samples were found to be below the LODs, different approaches were used to provide average consumption estimates and account for the induced uncertainty (see 3.6.1).

Meta-data of all individual samples (i.e. concentrations of drugs, sampling date, wastewater flow data and water quality parameters), characteristics of the WWTPs and subsequent back-calculations, as described in section 2.3, can be found in the SI (**Table S1**). The estimation of the population size represents one of the largest uncertainties (7-55%) typically reported in WBE studies (Castiglioni et al., 2013). So, in this study we use all information available to try to lower the uncertainty (i.e. census data, number of houses connected to the sewage system and chemical parameters routinely determined in the WWTPs such as BOD, COD, N and P). The most reliable estimation, or a combination of estimates, was selected on a case-by-case basis, together with the expert judgment of the local WWTP operators, as in former SCORE campaigns (see **Table 1** and **Table S1** for population estimates selected for each WWTP).

3.1 Cocaine

Both, COC and BE, were quantified in all wastewater samples collected (**Table S1**). Subsequent back-calculations to COC consumption by using population-normalized BE loads are shown in **Figure 1**. COC use was high in all cities monitored, with average consumption estimates ranging from 1.1 g/day/1000 inh. in Castellón to 2.8 g/day/1000 inh. in Reus (*i.e.* BE loads ranged from 296.5 to 791.6 mg /day/1000 inh.).

These data position Spanish cities at the top of the COC consumer markets compared with other European cities also investigated through WBE (González-Mariño et al., 2020; Thomas et al., 2012).

Although COC use is high in big cities such as Barcelona and Valencia, a trend related to a higher COC use in larger urbanized cities, as reported in other countries (Been et al., 2016a; Kankaanpää et al., 2016; Ort et al., 2014; Van Nuijs et al., 2009), was not observed in the present study. Levels of consumption are likely to depend on factors such as type, main activities or life-style of a city (i.e. reparties' location), geography (ways of drug supply), socio-demographic aspects and wealth. In the absence of evidence on these factors, population size could be assessed as a proxy, since some of these factors may correlate with the size of urban cities. Historically, drug use has been conceptualized as an urban problem. The higher availability of certain illicit drugs may be one determinant of their greater use in large urban areas (Banta-Green et al., 2009; Galea et al., 2005; Irvine et al., 2011). In this study, no relationship between the size of urban city and cocaine use was observed (Spearman test p-value: 0.39)(**Table S3**). As an example, Santiago de Compostela, a city of approximately 136.500 inhabitants, showed population-normalized estimates similar to those of Madrid (1.3 and 1.4 g/day/1000 inh., respectively). Moreover, if the different Spanish regions are considered (represented as different colors in **Figure 1**), no clear differences between cocaine use and the regions are observed. Since the Spanish GPS carried out by the Spanish Observatory of Drugs and Drug addiction (Observatorio Español de las Drogas y las Adicciones, OEDA) does not provide regionally disaggregated data and not all regions publish their own data, an in depth comparison with such indicator cannot be performed.

The increasing trend in COC consumption observed for Barcelona between 2014 and 2017 (González-Mariño et al., 2020), which could be also related to increased purity (Observatorio Español de las Drogas y las Adicciones, 2019), seems to have been stabilized based on the 2018 and 2019 data released by the EMCDDA, with overall means of 717 mg/day/1000 inh. of BE loads in wastewater (EMCDDA, 2020).

Population-normalized BE loads of 2018 of all Spanish cities participating in the present study can be found in **Figure S1**.

3.2 Cannabis

Cannabis use, estimated from the analysis of THC-COOH in wastewater, is shown in **Figure 2**. The population-weighted average consumption of cannabis in the Spanish cities monitored, back-calculated from population-normalized THC-COOH loads, ranged from 4.1 to 20.6 g/day/1000 inh. taking into account the different CFs (**Table 2**). The overall population-weighted mean of THC-COOH loads in wastewater was 113 mg/day/1000 inh. (Figure S2), which is higher than the overall mean of European cities (80 mg/day/1000 inh.) participating in a study performed in 2013 (Ort et al., 2014). The highest measured per-capita loads were found in Barcelona (231 mg/day/1000 inh.) (**Figure S2**), which corresponded to cannabis use of 8.4 to 42 g/day/1000 inh. Data generated from wastewater related to cannabis consumption has always been controversial, due to the uncertainties that exist around the analytical measurements, stability in the sewage system, possible adsorption to particulate matter, and poorly understood excretion rates (Causanilles et al., 2017). However, if longitudinal monitoring occurs within the same catchment during similar weather conditions, and when analysis are performed by the same laboratory using the same validated methodology, the relative trends in use could be evaluated even without knowledge of the exact sorption to particles, the potential degradation or the average excretion rates, because these parameters are expected to remain relatively constant over time (Bijlsma et al., 2020a; Burgard et al., 2019). When comparing wastewater data from 2011, reported by IDAEA-CSIC for the same WWTP of Barcelona, population-normalized THC-COOH loads increased by approximately a factor of two (109 mg THC-COOH/day/1000 inh. reported in 2011 (Thomas et al., 2012) vs 231 mg THC-COOH /day/1000 inh in 2018). A similar comparison, 2011 vs. 2018 wastewater data, could be made for other cities participating in the annual monitoring coordinated by SCORE, like Santiago de Compostela (79 vs. 65 mg THC-COOH /day/1000 inh.), Castellón (100 vs. 64.2 mg THC-COOH /day/1000 inh.) and Valencia

(15 vs. 26.4 mg THC-COOH /day/1000 inh.). The increasing trend observed in big cities, such as Barcelona or Valencia, was not observed in smaller cities, such as Castellón or Santiago de Compostela. Although variation of THC-COOH loads in wastewater was observed between 2011 - 2013 (Ort et al., 2014), the increase in cannabis consumption in Barcelona is notable. Nevertheless, as in the case with COC, the Spearman rank correlation analysis did not show a significant correlation between the size of the city and cannabis consumption (p-value: 0.22).

Spanish GPS from 2017 is disaggregated in the case of cannabis. Average prevalence data of cannabis consumption during the last-30 days by the Spanish population between 15 and 64 years was 9.1% in 2017, with high prevalence values reported for the autonomous regions Catalonia, Valencian Community, Balearic Islands and the Community of Madrid (11.9, 11.0, 10.5 and 10.1%, respectively), followed by the Basque Country (9.1%), Galicia (7.0%) and Castile-La Mancha (6.7%)(Observatorio Español de las Drogas y las Adicciones, 2019). Comparing these data in **Figure 2**, no clear match between WBE-derived values and official GPS values is found. Although Barcelona (the capital of Catalonia) and Palma de Mallorca (capital of Balearic Islands), also present high consumption values as derived from WBE, and similarly low WBE-derived consumption is detected in Santiago de Compostela (Galicia) and the two towns from Castile-La Mancha (Guadalajara and Toledo), the two cities of the Valencian Community (Valencia and Castellón) showed a WBE-derived consumption much lower than what would be expected from the GPS data. These findings may point towards a relevant localized effect rather than regional.

