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DOI

[10.1016/j.scitotenv.2012.06.069](https://doi.org/10.1016/j.scitotenv.2012.06.069)

Publication date

2012

Document Version

Final published version

Published in

Science of the Total Environment

[Link to publication](#)

Citation for published version (APA):

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Comparing illicit drug use in 19 European cities through sewage analysis

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HIGHLIGHTS

- First Europe-wide study of illicit drug use through sewage biomarker analysis.
- First application of a harmonized protocol to report and evaluate sampling, analysis and data handling.
- First inter-laboratory comparison of the analysis of illicit drugs.
- Comparable illicit drug use data for 19 European cities.
- Extrapolated total daily use of cocaine in Europe during the study period was equivalent to 356 kg/day.

ARTICLE INFO

Article history:

Received 11 May 2012

Received in revised form 19 June 2012

Accepted 20 June 2012

Available online 25 July 2012

Keywords:

Sewage biomarker analysis

Cocaine

Methamphetamine

Amphetamine

MDMA

Cannabis

ABSTRACT

The analysis of sewage for urinary biomarkers of illicit drugs is a promising and complementary approach for estimating the use of these substances in the general population. For the first time, this approach was simultaneously applied in 19 European cities, making it possible to directly compare illicit drug loads in Europe over a 1-week period. An inter-laboratory comparison study was performed to evaluate the analytical performance of the participating laboratories. Raw 24-hour composite sewage samples were collected from 19 European cities during a single week in March 2011 and analyzed for the urinary biomarkers of cocaine, amphetamine, ecstasy, methamphetamine and cannabis using in-house optimized and validated analytical methods. The load of each substance used in each city was back-calculated from the measured concentrations. The data show distinct temporal and spatial patterns in drug use across Europe. Cocaine use was higher in Western and Central Europe and lower in Northern and Eastern Europe. The extrapolated total daily use of cocaine in Europe during the study period was equivalent to 356 kg/day. High per capita ecstasy loads were observed in Dutch cities, as well as in Antwerp and London. In general, cocaine and ecstasy loads were significantly elevated during the weekend compared to weekdays. Per-capita loads of methamphetamine were highest in Helsinki and Turku, Oslo and Budweis, while the per capita loads of cannabis were similar throughout Europe. This study shows that a standardized analysis for illicit drug urinary biomarkers in sewage can be applied to estimate and compare the use of these substances at local and international scales. This approach has the potential to deliver important information on drug markets (supply indicator).

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1. Introduction

Illicit drug use and trafficking are international issues that have negative impacts across the social and economic spectrum, from the public health of individuals to the large-scale stability of national borders. Statistics show that around a third of European citizens have tried an illicit drug, while overdose claims the life of at least one citizen every hour (European Monitoring Centre for Drugs and Drug Addiction, 2010a). Ever-changing patterns in illicit drug production, demand and supply necessitate a program of frequent monitoring. Independent, objective and timely information on the type, scale and demographics of illicit drug use is essential in order to fully understand drug use and develop better methods and actions to respond to them. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is responsible for collating such information in Europe and providing these data to policy makers so that they can design management strategies and appropriate laws. This information is also vital for measuring the success of existing management strategies and laws in view of a dynamic drug culture and emerging trends.

Illicit drug use is a socially stigmatized and often hidden activity, so traditional survey methods such as general population surveys and interviews can be inaccurate and prone to conjecture. The possibility of adding a new technique that overcomes these challenges to the existing repertoire of research methods is therefore an exciting prospect (European Monitoring Centre for Drugs and Drug Addiction, 2008). On average between 80 and 90% of the population is connected to the sewer network with much lower rates (40–65%) connected to primary sewage treatment in Eastern Europe. Sampling sewage at the inlet of a sewage treatment plant (STP) can therefore provide a diluted and pooled community urine sample that can deliver objective near real-time estimates of the total quantities of illicit drugs being used by the connected population (Zuccato et al., 2008; van Nuijs et al., 2011a; Daughton, 2011) (Fig. S1). The quantitative analysis of sewage for the estimation of illicit drug use is complementary to existing epidemiologically based approaches and can provide additional, evidence-based information. The potential of this approach to provide information regarding drug use at local level has previously been shown by Zuccato et al. (2008) where reproducible and characteristic profiles of illicit drug use were obtained in three cities, quickly revealing any short-time changes. Subsequently this approach has been used to estimate local (i.e. city or small town) and national use (van Nuijs et al., 2011b), monitor use trends with time (short-term and long-term; Harman et al., 2011), identify changing trends and new habits (Reid et al., 2011a; Castiglioni et al., 2011a) and identify the use of new substances (Zuccato et al., 2008; van Nuijs et al., 2011a; Daughton, 2011). The conceptual advantages of estimating illicit drug use based on sewage analysis are that it provides an aggregated estimate of all the people contributing to the sewage in a catchment over the sampling period, is non-intrusive and ethical approval from individuals is of no concern, it is objective and does not have the problems of surveys that may suffer from a limited number of subjects and self-reporting bias, and the results can be obtained almost in real-time, which means within days or weeks compared to surveys that may take years before publication.

The approach has been applied in Australia, Europe, and North America yielding promising results (van Nuijs et al., 2011a, 2011b; Daughton, 2011; Reid et al., 2011a; Castiglioni et al., 2011a; Irvine et al., 2011). However, coordinated international studies have yet to be performed, and the direct comparison of these data is not trivial. Challenges arise from uncertainties associated with the sampling of sewage, behavior of the selected biomarkers in the sewer, reliability of inter-laboratory analytical measurements, different back-calculation methods, and different approaches to estimate the size of the population being tested.

The objective of the present study was for the first time to apply sewage analysis simultaneously in 19 European cities over a single

week, following a harmonized protocol to report and evaluate sampling, analysis and data handling. In this way, it was possible to compare patterns of illicit drug use across Europe in a sound and reliable way. Sewage samples were analyzed for biomarkers of cocaine (COC), amphetamine (AMP), methamphetamine (METH), ecstasy (as 3,4-methylenedioxymethamphetamine, MDMA), and cannabis (CAN). To evaluate the analytical performance of each participating laboratory, an inter-laboratory comparison exercise was performed. The resulting estimates of illicit drug use were then further compared with official national statistics as compiled and reported by the EMCDDA (European Monitoring Centre for Drugs and Drug Addiction, 2010a).

The approach applied on a Europe-wide scale can reveal if trends of drug consumption can be promptly monitored at an international scale, using a tool which is complementary to more focused survey methods, and which has the potential to become an additional source of real-time epidemiological information.

2. Methods

2.1. Sewer system characterization (questionnaire)

To assess the potential of sewage drug testing under normal operating conditions, we purposely relied on existing sampling procedures at the individual STPs. It is worth noting that the catchments and sewer systems cannot be controlled by the investigators and that both sewage flows and biomarker concentrations vary during a day; not only at the time-scales of hours but also minutes (Ort et al., 2010a). This has been neglected in most previous studies and relevant information, to evaluate the data quality from routinely collected sewage samples, has not been reported (Ort et al., 2010b). Therefore, we developed a specifically tailored questionnaire. In close cooperation with local sewer and STP operators it allowed project partners to gather important catchment and sewer system characteristics in a formalized manner. Information such as the presence and operation of lift stations (generating short hydraulic pulses), structural state of sewers (loss of sewage via exfiltration), variability of population size (commuters) and details on sampling procedures was considered to facilitate a meaningful data quality evaluation. Data were flagged when either excessive sewage leakage was reported or the sampling uncertainty was larger than inter-laboratory analytical variation.

2.2. Sampling

Raw sewage was collected from the inlet of 21 STPs spread over 11 European countries, representing nineteen cities (Valencia is served by three STPs), and servicing a combined population of approximately 15 million inhabitants (Fig. 1). Samples were collected from each location over seven consecutive days, starting on Wednesday 9th March 2011 and ending on Tuesday 15th March 2011. In five STPs, interruptions due to technical problems caused smaller sample sizes. In Barcelona, the sampling campaign was performed one week later, between the 16th and 22nd March 2011.

Samples were collected using the operational equipment of the individual STP. At all locations, 24-h composite samples were collected, while mode of sampling was either time-, volume- or flow-proportional (Ort et al., 2010a). Automated equipment was used at all locations with the exception of Barcelona. All samples were stored in silanized glass, polyethylene terephthalate (PET) or high-density polyethylene (HDPE) containers and analyzed within 12 h of collection. If this was not possible, the samples were immediately frozen at -20°C until analysis to prevent degradation of the illicit drug target residues. Based on published data on analyte stability there would not be a substantial difference between the two sample storage scenarios (i.e. 4°C for 12 h or longer at -20°C) (Baker and Kasprzyk-Hordern, 2011; Reid et al., 2011b). For each sample, the flow rate (L/day) of the sewage stream was recorded and where possible, water quality parameters such as total nitrogen (N), total

phosphorus (P), chemical oxygen demand (COD) and biological oxygen demand (BOD) were measured in order to support estimating the contributing population and normalize for it (Andreottola et al., 1994; Zessner and Lindtner, 2005; Garnier et al., 2006).

2.3. Analysis

2.3.1. Analytical methodology

The physical and chemical composition of sewage is rather complex and includes large loadings of suspended particulates as well as the presence of relatively high concentrations of compounds that can potentially interfere with the analysis of the target drugs. Therefore the sewage samples were filtered (filter type GFC, 0.45 μm), concentrated and cleaned-up using solid phase extraction (SPE) prior to analysis. All participants used SPE for the preconcentration of samples typically using polymeric cartridges (e.g. Oasis HLB) in off-line or on-line mode (Table S1). More details on analytical methodology can be found elsewhere (Castiglioni et al., 2006; Kasprzyk-Hordern et al., 2008; Postigo et al., 2008; Bijlsma et al., 2009; Hogenboom et al., 2009; van Nuijs et al., 2009; Terzic et al., 2010; Vazquez-Roig et al., 2010; Reid et al., 2011a; González-Mariño et al., 2012). The analytical technique of choice for the quantitative analysis of the target drug residues was liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). Most participating groups used triple quadrupole or hybrid quadrupole-linear ion trap mass analyzers, with the exception of KWR (The Netherlands) and the University of Santiago de Compostela (Spain) who used high-resolution mass spectrometry (LTQ-Orbitrap and time-of-flight MS, respectively).