3.3 Amphetamine and methamphetamine

3.3.1 Loads and consumption estimates

Population-normalized mass loads of AMP and METH are reported in the SI (**Table S1 and Figures S3**) while **Figure 3** illustrates the back-calculated consumption of AMP and METH in the Spanish cities monitored. In several wastewater samples collected from Castellón, Guadalajara, Lleida, Madrid-2, Santiago de Compostela and Toledo AMP and/or METH could not be quantified (**Table S1**). This might be related to the slightly higher LOQs of the analytical methods when applied to these samples (**Table S2**) and/or to a lower consumption in these cities. In any case, it seems that the consumption of AMP and METH is not largely extended in most of the cities monitored. However, the consumption of AMP in Bilbao stands out and seemed extremely high (consumption of 693 mg/day/1000 inh.; weekly population-normalized mean load of 250 mg/day/ 1000 inh.) compared to the rest of cities included in this work, which were comparable to mass loads found in some Belgian and Dutch cities (González-Mariño et al., 2020). Matching these figures to Spanish GPS data (from 2017), the last-year and last-30 days prevalence of AMP were 0.5 and 0.2%, respectively (Observatorio Español de las Drogas y las Adicciones, 2019). The Basque Country, of which the metropolitan area of Bilbao represents almost half of its population, also published GPS data, where AMP last-year and last-30 days prevalence was 1.0 and 0.4%, *i.e.* twice the Spanish average (Gobierno Vasco, 2018). Even with the drug checking services data (**Table S7**) indicating that AMP purity was ca. 25% higher in the Basque Country (Ailaket, 2020) than that of the other regions of Spain (EnergyControl, 2020), WBE figures may still look high. Given that the high loads measured in Eindhoven (the Netherlands) were ascribed to direct disposal of production waste (González-Mariño et al., 2020) additional enantiomeric analyses were performed to determine whether these high values were due to illicit and licit (i.e. prescribed) use, or direct dumping of the unused drug into the sewer network (this issue is further discussed in Section 3.3.2).

High consumption of both AMP and METH was observed in Barcelona with 97.5 and 116.8 mg/day/1000 inh., respectively. Spanish GPS last-year prevalence of METH in 2017 was 0.2% (Observatorio Español de las Drogas y las Adicciones, 2019), whereas regional data of Catalonia from the same year estimated its last-year prevalence on 0.4% (Generalitat de Catalunya, 2019). However, looking at **Figure 3**, it looks that this is a rather localized phenomenon in the area of Barcelona, since WBE-derived consumption estimates of other cities from the same region (**Table 1** and **Figure 3** yellow boxplots) are closer to most of the Spanish cities. Compared to Barcelona, the consumption of these substances was approximately 4 times lower in Madrid, the capital of Spain, where ca. 1 million inh., corresponding to 30% of the total city population, were monitored (similar population as covered by the WWTP of Barcelona). However, it is important to mention that differences in consumption were observed within the city of Madrid itself, with higher AMP and METH WBE-derived consumption values found in Madrid-1. This could be related to the fact that the catchment area of Madrid-1 covers the city Centre, whereas Madrid-2 receives urban wastewater from districts located in the North of the city (**Table S1**). Hence, information on the demographics of the WWTP watershed is essential for data interpretation. The Spearman rank correlation test showed that AMP consumption is not related to the size of the city (p-value: 0.13); however, a positive correlation was observed for METH (p-value: 0.021), as shown in **Figure S4 (top)** and **Table S3**.

The international wastewater monitoring campaigns yearly performed by SCORE report the highest mass loads of AMP in cities from central and northern Europe, whereas the highest METH loads are found mostly in eastern countries (EMCDDA, 2020). Weekly population-weighted mean loads reported by the Spanish cities herein monitored in 2018 for AMP (51.2 mg/day/1000 inh.; 17.5 mg/day/1000 inh. when excluding Bilbao and its metropolitan area) and METH (12.0 mg/day/1000 inh.; 3.42 mg/day/1000 inh. when excluding Barcelona) were lower than those reported by the European cities that provided data in the same year (52.1 mg/day/1000 inh. and 30.2 mg/day/1000 inh., for AMP and METH respectively), when these two specific areas (Bilbao for AMP, and Barcelona for METH) are not considered. An increasing trend of AMP loads in wastewater was observed within 2011-2016 in Barcelona, but in contrast to most European cities, AMP consumption in Barcelona seems to be stabilized or even slightly decreased based on the data reported in most recent years (EMCDDA, 2020). The opposite, however, occurs with METH use in Barcelona, which has increased considerably since 2016 from an overall mean of 24.1 mg/day/1000 inh. to up to 106.8 mg/day/1000 inh. of METH loads in 2019 (EMCDDA, 2020). The latter corresponds to

260.6 mg/day/1000 inh. of consumed METH. This trend is not observed in other Spanish cities, but 17 out of the 42 European cities monitored in 2018 and 2019 reported an increase of METH use. However, in contrast to COC, AMP and MDMA, METH concentrations detected were from very low to below the LOD in most Spanish locations.

According to the EMCDDA, AMP is more commonly used than METH in most EU countries, although there are indicators that signaled that METH production and use are spreading (EMCDDA, 2019a). This is in good agreement with the results of our study, which show that AMP has been found at higher concentrations compared to METH in most of the cities monitored, except for Barcelona and Madrid. Relatively high METH use is known in Barcelona, where consumption is related to "chemsex", which has been declared a public health problem by Barcelona authorities. In 2016, 193 specific cases of the problematic use of drugs (including mephedrone, ketamine and GHB) for sexual purposes were treated in Barcelona (Mouzo Quintáns, 2017).

3.3.2 Enantiomeric analysis of amphetamine

Complementary enantiomeric analysis helps in differentiating whether a drug residue present in wastewater results from its actual consumption or direct disposal of unused drug or production waste in the sewer network (Emke et al., 2014; Kasprzyk-Hordern and Baker, 2012a, 2012b). This is possible for chiral drugs such as AMP, which is commonly synthesized as a racemic mixture of R(-)-AMP and S(+)-AMP via the Leuckart method (King, 2009). However, the pathway and rate of metabolism of AMP is different as the S-enantiomer is much more active and, therefore, metabolizes faster (Cody and Schwarzhoff, 1993). This, consequently, results in a change of the enantiomeric ratio towards the enrichment of R(-)-amphetamine. In this work, additional enantiomeric analyses were performed for the wastewater samples collected from Bilbao to determine the origin of the exceptionally high loads of AMP found in wastewater, *i.e.* mean load 250 mg/day/ 1000 inh. (Figure S3).