These highly sensitive analyzers, together with steps to concentrate and clean-up the sewage samples enable analysis at a low concentration level (ng/L) in sewage (van Nuijs et al., 2011a; Castiglioni et al., 2011a). The use of mass labeled internal standards is mandatory to compensate for the potential analytical errors associated with the sample manipulation and due to matrix effects (increase or decrease of analytical signal due to matrix interferences). Internal standards were added to the samples prior to sample treatment. Each participating laboratory used an in-house and fully validated analytical method. The methods comply with identification and confirmation criteria (The Commission of the European Communities, 2002) used in this type of LC–MS/MS analysis to avoid the reporting of false positives or negatives. The analytical quality assurance was evaluated by an inter-laboratory study of two external quality control solutions (50 ng/mL and 500 ng/mL). The mean concentrations reported by participating laboratories ($n = 11$, except for cocaine, $n = 12$, and THC $n = 9$) varied between 49 and 60 ng/mL for the low-level solution and between 460 and 530 ng/mL for the high-level solution. Inter-laboratory variation (relative standard deviation (RSD)) varied between 26 and 38% for the low-level spike and between 12 and 26% for the high-level spike. With regard to the sigma difference of the two standard solutions (450 ng/mL), the average recovery of the spike in both samples ranged between 91 and 111% for all laboratories. The recovery of the spike, the systematic errors of the measurements and the between-laboratory systematic errors represent aspects of accuracy. The precision is represented by the coefficient of variation of reproducibility and the outliers of the sigma difference. Combining the accuracy and precision, a report mark per parameter was awarded. The average report mark was 8.3 out of 10 (lowest

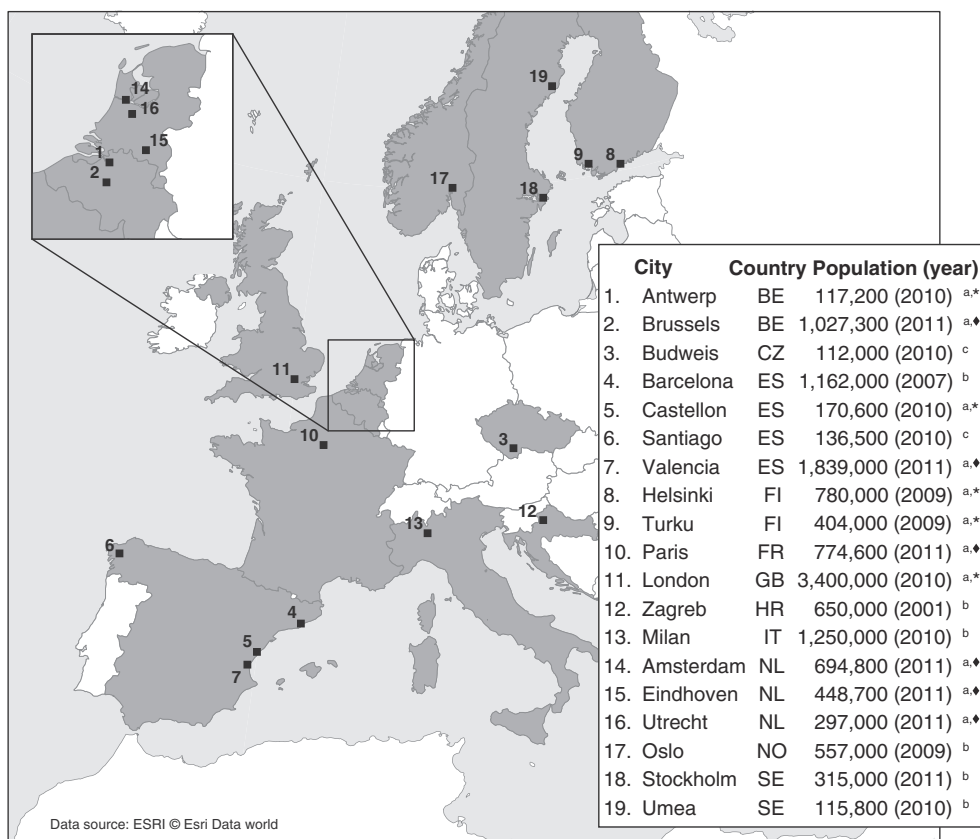


Fig. 1. Cities that participated in the study. Population estimates in the catchment of the corresponding sewage treatment plants are based on the method that was deemed the most appropriate by the sewage treatment plant operators (see footnotes and text for more details). Population estimated based on: ^aMeasured influent load; ^bBOD/COD/N/P (average of year calculated by sewage treatment plant operators); ^cBOD (7-day average during sampling week, 60 g BOD person⁻¹ day⁻¹). ^dCensus data. ^eNumber of house connections or drinking water subscribers.

for amphetamine 7.0 and the highest for MDMA and benzoylecgonine 9.0).