The enantiomeric fraction (EF) of AMP, expressed as the ratio of R(-)-AMP with respect to the sum of the two enantiomers EF_R, was 0.53 ± 0.03 (mean of the whole week ± standard deviation). An example chromatogram is presented in **Figure S5**. This value implies a slight prevalence of the inactive R(-)-AMP with respect to the more active S(+)-AMP, which would indicate a prevalence of illicit consumption as being similar to what is found in most European countries (Kasprzyk-Hordern and Baker, 2012b). However, AMP can also be excreted as a result of metabolism of prescription drugs to treat attention deficit hyperactive disorder (ADHD), narcolepsy and even weight loss (Cody, 2002). In Spain, the only licit prescribed source of AMP is lisdexamphetamine (CIMA, 2020). Yet, the contribution owing to the use of this medicine was considered negligible, as being enantiomerically pure, it is excreted only as dexamphetamine *i.e.* S(+)-AMP, after hydrolysis in the human body (Pennick, 2013). Thus, more prevalence to the S-enantiomer would have been expected in that case.

Although a direct disposal of racemic AMP in the sewer system cannot be discarded (i.e. the EF_R value is not statistically different from 0.5), it seems unlikely since the mass loads of AMP were high during the whole week (**Table S1**) and the EF_R value obtained was similar to that generally found in wastewater across Europe (Castrignanò et al., 2018). A direct dumping of unused chiral drug or drug manufacturing waste, normally coincides with a high peak in mass loads, as it was observed for instance in the Netherlands for MDMA (Emke et al., 2014), but not in our study. Further research with a higher number of sampling campaigns is however recommended.

3.4 MDMA

MDMA consumption in Spain, back-calculated from population-normalized MDMA loads, is shown in **Figure 4**. Average consumption estimates ranged from 10.6 mg/day/1000 inh. for Castellón to 205.8 mg/day/1000 inh. for Barcelona with an overall population-weighted average consumption estimate of 90.7 mg/day/ 1000 inh. (**Table 2**) in all Spanish cities monitored (resulting from an average MDMA load of

20.6 mg/day/ 1000 inh. in wastewater) (**Figure S6**). The highest values were observed in Barcelona, Palma de Mallorca and Madrid, specifically in WWTP Madrid-1 which covers the city Centre (**Table S1**). This is in agreement with other countries where occurrence of MDMA seems to be predominant in larger urban areas (Been et al., 2016a; Lai et al., 2013; Mackulak et al., 2014; Nefau et al., 2013). Furthermore, higher MDMA use was also associated to vacation areas (Lai et al., 2013), and Palma de Mallorca has a long-standing tourist tradition reflected in the wide range of nightlife settings, facilities and services opened since spring every year. As is the case with METH, a positive correlation (**Figure S4 (bottom), Table S3**) was observed between the size of the city and MDMA consumption (Spearman rank correlation test p-value: 0.026).

In general, Spain can be considered a low-MDMA-usage country, but wastewater data reported to EMCDDA indicates an increasing trend in MDMA use over the years for the four cities participating in SCORE *i.e.,* Barcelona, Castellón, Santiago de Compostela, and Valencia. This may be related also to an increase in purity of MDMA in the last years (i.e. MDMA purity raised in the period 2011-2018 from 71 to 81% in crystal MDMA, and MDMA content in pills from 93 to 180 mg in the same period, data from Energy Control drug checking service (EnergyControl, 2020)). Nevertheless, the overall weekly population-normalized loads of MDMA of the Spanish cities monitored in this study (20.6 mg MDMA /day/1000 inh.) (**Figure S6**) are still below the average population-normalized loads reported by the European cities in 2018 (29.8 mg MDMA /day/1000 inh.) (EMCDDA, 2020). Yet, in higher-prevalence cities, such as Barcelona, the increase may reflect that MDMA is no longer a niche or subcultural drug limited to dance clubs and parties, but now may also being used by a broad range of young people in mainstream nightlife settings (EMCDDA, 2019a).

3.5 New Psychoactive substances

Analysis of wastewaters collected in this study throughout Spanish cities revealed the presence of only few NPS (**Table S4**). However, these NPS could not be quantified, since they were observed below the LOD of the methodology applied, except for ketamine (19.1 – 79 ng/L, **Table S1**). Although it might be disputable if ketamine can still be considered as an NPS, it is included here as it was determined by the same method applied to NPS and in weekend samples only. This substance was detected in 7 out of the 13 cities, and generally in the larger cities investigated. Ketamine was also found at relative high per-capita loads in wastewater samples collected during a festival celebrated in Spain (Bijlsma et al., 2020b) and is raising concerns by EU member states because of the apparent growing in importance in the drug market (EMCDDA, 2019a). Ketamine was also often detected in 2018 by the drug checking service of the Spanish Association "Energy Control" *i.e.* 142 times, followed by 3-methylmethcathinone (15 times) and mephedrone (9 times) (data not published). Furthermore, mephedrone was detected in Barcelona and Madrid, and dipentylone and methedrone were found in samples taken from Valencia and Móstoles (a suburb of Madrid), respectively.

NPS have a much lower consumption prevalence than the traditional illicit drugs (Observatorio Español de las Drogas y las Adicciones, 2019). The majority of the Spanish population (73.8%) has never heard of these type of substances and, from survey data, the prevalence of NPS use (between 15 and 64 years) is 1.1%, where 0.5% admitted to have consumed ketamine, 0.4% synthetic cannabinoids, 0.2% salvia, and 0.1% mephedrone once during their life (Observatorio Español de las Drogas y las Adicciones, 2019). Synthetic cannabinoids and salvia were not monitored in wastewater in this study, but consumption of ketamine and mephedrone was reflected by the wastewater data. Dipentylone and methedrone were also detected in a few wastewater samples, despite that these substances have not been reported by the Spanish National Focal Point (Observatorio Español de las Drogas y las Adicciones, 2019). Although more data would be required to support the possible consumption of these substances, their detection in some samples illustrates the potential screening capability of wastewater analysis, and the possibility to use WBE as an early warning system for NPS monitoring.

3.6 National consumption estimates based on wastewater data

National Spanish drug consumption was estimated by the extrapolation of wastewater data from the 13 cities monitored*,* equivalent to 12.8% of the Spanish population. Although (i) population size estimates, (ii) the short period of time investigated (one week), (iii) estimation of excretion rates used for back-calculations, and (iv) potential degradation of targeted biomarkers in sewers might further affect the accuracy of the WBE results (Been et al., 2016a; Castiglioni et al., 2013; McCall et al., 2016), the ESAR-net network has recently shown that wastewater data can lead to a good estimate of nicotine consumption when compared to tobacco sales records (Montes et al., 2020). Consequently, WBE data discussed below should be taken as a rough estimation, but useful orientation of illicit drug consumption by Spanish inhabitants.