3.2.3. Normalization and estimation of use

The use of the main classes of illicit drugs was assessed by measuring specific target biomarkers in urban sewage. The main requirements for the selection of a target residue were the presence in sewage at quantifiable concentrations, and the stability of the target residue from chemical and biological degradation in the sewer and during sampling and storage. Parent drugs were chosen as target residues for amphetamine-type stimulants (AMP, METH, and MDMA), while the main urinary metabolites were chosen for cocaine (COC) and cannabis (CAN), which were benzoylecgonine (BE) and 11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH) respectively (Zuccato et al., 2008; van Nuijs et al., 2011a). The amount (daily mass load) of each target residue that was excreted by a population was calculated by multiplying measured sewage concentrations (ng/L) by corresponding daily flow rates of sewage (L/day) during the sampling campaigns. Data were then normalized by population size (1000 inhabitants) to allow comparison among different cities. Different approaches were used to estimate the population served by each STP due to the specific characteristics of the individual sewer systems investigated (i.e. census data, number of house connections or drinking water subscribers, or using a measured biological parameter in sewage; BOD, COD, N, and P; Andreottola et al., 1994; Zessner and Lindtner, 2005; Daughton, 2012). The variability among the different population estimates obtained was found to be between 7 and 40% (%RSD) and is strictly dependent on the sewage composition (i.e. presence of industrial wastes which can increase the hydrochemical parameters) or the sewer system characteristics. It was therefore not possible to use a unique approach to estimate the population at all sites, and the most reliable estimation was selected on a case by case basis based upon the expert knowledge of the local STP operators.

The daily loads relative to population (mg/day/1000 inhabitants) calculated for target residues of AMP, METH, MDMA, and CAN were averaged over all sampling days to compare illicit drug use across the investigated cities. For COC, it was possible to back-calculate its consumption from the measured normalized daily loads of BE using the model suggested by Zuccato et al. (2008), which contains parameters for human metabolism and the subsequent excretion of BE as well as the molar ratio of COC/BE. A complete review of all COC pharmacokinetic studies available in the literature suggests a median excretion value of 38% of a cocaine dose and a correction factor of 2.77 of BE excretion (Castiglioni et al., 2011b).

3. Results

3.1. System characterization and sampling

The questionnaire had a very good response and all but one of the operators were very cooperative. The results show that 10 of 20 STPs sample volume proportionally with average sampling intervals of around 15 min, which resulted in generally acceptable uncertainties with only two potentially critical sites. Similarly, flow data quality also seems satisfactory since 10 of the 20 STPs reported that they calibrate their flow meters in periods of between 1 month and 6 years. Systematic flow measurement errors cancel out when population size is determined from wastewater parameters (e.g. BOD); i.e. if a too high illicit drug load was calculated due to a too high flow, this would similarly hold true for the BOD load resulting in an overestimation of the population directly proportional to the overestimation of illicit drug loads (Lai et al., 2011). Only for five STPs, for which the served population was not determined by means of a wastewater parameter, was the flow meter calibration not reported. Uncertainties of daily flow were reported to be between 0.3 and 8% (median: 1%), which are rather low. Sewage losses were

reported for four catchments; in two cases, a sewage loss of >20% may also imply a biomarker load loss of >20% (Fig. 2).

3.2. Patterns of illicit drug use in Europe

3.2.1. Cocaine (COC)

The COC use estimates (averaged for all sampling days), calculated from BE loads, are shown in Fig. 2A. COC use could be estimated for all investigated cities, since BE could always be quantified in sewage. The highest use of COC was observed in Antwerp followed by (in order of decreasing use) Amsterdam, Valencia, Eindhoven, Barcelona, London, Castellón, and Utrecht, where the average estimates were in the range of 987–1998 mg/day/1000 inhabitants. Milan, Santiago de Compostela, Paris, and Brussels showed estimates of COC use around 511–662 mg/day/1000 inhabitants, while the average estimates were lower in Budweis, Zagreb, Helsinki, Turku, Oslo, Stockholm, and Umeå, between 2 and 146 mg/day/1000 inhabitants.

3.2.2. Ecstasy (MDMA)

Results of the MDMA analysis are presented in Fig. 2B. The three Dutch cities (Amsterdam, Utrecht, and Eindhoven) showed the highest loads of MDMA relative to population size with average loads of between 67 and 615 mg MDMA/day/1000 inhabitants. Results from Antwerp and London also indicate large quantities of MDMA relative to population size (32–52 mg MDMA/day/1000 inhabitants). MDMA was not detected in Castellon, Umeå, Stockholm, and Oslo.

3.2.3. Amphetamines (AMP and METH)

METH was detected in the majority of sewage samples with the exception of Zagreb, Paris, and Castellón and the three cities included from the Netherlands (Amsterdam, Eindhoven and Utrecht). Measurements in both Helsinki and Turku showed the highest METH loads relative to population size with 376 and 300 mg METH/day/1000 inhabitants, respectively (Fig. 2C). Results from Oslo (245 mg METH/day/1000 inhabitants) and Budweis (175 mg METH/day/1000 inhabitants) also indicate large quantities of METH relative to their population size. The remaining cities show relatively low METH loads relative to population size (3–49 mg METH/day/1000 inhabitants). It should be noted that in the cities with relatively low METH use, relatively high use of AMP was observed, and vice versa.

AMP was the dominant amphetamine-like drug in Zagreb, Valencia, Barcelona, Castellón, Santiago de Compostela, Stockholm, Umeå, and London. Most notable, however, were the results from Belgium and The Netherlands with 33–3040 mg AMP/day/1000 inhabitants. The result from Eindhoven is extremely high in relation to all other cities, and is, in fact, the highest result ever recorded in the Netherlands.

3.2.4. Cannabis (THC-COOH)

The highest measured per-capita loads of THC-COOH (192 mg THC-COOH/day/1000 inhabitants) were observed in Amsterdam (Fig. 2D). Results for other cities were in the range of 14–124 mg THC-COOH/day/1000 inhabitants. The analysis of THC-COOH in sewage was not performed for Antwerp, Brussels, London, Oslo, Helsinki and Turku, due to validated methods not being available in all participating laboratories, and it was below the detection limit in Umeå and Stockholm.

3.3. Weekly patterns in illicit drug use

The day-to-day variation of illicit drug loads is charted in Fig. 3 for COC and MDMA as percentage of the measured total weekly load. The MDMA loads for Utrecht were excluded from the analysis since it is possible that they are related to the dismantling of an illegal production facility. Besides Utrecht the following cities were also excluded from the analysis of weekly MDMA pattern since all values were below the limit of quantification: Oslo, Stockholm, Umeå and

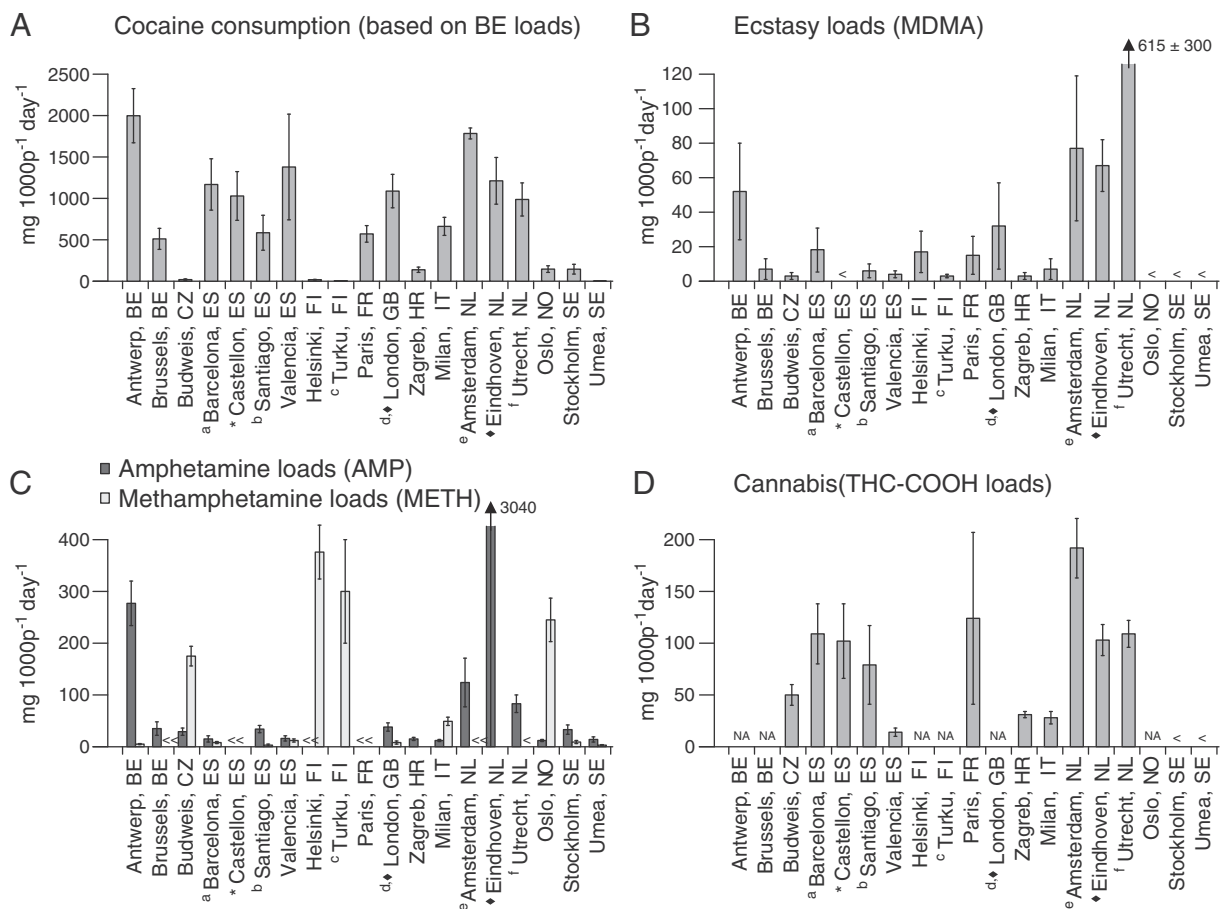


Fig. 2. Average estimates of cocaine (COC) consumption (back-calculated from benzoylecgonine (BE) loads, the main metabolite of COC) and population-normalized loads of amphetamine (AMP), methamphetamine (METH) in 19 selected European cities and cannabis (THC-COOH, all in mg/1000 inhabitants/day) in 13 of them between the 9th and 15th March 2011 (mean \pm SD from all sampling days, $n = 7$). ^aSampling one week later (16th–22th March 2011). ^b $n = 6$, sample of Monday 14th March missing. ^c $n = 6$, sample of Sunday 13th March missing. ^d $n = 6$, sample of Saturday 12th March missing. ^e $n = 6$, sample of Monday 14th and Tuesday 15th March missing. ^fSampling started one day later (10th–16th March 2011). ^{*}Sampling uncertainty estimated to be larger than variation of interlaboratory comparison of chemical analysis. [♦]Exfiltration of sewerage larger than 20%. ^{NA}Not analyzed. [<]All measured concentrations were below the limit of quantification.