3.6.1 Daily and annual consumption estimates

Mean population-normalized daily consumption estimated (mg/day/1000 inh.) measured in wastewater from the cities monitored (**Table 2**) were extrapolated to daily (kg/day) and annual (ton/year) consumption estimates of the entire Spanish population (**Table 3**). This extrapolation must be interpreted with care due to the uncertainties associated to this process, as only 13 cities, representing around 13% of the Spanish population, have been included in our study. Furthermore, AMP, METH and MDMA, could not be quantified in all wastewater samples analyzed, which makes the consumption estimation more complicated. Therefore, two different scenarios were considered for these compounds to better address uncertainty in national estimates: 1) under-estimative scenario, in which data below the LOD were replaced by zero, and data falling between the LOD and LOQ were replaced by the LOD, biasing the results low; 2) over-estimative scenario, in which data below the LOD were replaced by the LOD, and data between the LOD and the LOQ were replaced by the LOQ, biasing the results high. Moreover, as previously discussed, national cannabis consumption was estimated by applying two different CFs, where the application of CF 36.4 could be considered as under-estimative scenario and CF 182 as over-estimative scenario.

Consumption estimates of AMP in Spain (142 mg/day/1000 inh.; resulting in 7 kg/day; 2.61-2.96 ton/year once the two scenarios are applied, **Table 2**) were strongly influenced by the high levels found in the metropolitan area of Bilbao. Accordingly, by excluding these data, daily and annual consumption at national level would result in much lower estimates (49 mg/day/1000 inh.; 2.3 kg/day; 0.83 ton/year). This points to the fact that more research and further monitoring of AMP in wastewater from the same or from other Spanish cities, would be required to confirm these values. Furthermore, the under-estimative and over-estimative scenarios resulted in wider ranges for AMP, METH and MDMA (**Table 2 and Table 3**), especially for METH, for which annual consumption estimates varied between 0.50 and 0.80 ton/year, since this substance was below LOQ in more samples. Since BE and THC-COOH were quantified in all wastewater samples collected, the two scenarios considered had no effect on the estimations of COC and cannabis consumption. However, different excretion CFs, 36.4 and 182, were applied to cannabis, which resulted in an average population-normalized daily consumption of 4113 and 20567 mg/day/1000 inh, respectively, and annual consumption estimates of 69.6 and 350 ton/year, respectively.

Table 4 shows national consumption estimates derived from the data obtained in the 13 cities included in this study compared with estimates considering only the 4 cities that yearly participate in SCORE and report data to the EMCDDA (more details can be found in **Tables 2, 3**, **S5 and S6**). This comparison provides a better picture on the representativeness of the data of the four cities yearly reported. **Table 4** shows that national estimates of drugs with lower prevalence, such as AMP and METH, are strongly affected by high concentrations found in the samples from Bilbao (all cities) and from Barcelona (only SCORE), respectively. However, the estimates for COC and cannabis did coincide very well, indicating that estimates based on only few cities can be representative enough for high prevalent drugs. Obviously, it would be advisable to monitor more cities as uncertainty in national estimations tends to diminish taking into account spatial differences (Kankaanpää et al., 2016; Nefau et al., 2013; Van Nuijs et al., 2009). More intensive sampling (> 1 week) is also recommended to characterize the temporal variations more clearly (González-Mariño et al., 2020; Lai et al., 2013; Thomas et al., 2012).

3.6.2 Retail drug market estimates

By combining the national consumption estimates derived from wastewater data with purity and price data compiled in **Table S7**, the amount of mixed commercial ("cut") substance consumed and its value in the illicit market could be estimated (**Table 3**). Hence, in the case of COC, the estimation made in the previous section (36 ton/year) would translate in-between 53.6 and 102 tons of cut drug /year (using the purity ranges reported by OEDA (Observatorio Español de las Drogas y las Adicciones, 2019), see also **Table S7**). In relation to AMP and METH, the estimated amount of cut drug would range between 5.0 and 7.0 ton/year and between 0.78 and 1.2 ton/year, respectively. Regarding AMP, the average purities reported by the drug checking systems of Energy Control and Ai Laket!! were considered (Ailaket, 2020; EnergyControl, 2020). Although Energy Control analyzed by far the largest set of samples, this organization receives samples mainly from the areas of Madrid and the Mediterranean coast. The relevance of the metropolitan area of Bilbao, an area of the Basque Country, on AMP consumption in Spain was highlighted before, and therefore the purity data reported by Ai Laket!! was of interest since they are based in this region. MDMA was estimated as either being marketed only as tablets or only as crystal, resulting into 8.6 – 8.9 millions of tablets/year or 1.9 – 2.0 tons of crystal MDMA/year. Of course, the actual situation is a distribution between both formats, but this cannot be calculated since GPS did not account for MDMA format preferences.

Both national consumption and drug market estimates obtained in the present study are larger than those previously reported in a study conducted in Spain in 2007-2008 covering 7 WWTPs (representing approximately 3% of the total Spanish population) and using different CFs (coming from the by then available excretion rates), and purity and price values. The only exception to this trend is the national consumption estimate of amphetamine, which in that case was higher (8.8 kg/day and 3.2 tons per year) (Postigo et al., 2010).

By contrasting the data on annual consumed product with seizures (**Table S7**), it seems that the Spanish internal demand of COC is within the same order of magnitude than the amount seized, whereas the summed demand of AMP and METH (since seizure data aggregates both stimulants) would be 21-30 times higher than the amount seized. Similarly, the OEDA reported 300,571 tablets of MDMA seized in 2018 (Observatorio Español de las Drogas y las Adicciones, 2019). Assuming that all MDMA would be sold as tablets, the internal demand of MDMA is approximately 30 times higher than the amount seized. These findings are pointing that the relative amount of COC seized is higher than that of AMP, METH or MDMA. The number of drugs seized in Spain varies greatly from year to year (Observatorio Español de las Drogas y las Adicciones, 2019). This generally reflects police and customs activity rather than the amount of drugs consumed. These results are biased especially when the control is focused on some special type of drugs, which can make others become overlooked. It should be borne in mind that Spain is one of the main entrance routes of COC and cannabis in Europe, which is not the case for the other three drugs (EMCDDA, 2019b).

Annual WBE-derived consumption can also be translated into an estimation of the Spanish retail illicit market (**Table 3**) based on the prices published by the OEDA (**Table S7**)(Observatorio Español de las Drogas y las Adicciones, 2019). Based on the data compiled in this work, a retail market size of approximately 3200 - 6000 million € for COC, 102 - 145 million € for AMP, 16 - 26 million € for METH, and 88 - 92 million € for MDMA was estimated. The latter was estimated assuming that all MDMA would be sold as tablets, since OEDA provides no price data for crystal MDMA. The Spanish gross domestic product (GDP) in 2018 was 1,202,193 Million € (INE, 2020). Thus, the illicit market of COC consumed in Spain would represent about 0.2 - 0.5% of the GDP, which is in the range reported by the EMCDDA in Europe (drug illicit market ≥0.4% in half of the EU countries). As a benchmark, the sales of tobacco in 2018 represented 11,753 million € (ca. 1% of the National GDP) according to the Spanish Tobacco Market Commission (Hacienda, 2020).