Castellón. Cocaine use (based on BE loads) and MDMA loads show a clear weekly pattern characterized by an increase of use during the weekend compared to weekdays: 14 out of 19 cities had highest COC use on Saturday or Sunday. The increased BE loads on Saturday and Sunday indicate elevated COC use on Friday and Saturday nights (including the early hours of Sunday morning). The use over all cities on an individual weekday (MON-FRI) was approx. 13% (median) of the total weekly use, increasing significantly to 18% on a weekend-day (SAT/SUN): $p = 1.2e-6$, Wilcoxon test *weekend-day (SAT/SUN) vs. weekday (MON-FRI)*. If the weekend was extended and includes Friday the effect is still significant, but slightly weaker: $p = 3.2e-6$, Wilcoxon test *weekend-day (FRI-SUN) vs. weekday (MON-THU)*. Median MDMA loads more than doubled on a weekend-day compared to a weekday, from 10% (MON-FRI) to 24% (SAT/SUN) of the total weekly load: $p = 1.3e-8$, Wilcoxon test *weekend (SAT/SUN) vs. weekdays (MON-FRI)*. Even if Sunday alone was tested against all other days as weekdays (MON-SAT) the increase is still significant: $p = 1.6e-5$, Wilcoxon test *SUN vs. weekdays (MON-SAT)*.

4. Discussion

4.1. System characterization and sampling

Our results show that a thorough analysis of sewage systems, which requires close collaboration with local STP operators, and sampling design are necessary to obtain good, reliable data. The results of

this study revealed only a few cases where the level of confidence associated with the STPs' own sampling equipment and protocol was unacceptable. This supports the recent findings of Mathieu et al. (2011) who suggest that the STPs' own sampling equipment is usually suitable for this type of study where the catchments houses more than 100,000 inhabitants. Given the observed variability across Europe (Fig. 2), an estimated additional variability of 10–30% from discharge measurements would not affect the general conclusions of this study. The consequences of sewage losses, for example from re-use or sewer leakage are generally difficult to assess and need to be factored in on a case by case basis.

4.2. Cocaine

COC use estimates in 19 European cities were in the range of 2–1998 mg COC/day/1000 inhabitants (Fig. 2A). A notable trend was that COC use is higher in more urbanized towns/cities within the same country. For example, COC use in the Swedish town of Umeå (population 75,000) resulted in population-normalized loads of 2 mg COC/day/1000 inhabitants, while results from the Swedish capital of Stockholm (population 850,000) were substantially higher at 145 mg COC/day/1000 inhabitants. The same trend was observed in Finland where the smaller city of Turku (6 mg COC/day/1000 inhabitants) showed much lower population-normalized loads than the capital Helsinki (18 mg COC/day/1000 inhabitants). Similar

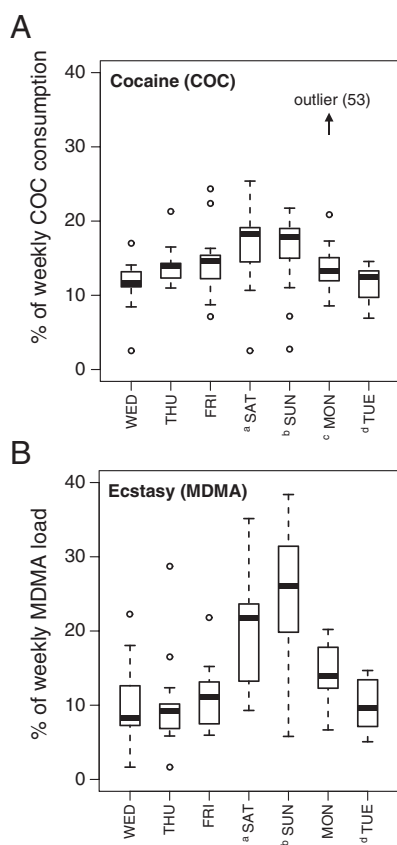


Fig. 3. Day-to-day variation of cocaine (COC) consumption (based on the loads of the main metabolite benzoylcegonine (BE)) in 19 cities and ecstasy (MDMA) in 15 cities. Medians are significantly different on the weekend compared to weekdays. ^aSample from London missing (COC n = 18, MDMA n = 14). ^bSample from Turku missing (COC n = 18, MDMA n = 14). ^cSample from Amsterdam and Santiago missing (COC n = 17, MDMA n = 13). ^dSample from Amsterdam missing (COC n = 18, MDMA n = 14).

patterns have been observed in The United States with higher COC use in the more urbanized cities (Banta-Green et al., 2009).

Any comparison between national annual prevalence data and a city-specific, one-week snapshot based on sewage analysis, must be treated with caution due to intra-country temporal and spatial variability. However, a general assessment as to whether the sewage-based data reflect the known information obtained via socio-epidemiological surveys is of value. The highest European prevalence of COC use among the general population (aged 15–64) and young adults (aged 15–34) is reported to be in Spain, the United Kingdom, Italy, Ireland and Denmark (European Monitoring Centre for Drugs and Drug Addiction, 2010a). Among the countries included in our study, the lowest prevalence data are reported for Norway, Sweden, Finland, the Czech Republic, France and the Netherlands, with no data available for Belgium and Croatia. The COC use estimates based on sewage analysis in general agree with these data, except for a few cases (Fig. 2). Sewage analysis found that COC use in Dutch cities is higher than would be expected from the 2010 prevalence data. Furthermore, prevalence data for Italy are among the highest in Europe, while estimates for Milan from sewage analysis show lower than expected use (Fig. 2). A possible explanation for this discrepancy may be the reduction in COC use in Italy, which has been observed between 2008 and 2009 through sewage analysis (Zuccato et al., 2011), and subsequently confirmed in 2010 by national epidemiological surveys (Dipartimento Politiche Antidroga, 2010).

There is clearly a need to better understand how much COC is being used in Europe (European Monitoring Centre for Drugs and Drug Addiction, 2010a) and sewage analysis can be applied as a supply indicator to assess the size of the European consumer market for

illicit drugs. Using the mean daily COC estimate (708 mg/day/1000 inhabitants) and a total European population of 502 million people, it appears that during the study period, approximately 355 kg/day of pure cocaine was used in Europe. It is clear that this is only a crude estimate, based upon the testing of only 2% of the population from a selection of cities and countries over a limited period. Further refinement of the calculation methodology and the wider application of the technique, possibly on a country basis, would provide a valuable insight into the amounts of COC used across Europe.

4.3. MDMA

The Czech Republic and the United Kingdom are reported to have the highest prevalence of ecstasy use among the general population (aged 15–64). In the young adults group (aged 15–34), the Netherlands is also reported to be a country with a high prevalence of MDMA use (European Monitoring Centre for Drugs and Drug Addiction, 2010a). Additionally, as already discussed in the previous paragraph, Belgium and the Netherlands are reported to be the main countries where MDMA is produced. Sewage analysis results from the present study reflect the published prevalence data with high per capita loads detected in Eindhoven, Amsterdam and London. The per capita MDMA loads detected in Utrecht were substantially higher than any other European city and more than 10 times higher than previously measured in Utrecht (Bijlsma, pers. comm.). It is possible that a police raid on an illegal MDMA manufacturing facility in Utrecht two days prior to the study resulted in MDMA being released into the city's sewer network resulting in the high levels that were measured. If this is the case, then the signal does not reflect the actual use of MDMA in Utrecht during this study.

Other high per capita MDMA loads were detected in Antwerp, situated close to the Dutch border, and in Barcelona, compared with other locations sampled in both countries. The fact that MDMA was not detected in Swedish sewage is in agreement with MDMA prevalence data, which states that Sweden is a low-prevalence country, along with the other Nordic countries of Norway and Finland. The discrepancy between the low levels of MDMA detected in Czech sewage and the reported highest national prevalence of ecstasy use in Europe may possibly be explained by the local situation that may not be representative of the Czech Republic. The MDMA loads measured in the present study compared well with previous European reports using the sewage analysis approach, both in terms of extent of the average load and of weekly variation profile (van Nuijs et al., 2011a). As observed for COC and MDMA, in the majority of cases the use was higher in highly populated urban centers.

4.4. Amphetamines

The results from sewage analysis indicate that Finland, Norway and the Czech Republic have the highest rates of METH use per-capita. These results correspond well with reported statistics that highlight the significant amount of METH that is regularly seized in the Nordic region and North East Europe (European Monitoring Centre for Drugs and Drug Addiction, 2010a). Interestingly, the same three nations have notably low rates of COC use (Fig. 2), which may indicate an inverse relationship between the two drugs. One simple explanation may be price and availability, whereby the use of COC is diminished in the face of a readily available supply of the substantially cheaper drug METH. However, Finland, Norway and the Czech Republic represent only a small fraction of the total European population.

Our results indicate that AMP dominates the amphetamine-like drug class on a European-wide perspective with respect to per-capita drug levels in sewage. Of particular interest are the per-capita loads of AMP in the cities of Northern Belgium (Antwerp) and the Netherlands which are the highest in Europe. These results are in contrast to the

published drug-use prevalence data which suggest that the use of AMP in these nations is actually a factor of 2–3 lower than in the rest of Europe. One possible explanation is that the Netherlands, Poland and Belgium are major producers of AMP (European Monitoring Centre for Drugs and Drug Addiction, 2010b). In 2008, 38% of the sites identified in the EU member states as being involved in production of AMP were discovered in the Netherlands. This is important for the interpretation of sewage analysis results, since the AMP and METH loads in these countries may reflect both the use of these substances and also release from drug production facilities. AMP and METH can also arise in sewage systems from the metabolism of prescribed medicines (e.g. Selegiline) which may add to the signal being measured, however prescription rates suggest that any contribution would be <1% of the total amphetamine signal.

4.5. Cannabis

During 2010, cannabis was used by around 7% of the population aged between 15 and 64 making it the most popular drug in Europe (European Monitoring Centre for Drugs and Drug Addiction, 2010a). In the smaller cities of the Netherlands (Eindhoven and Utrecht) comparably high loads of cannabis were found in the sewage with a maximum observed in Amsterdam. This may be explained by the liberal Dutch drug policy on cannabis use, which is permitted for every citizen over the age of 18 years. However, the prevalence of cannabis use is reported to be greatest in the Czech Republic, Italy, Spain and France. Spain, due to its strategic trafficking location, is an important cannabis market, and besides Barcelona, the smaller cities of Castellón and Santiago de Compostela also show high rates of use. Yet, per capita THC loads measured in Valencia are much lower than would be expected from published prevalence data. In Milan, the determined loads are also relatively low, and not in line with the cannabis use in Italy reported by the monitoring authorities. When interpreting these results the THC content of resin and herbal cannabis should be considered, since it can vary strongly from country to country, ranging from 3 to 17% (resin cannabis) and from 1 to 15% (herbal cannabis).