In the case of cannabis, estimations are far more uncertain as previously commented (Causanilles et al., 2017). That said, calculations were performed (**Table 3**) contemplating the different CFs for excretion, and consumption being either 100% herbal vs 100% resin cannabis (again the situation is somewhere in between), in order to account for such uncertainty. Annual consumption estimations as either herb or resin are 3465 and 1891 ton/year, respectively, when applying a CF of 182, whereas a CF of 36.4 leads to approximately 5-times lower values, *i.e.* 689 - 376 tons (herb or resin)/year. By contrasting these estimates to seizures (**Table S7**) the internal demand is 5.1 to 9.4 (CF 182), or 1 to 1.9 (CF 36.4), times higher than the amount of drug seized. As for COC, Spain is on one of the main trafficking routes of cannabis entering Europe (EMCDDA, 2019b), so the internal demand to seized amount ratio is expected to be lower than in the case of AMP, METH and MDMA. In economic terms, the retail market size of cannabis is estimated to be in between 10,570 - 18,087 million € (CF 182) or 2101-3596 million € (CF 36.4). The latter figures may be closer to reality given the fact that EMCDDA estimated cannabis and COC markets contribute in a similar amount to the EU illicit retail market (39% vs. 31%, respectively) (EMCDDA, 2019b) and that analytical uncertainty of cannabis is generally associated to negative bias (Causanilles et al., 2017).

Several authors have tried to derive the amount of cannabis consumed, i.e., its main psychoactive component tetrahydrocannabinol (THC), into a hand-rolled joint. For instance, Casajuana-Kögel et al. (Casajuana Kögel et al., 2017) deduced from the analysis of 315 joints provided by volunteers in Barcelona in 2015-2016, that each joint of cannabis (independently whether it was prepared from herb or resin) would contain an average of 7 mg of THC. Thus, from the average amount of cannabis daily consumed in

Spain (**Tables 2 - 3**), a consumption of 2.9 joints/day/inhabitant and 0.58 joints/day/inhabitant would be estimated using the CFs of 182 and 36.4, respectively. The latest Spanish GPS data from 2017, (Observatorio Español de las Drogas y las Adicciones, 2019, 2017) estimated an average consumption of 2.7-3.1 joints/day among the consumers (note: not per Spanish inhabitant). The data obtained from WBE seems to overestimate, when combined to GPS daily consumers' prevalence of 2.1% and 9.1% during the last month, or even when using the data related to the younger population (14-18 year, 19.3% last-month prevalence, which would correspond to an average of 3.4 of joints/day/consumer) (Observatorio Español de las Drogas y las Adicciones, 2018).

Yet, other values of standard joint content have been proposed in the literature (Freeman and Lorenzetti, 2020). Moreover, Casajuana-Kögel et al. (Casajuana Kögel et al., 2017) also reported that the standard joint would be equivalent to 0.25 g of cannabis. That would lead to a 2.8% THC potency, which seems to be far lower than the potency compiled in **Table 3** of 10.1 - 18-1%. If the 0.25 g/joint would be combined with such higher THC content, then the calculated amount of joints would decrease down to the range of 0.45-0.74 joints/day/inhabitant (CF 182) or 0.090-0.16 joints/day/inhabitant (CF 36.2), which would agree better with WBE data, particularly when using the second CF value. This clearly demonstrates that interpretation of data from different sources is complicated and should be performed with care, it also illustrates that further research is needed to improve the estimation of cannabis consumption through WBE, and that the CF applied probably plays a relevant role as suggested by Burgard et al. (Burgard et al., 2019). From the back-calculation performed in this study, a CF of 182 seems to overestimate cannabis consumption. Stability is still difficult to interpret because there are few studies and results are not entirely consistent. Furthermore, biliary excretion of THC and its metabolites is relevant and, thus, faeces are an important route of elimination of cannabinoids conjugates. Several studies (Been et al., 2016b; Bijlsma et al., 2020a; Khan and Nicell, 2012), estimated the amount of THC and its metabolites excreted in faeces based on a study by Wall et al. (Wall et al., 1983) and established that between 3.1 and 5% of the cannabis smoked is excreted this way. More recently, Fabritius et al. (Fabritius et al., 2012) provided data on the biliary and urinary excretion of cannabis: making a very rough back-calculation and using the median values, they estimated an elimination rate by feces of 3%, very similar to the value previously reported.

4 Conclusions

WBE is herein demonstrated as an invaluable tool for the near real-time assessment of trends and spatial variations in illicit drug consumption. Cannabis and COC are the most consumed drugs in Spain, while the use of AMP, METH, MDMA, and especially NPS is less common. Our results show that, especially in the case of COC, its consumption in Spain is not clearly related to city and population size, unlike in other countries. On the contrary, its consumption is quite homogeneous in the whole territory. This could be explained, because Spain is a country where COC is brought in and, thus, there is a more homogeneous availability of this drug within the country. An advantage of this type of analysis is that it allows for clear geographical variations such as the high concentration of METH in Barcelona, which corroborates already known data, or the high amount of AMP detected in Bilbao, which could not be satisfactorily explained yet. Another advantage of WBE is the ability to follow the changes in drug of abuse consumption rates by using a continuous sampling strategy.

This is the first study performed in Spain to investigate drugs use based on wastewater analysis at the national level and to compare the results obtained with other indicators of consumption. Although calculations in WBE can still be improved (particularly for cannabis), WBE data are complementary to other information sources commonly used for drug use statistics. Population-normalized data allows the comparison between different cities as well as the evaluation of spatial and temporal trends. The extrapolation to national consumption estimates from the results obtained in a limited number of cities and the corresponding retail drug market needs to be taken with caution, as several assumptions had to be made. Despite these limitations, data reported and trends observed in this first monitoring covering a notable number of cities in Spain are of interest and correlate well with other sources of information. Thus, the cannabis and COC use in Spain can be established in a similar way using WBE data, seizure data, surveys, and treatment and intoxication data. However, WBE has advantages over other illicit drug consumption monitoring methods such as the immediacy of the results and the large percentage of the population that can be covered. However, no single indicator is reliable on its own and each indicator has its advantages and limitations. Hence, the application of various indicators, including WBE data, is the way forward to get more insight on drug consumption at community level.