4.6. Weekly patterns of drugs use

Daily changes in illicit drug use were reflected in changes in STP influent sewage loads. The patterns in COC use showed clearly that there was increased use during the weekend compared to weekdays (Fig. 3), reflecting the recreational use of this drug. A trend in the MDMA loads along the week could also be observed, with statistically higher loads during the weekend (Sunday). The higher ecstasy loads measured during the weekend are in agreement with the recreational character of this substance and its popularity in the dance and music scene. For AMP, METH and THC-COOH, no weekly patterns in their loads could be observed, which is in agreement with the known pattern of use of these substances.

5. Conclusions

For the first time sewage analysis using a uniform protocol has been simultaneously applied in 19 European cities to estimate and compare the use of illicit drugs across Europe. The quantitative, non-intrusive, objective and rapid analysis of the illicit drug use of 15 million individuals was determined over a 1-week period, providing the most current data on illicit drug use in Europe with the results generally being in good agreement with officially reported prevalence data. In addition the approach also has the potential to be used as a supply indicator of the international illicit drug market. The present study clearly reinforces the conceptual strengths of analyzing biomarkers in sewage to produce objective and updated data on the use of illicit drugs and their market at local, national and international scales.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2012.06.069>.

Acknowledgments

The following scientists contributed to the study: Marijan Ahel, Aasmil Asgadaouan, Damià Barceló, David R Baker, Leon Barron, Jean-Daniel Berset, Cristina Blasco, Jenny-Maria Brozinski, Ganna Fedorova, Jerker Fick, Iria González-Mariño, Leif Kronberg, Katherine Langford, Ana Masia, Nicola Mastroianni, Thomas Nefau, Brett Paul, Isaac Rodríguez, Andreas Scheidegger, Ivan Senta, Mauro Tettamanti, Mats Tysklind and Ettore Zuccato. We thank Paul Griffith, Jane Mounteney and Ana Gallegos from EMCDDA for helping facilitate the study. We thank the people and agencies who assisted in collecting data. In particular, Pia Ryrfors and colleagues at Vestfjorden Avløpselskap (VEAS, Oslo, Norway), Jean-François Mougel (Aquiris, Belgium), Alain Vandelanootte (Aquaflin, Belgium), Alex Veltman, Waternet (Amsterdam, the Netherlands), Peter van Dijk and Stefan Wijers, Waterschap de Dommel (Eindhoven, the Netherlands), Mark Stevens and Alexander de Kruijff, Hoogheemraadschap de Stichtse Rijnlanden (Utrecht, the Netherlands), Paolo Camporini and Manuela Melis (Milan, Italy), Carlos Ferrer and colleagues at FACSA (Castellón, Spain), Fernando Llavador (EPSAR, Valencia, Spain), Vicente Fajardo Montañana (Aguas de Valencia, Spain), Julio Vasco, Pere Aguilo and Begoña Martínez from EMSSA (Barcelona, Spain), Ulla-Maija Laakkonen and Laura Aalberg, Rikospoliisi (Helsinki, Finland), Mirva Levomäki, Lounais-Suomen vesi-ja ympäristötutkimus Oy (Turku; Finland), Raimo Laakkonen, Turun seudun puhdistamo Oy (Turku, Finland), Kari Murtonen and Eija Lehtinen, Helsingin seudun ympäristöpalvelut – kuntayhtymä HSY (Helsinki, Finland), Jiri Stara and other colleagues at CEVAK a.s. (Budweiss, Czech Republic), Bruno Marion and Roland Nedelec (Seine Centre, SIAAP, France), Ana Lago (Aquagest, Santiago, Spain), Cajsa Wahlberg (Svenskt Vatten, Sweden), Britta Bristav, Johanna Westlund and other colleagues at UMEVA and Pia Dillner and other colleagues at STP Henriksdal (Sweden). Funding for the study was provided by the Research Council of Norway (185523/V50), Dublin City University (personal travel award to KVT), the European Centre for Drugs and Drug Addiction (EMCDDA), Research Foundation Flanders (fellowships to AvN and AC), the Joint Research Program (BTO) of the Dutch Water companies (EE and PdV), Fondazione Cariplo (grant 2009-3468-2009-3513) and Regione Lombardia (grant “Ricerca Innovativa”) (SC), the Spanish Ministry of Science and Innovation (Consolider-Ingenio 2010 CSD2009-00065) as well as by the Ministry and the European Regional Development Funds (ERDF) (project CTQ2010-16448), Ledningsgruppen för Åbo Akademis allmänna miljöprofilering (AM), University of Huddersfield Research Fund (BKH), CENAKVA no. CZ.1.05/2.1.00/01.0024, the GAJU no. 047/2010/Z (RG), the Spanish Ministry of Science and Innovation (project no. CTQ2010-18927; and Ramón y Cajal (JBC) funding), and the Spanish Ministry of Education (FPU IGM) grant.

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