CRediT authorship contribution statement

Lubertus Bijlsma: Resources, Investigation, Formal analysis, Visualization, Writing - original draft. **Yolanda Picó**: Supervision, Visualization, Writing - review & editing. **Alberto Celma**: Investigation, Formal analysis, Writing - review & editing. **Vicente Andreu**: Resources, Formal analysis, Writing - review & editing. **Andrea Estévez-Danta**: Investigation, Formal analysis, Writing - review & editing. **Iria González-Mariño**: Resources, Writing - review & editing. **Félix Hernández**: Supervision, Visualization, Writing – review & editing. **Miren López de Alda**: Resources, Writing - review & editing. **Ester López García**: Formal analysis, Writing - review & editing. **Rosa María Marcé**: Resources, Writing - review & editing. **Manuel Miró**: Resources, Writing - review & editing. **Rosa Montes**: Investigation, Formal analysis, Writing - review & editing. **Elena Pitarch**: Investigation, Formal analysis, Visualization, Writing - review & editing. **Eva Pocurull**: Resources, Writing - review & editing. **Cristina Postigo**: Resources, Formal analysis, Writing - review & editing. **Ailette Prieto**: Resources, Writing - review & editing. **Andreu Rico**: Resources, Writing - review & editing. **Rosario Rodil**: Resources, Writing - review & editing. **Yolanda Valcárcel**: Resources, Writing - review & editing. **Mireia Ventura**: Resources, Writing - review & editing. **Unai Pérez de San Román-Landa**: Resources, Writing - review & editing. **José Benito Quintana**: Visualization, Supervision, Funding acquisition, Writing - review & editing.

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.

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Table 1: Overview of the locations and main characteristics of the WWTPs included in this study.

City	Region	Population	Population (date)[a]	Covering the city (%)	Average flow (m^3/day)	Sampling date	Sampling mode[b]
Barcelona	Catalonia	1.163.154	C (2017)	35	270.672	14 - 20.03.2018	T (50 mL / 10 min)
Bilbao	Basque Country	860.237	C (2016)	100	263.818	17 - 23.04.2018	T (100 mL / 60 min)
Castellón	Valencian Community	171.669	C (2015)	100	34.285	11 - 17.04.2018	T (100 mL / 15 min)
Guadalajara	Castile-La Mancha	94.755	BOD (Jan-Apr 2018)	100	29.490	02 - 08.05.2018	T (200 mL / 60 min)
Lleida	Catalonia	143.612	C (2017)	100	42.264	07 - 13.03.2018	T (200 mL / 60 min)
Madrid-1	Community of Madrid	727.176	COD (May 2018)	30	108.901	16 - 22.05.2018	T (400 mL / 30 min)
Madrid-2		227.869	BOD (2016)		43.563	20 - 26.06.2018	T (100 mL / 60 min)
Móstoles		187.281	H x 3.5	90	26.891	17 - 23.05.2018	T (100 mL / 60 min)
Palma de Mallorca	Balearic Islands	454.453	C (2017)	100	47.572	10 - 24.04.2018	T (100 mL / 15 min)
Reus	Catalonia	115.000	C (2017	100	17.217	17 - 23.04.2018	F
Santiago de Compostela	Galicia	136.500	H x 2.5	100	106.627	13 - 19.03.2018	T (150 mL / 10 min)
Tarragona	Catalonia	142.635	C (2017)	100	23.985	17 - 23.04.2018	T (450 mL / 60 min)
Toledo	Castile-La Mancha	79.793	BOD (Apr-May 2018)	100	14.017	17 - 23.04.2018	T (100 mL / 15 min)
Valencia-1	Valencian Community	527.222	COD	100	124.587	10 - 16.04.2018	T (100 mL / 60 min)
Valencia-2		788.242	COD		204.014	10 - 16.04.2018	T (100 mL / 60 min)
Valencia-3		162.249	COD		29.593	10 - 16.04.2018	F

[a] Method to estimate the population: C = Census, BOD = Biological Oxygen Demand, COD = Chemical Oxygen Demand, H = number of houses connected to the sewage system

[b] Sampling mode: T = Time proportional (volume sampled/frequency of sampling), F = Flow proportional

Table 2: Population-normalized daily consumption estimates of AMP, METH, MDMA, COC and Cannabis by the Spanish population (mg/day/1000 inh.) using 2018 data of all cities monitored in this study

Day	AMP	METH	MDMA	COC	Cannabis (CF 182)	Cannabis (CF 36.4)
Tuesday	127	27	68	1789	22799	4529
Wednesday	137	29	54	1810	20436	4065
Thursday	144	34	44	1830	17842	3548
Friday	122	30	51	2024	17983	3571
Saturday	160	30	120	2801	20156	4008
Sunday	130	32	176	2465	22771	4532
Monday	173	25	123	2154	21980	4359
Average	***142***	***29***	***91***	***2125***	***20567***	***4113***
SD	***19***	***3***	***50***	***384***	***2087***	***414***
Scenario 1 and 2 range*	***141– 160***	***29 – 47***	***91 – 95***	***-***	***-***	***-***

*Scenario 1) underestimation; Scenario 2) overestimation (see section 3.6.1 for details). Not applicable in the case of COC and THC since they were above LOQs in all samples.

Table 3: Spanish daily (kg/day) and annual (ton/year) consumption estimates of pure substance (AMP, METH, MDMA, COC and Cannabis) based on wastewater data and extrapolation to consumed cut product and retail market size using 2018 data of all cities

	Average daily consumption of pure substance (kg/day ± SD[a])	Annual consumption of pure substance (tons/year)[b]	Purity/Potency[c]	Consumed product /year	Price [d]	Retail market size (millions €)
AMP	6.6 ± 1.0	2.40 – 2.72	38.6 - 48.4%	5.0 - 7.0 tons	20.61 €/g	102 - 145
METH	1.4 ± 0.2	0.50 - 0.80	64%	0.78 - 1.2 tons	20.61 €/g	16.1 – 25.8
MDMA (as tablets)	4.2 ± 1.5	1.54 – 1.60	179.97 mg/tablet	8.6 – 8.9 million tablets	10.29 €/tablet	88.5 – 91.5
MDMA (as crystal)			81.2%	1.9 - 2.0 tons	NA	NA
COC	99 ± 18	36.2	35.5 - 67.5%	53.6 - 102 tons	59.21 €/g	3173 - 6039
Cannabis (CF 182)	960 ± 97	350	Herb 10.1 % Resin 18.5%	Herb 3465 tons Resin 1891 tons	Herb 5.22 €/g Resin 5.59 €/g	Herb 18087 Resin 10570
Cannabis (CF 36.4)	191 ± 19	69.6	Herb 10.1 % Resin 18.5%	Herb 689 tons Resin 376 tons	Herb 5.22 €/g Resin 5.59 €/g	Herb 3596 Resin 2101

[a] Standard deviation of average daily consumption estimates of 7 consecutive days of all cities monitored in this study

[b] Ranges account for scenarios mentioned in Table 2 and section 3.6.1

[c] See details and references on purity/potency in Table S7

[d] Price data (2018) from (Observatorio Español de las Drogas y las Adicciones, 2019)

Table 4: Daily population-normalized (mg/day/ 1000 inh.) and non-normalized (kg/day) consumption estimates by the Spanish population in 2018 using all cities studied herein and only SCORE cities (*i.e.* Barcelona, Castellón, Santiago de Compostela and Valencia)

	All cities Population-normalized estimates (mg/day/ 1000 inh.)	**SCORE cities Population-normalized estimates (mg/day/ 1000 inh.)**	**All cities Non-normalized estimates (kg/day)**	**SCORE cities Non-normalized estimates (kg/day)**
AMP	142	59	6.6	2.8
METH	29	48	1.4	2.2
MDMA	91	109	4.2	5.1
COC	2125	2216	99	103
Cannabis (CF 182)	20567	20208	960	973
Cannabis (CF 36.4)	4113	4040	191	197

Figure captions

Figure 1: Average consumption estimates of cocaine (g/day/1000 inh.), back-calculated from population-normalized benzoylecgonine loads (***Figure S1***). Box colors indicate the region (Autonomous Community): Catalonia = yellow, Basque country = violet, Valencian Community = brown, Castile-La Mancha = orange, Community of Madrid = blue, Balearic Islands = green, Galicia = fuchsia.

Figure 2: Average consumption estimates of cannabis (g/day/1000 inh.) applying an excretion correction factor (CF) of 182 (left scale) and 36.4 (right scale), back-calculated from population-normalized THC-COOH loads (***Figure S2***). Box colors indicate the region (Autonomous Community): Catalonia = yellow, Basque country = violet, Valencian Community = brown, Castile-La Mancha = orange, Community of Madrid = blue, Balearic Islands = green, Galicia = fuchsia.

Figure 3: Average consumption estimates of amphetamine (top) and methamphetamine (bottom) (mg/day/1000 inh.) (A). A larger scale on the right side of the figure (B) to visualize amphetamine consumption in Bilbao and methamphetamine consumption in Barcelona. Consumption estimates were back-calculated from corresponding population-normalized loads (***Figure S3***). Box colors indicate the region (Autonomous Community): Catalonia = yellow, Basque country = violet, Valencian Community = brown, Castile-La Mancha = orange, Community of Madrid = blue, Balearic Islands = green, Galicia = fuchsia. ***** = amphetamine or methamphetamine could not be quantified in any sample collected.

Figure 4: Average consumption estimates of MDMA (mg/day/1000 inh.), back-calculated from population-normalized MDMA loads (***Figure S6***). Box colors indicate the region (Autonomous Community): Catalonia = yellow, Basque country = violet, Valencian Community = brown, Castile-La Mancha = orange, Community of Madrid = blue, Balearic Islands = green, Galicia = fuchsia.

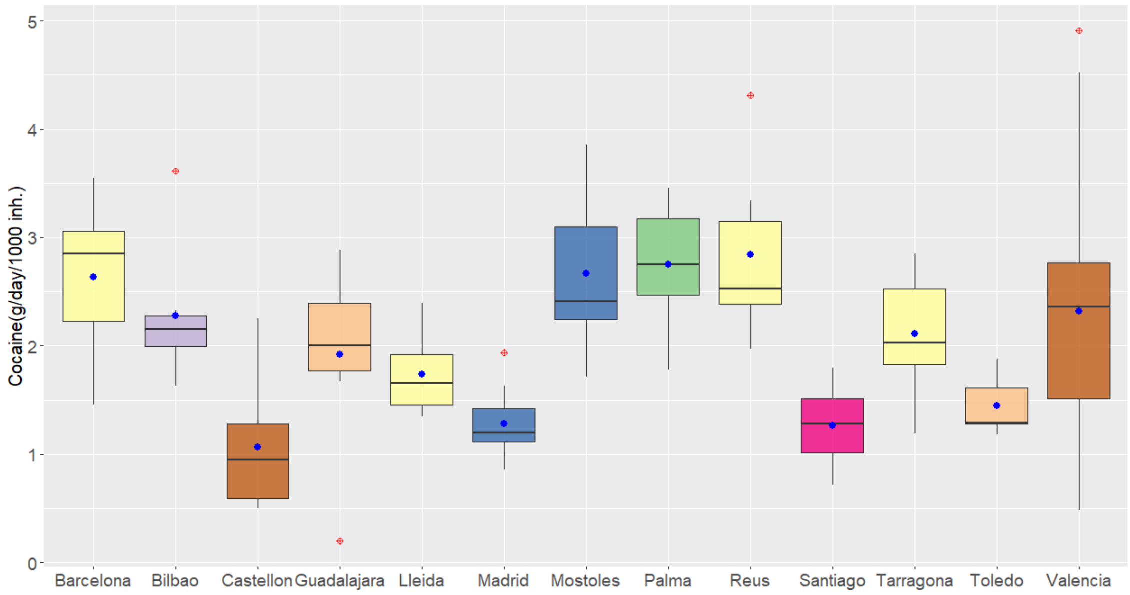
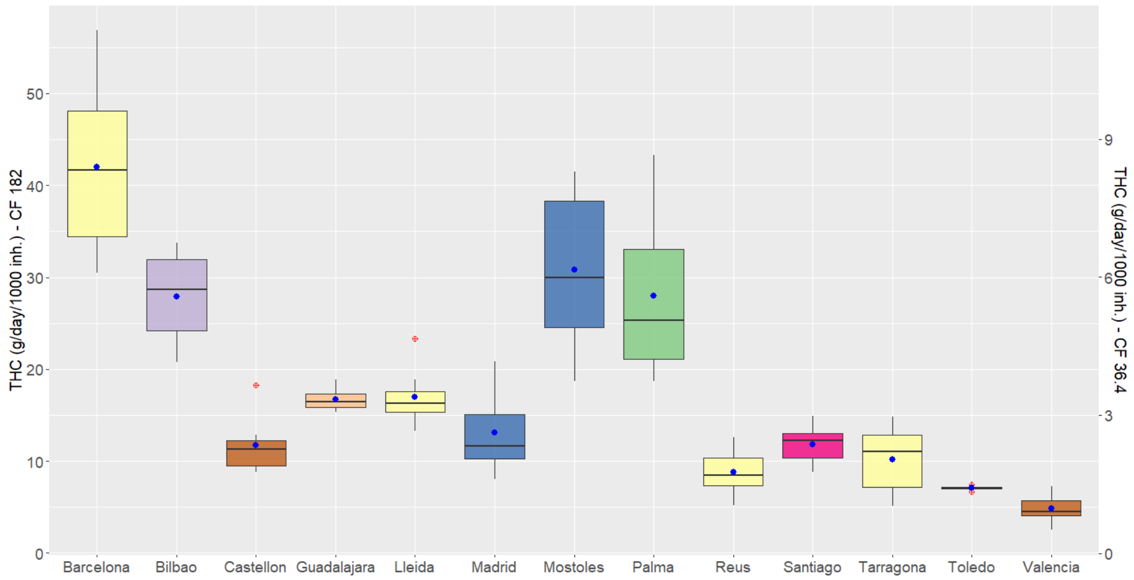

Cocaine(g/day/1000 inh.)
0
1
2
3
4
5
Barcelona
Bilbao
Castellon
Guadalajara
Lleida
Madrid
Mostoles
Palma
Reus
Santiago
Tarragona
Toledo
Valencia

Figure 1



THC (g/day/1000 inh.) - CF 182
50
40
30
20
10
0
THC (g/day/1000 inh.) - CF 36.4
9
6
3
0
Barcelona
Bilbao
Castellon
Guadalajara
Lleida
Madrid
Mostoles
Palma
Reus
Santiago
Tarragona
Toledo
Valencia

Figure 2

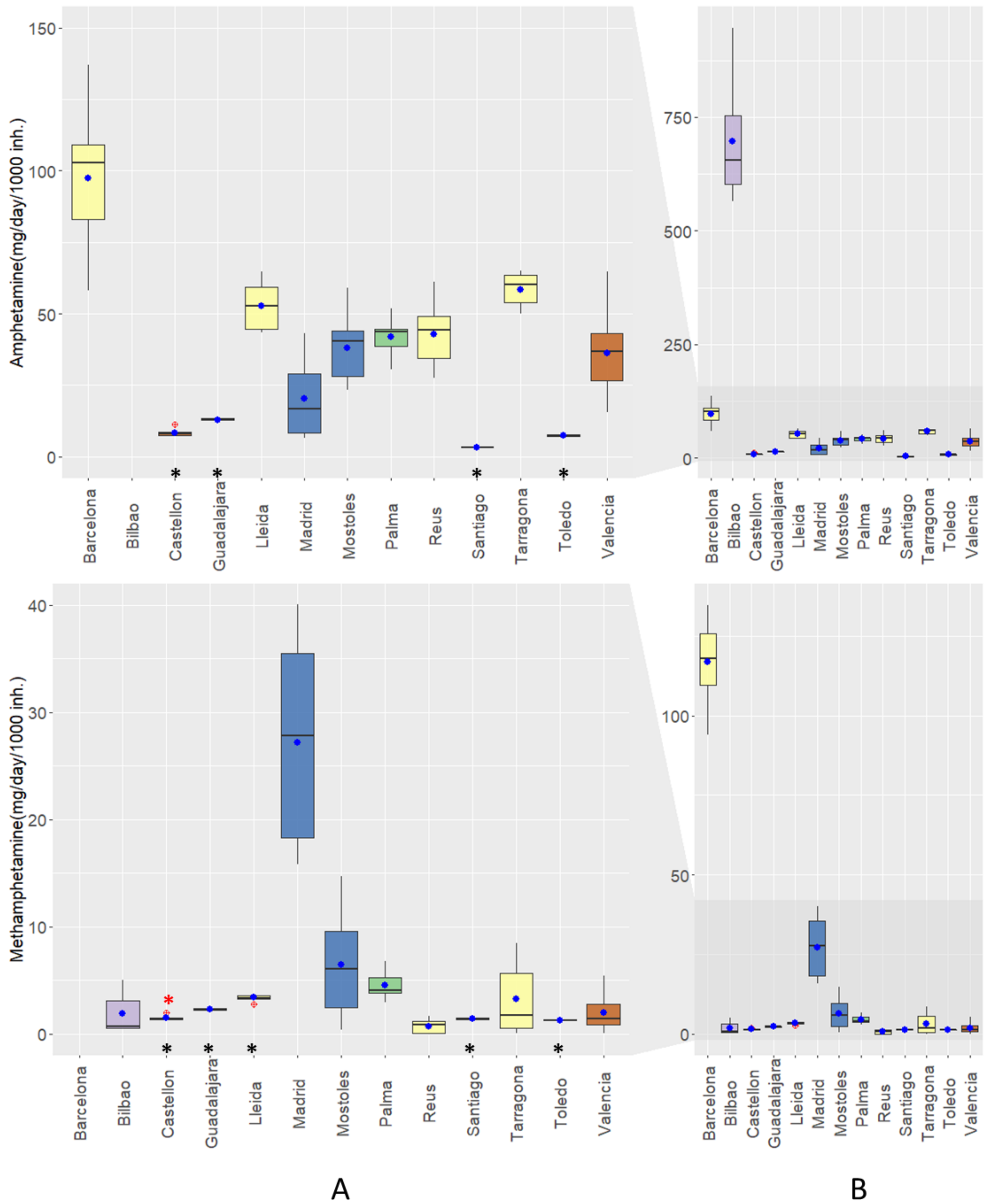

Amphetamine(mg/day/1000 inh.)
Methamphetamine(mg/day/1000 inh.)
Barcelona
Bilbao
Castellon
Guadalajara
Lleida
Madrid
Mostoles
Palma
Reus
Santiago
Tarragona
Toledo
Valencia
A
B

Figure 3

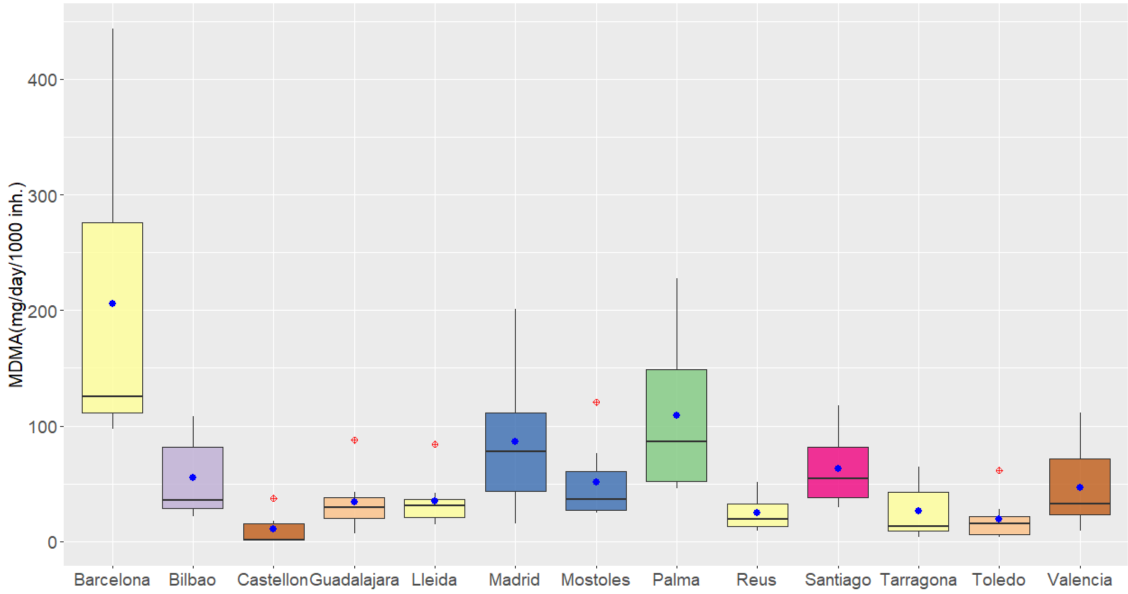

MDMA(mg/day/1000 inh.)
400
300
200
100
0
Barcelona
Bilbao
Castellon
Guadalajara
Lleida
Madrid
Mostoles
Palma
Reus
Santiago
Tarragona
Toledo
Valencia

Figure 4