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Illicit drugs consumption evaluation by wastewater-based epidemiology in the urban area of Palermo city (Italy)

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Abstract
Introduction. A wastewater-based epidemiology approach was performed to estimate the drug consumption in Palermo city, the fifth largest city of Italy with a population of 671 696 inhabitants, and to investigate the monthly variability of drug loads in wastewater from different areas of the city. A seven-months detection campaign was conducted at the two wastewater treatment plants of the city.
Methods. Following a pre-treatment, 32 samples of wastewater were analyzed by liquid chromatography-tandem mass spectrometry.
Results. We estimated a mean cocaine use in Palermo of 0.19 g/day/1000 people, corresponding to 1.90 doses/1000 people and cannabinoids use of 2.85 g/day/1000 people, corresponding to 35.62 doses/1000 people. Amphetamines residues in wastewater were always recovered in concentrations lower than the limit of quantification.
Conclusion. Our findings showed that drugs consumption in Palermo is in line with those of other Italian cities and that no significant differences on prevalence on cocaine and cannabinoids consumption were recorded in the different months of the survey, except for the summer period in a wastewater treatment plant of the city.

Key words
- drugs consumption
- wastewater analysis
- wastewater-based epidemiology
- cannabinoids
- cocaine
- amphetamines

INTRODUCTION

Monitoring illicit drug use is very difficult because of hidden and complex nature of drug-using behaviours, and also because, to date, no adequate indicator has been defined to evaluate the drug consumption in a population. True estimate of the illicit drugs is a central component of the work carried out by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [1]. Different indicators of drugs consumption have been used over time [2-7]. The direct questionnaires to the population, which represent the main element of a survey, are potentially affected by objective factors, namely the propensity of consulters to respond honestly to questions that constitute an illegal or socially reprehensible behaviour. Integration with other indirect consumers' data, including seizures by law enforcement, hospital admissions for specific causes or enrolment in addiction recovery centres, can also introduce an additional error in the temporal assessment of consumption. All indicators proposed alone are inadequate and underestimate the real consumption of drugs in populations. In fact, no indicator itself can be considered a complete evaluation tool, so that a multidisciplinary approach is required for the development of new fields of study. The concept of wastewater analysis to evaluate chemicals excretion in a community is far away in time [8-10]. In recent years, it has been defined a new method of investigation of illicit drug consumption in the population based on the analysis of urban wastewater that is called wastewater-based epidemiology (WBE). The WBE approach consists in the collecting and pooling by the sewage system of human excretion products of external or internal bodily origin (biomarkers) resulting from exposure to foreign chemical compound. EMCDDA has pointed out that this fast-developing discipline has the potential to complement and extend the existing epidemiological tools, providing evidence-based estimates of illicit drug use with

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relatively accurate detail as to time and location [11]. WBE detects geographical differences in drug use patterns, which were mostly consistent with data obtained by other approaches. Moreover, wastewater analysis has proven able to detect local and temporal patterns of drug use, demonstrating its potential to provide complementary information that is complementary to that provided by standard techniques. By analysing the raw wastewater composition (in terms of drugs concentration) it is possible to identify the main illicit substances consumed within an urban area. WBE can help also governments and local authorities to better struggle with the spread of drugs. More precisely, on the basis of the results of WBE, drugs prevention programs can be planned and implemented thus positively contributing to the economy (the illegal drugs trade in the 28 members European Union is worth at least € 24bn a year) and to the public health (drug use is one of the major causes of mortality among young people in Europe, both directly through overdose and indirectly through drug-related diseases, accidents, violence and suicide) [12, 13]. In recent years, wastewater-based epidemiology has been applied worldwide by several research groups at local and national scales, demonstrating the potential of the approach for quantifying illicit drug use at community level [14-16]. Few studies were conducted in South of Italy [17, 19-22] and no recent data were avaible to clarify the diffusion of the major drugs of abuse in Palermo, the fifth largest city of Italy with a population of 671 696 inhabitants, also in relation to the different area of the city and to the different periods of the year. Therefore, the aim of the study was to use a WBE approach to estimate the drug consumption in Palermo and to investigate the monthly variability of drug loads in wastewaters.

METHODS

Selection of drug target residues

The target residues were selected according to current knowledge of the metabolic fate of each active drug [23, 24], and the stability of candidate residues in wastewater [25, 26]. In particular, we evaluated cocaine and its metabolite benzoylecgonine (BEG), cannabinoids (11-nor-9-carboxy-delta9-tetrahydrocannabinol, or THC-COOH) and 3,4-methylenedioxy-N-methylamphetamine, or ecstasy (MDMA), methylenedioxyethylamphetamine (MDE), 2,3-methylenedioxyamphetamine (MDA) and methamphetamine for consumption of amphetamines. The following deuterated molecules were used as internal standards (IS): benzoylecgonine-d3, amphetamine-d6, MDMA-d5 and 11-nor-9-carboxy-Δ9-THC-d3. All reference standards were acquired from Cerilliant Corporation (Round Rock, Texas). The standards, available as solutions in methanol or acetonitrile (1 or 0.1 mg/mL), were diluted up to 1 μg/mL with methanol and stored at -20 °C in the dark. Working solutions containing all the substances to be analyzed were prepared before each analytical run. High-performance liquid chromatography methanol HPLC-grade, acetonitrile HPLC-grade, hydrochloric acid (37%), formic acid (98%), acetic acid (> 99%), and ammonium hydroxide solution (30%) were supplied by Sigma-Aldrich (Steinheim, Germany). Ultrapure water was obtained by purifying water in a Milli-Q Gradient A-10 system (Millipore, Bedford, MA).

Wastewater sampling

Samples were collected from the two Palermo full-scale wastewater treatment plants (namely, Fondo Verde – WWTP1 and Acqua dei Corsari – WWTP2). Both WWTPs treat the urban wastewater produced from the Palermo population by adopting a conventional activated sludge treatment. WWTP1 treats wastewaters produced within the northwestern catchment of the city (average influent flow rate equal to 17 149 m^3/day corresponding to around 45 000 inhabitants equivalent), while WWTP2 treats wastewater produced within the southeastern catchment of the city (average influent flow rate equal to 102 450 m^3/day corresponding to around 340 000 inhabitants equivalent). *Figure 1* represents the location of the two aforementioned catchments, the main sewer line, the submarine outfalls and the WWTPs. For each WWTP, adopting an automatic sampling device, 2 liters of 24-h composite samples of raw wastewater were collected between March and November 2015, with the exception of May and August. Sample were stored in bottles at 4 °C and filtered the same day of collection and processed within 2 days.

Sample processing and solid-phase extraction

Prior to extraction, samples were filtered preliminary with an 8 μm cellulose filter (Whatman, Kent, UK) and then with a 0.45 μm nitrate cellulose filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Then, drugs and their metabolites, except for THC-COOH, were extracted using mixed reversed-phase/cation-exchange cartridges (Bond Elut Plexa PCX Agilent, CA, USA), as described by Castiglioni *et al.* [12]. Briefly, wastewater samples (50 mL) were spiked with 50 μL of a mix solution of IS (1 μg/mL) and the pH was adjusted to 2.0 with 37% HCl. The SPE cartridges were conditioned before use by washing with 6 mL of methanol, 3 mL of Milli-Q water, and 3 mL of water acidified to pH 2. Samples were then passed through the cartridges under vacuum at a flow rate of 5 mL/min. Cartridges were vacuum-dried for 5 min, washed with 3 mL of methanol and eluted with 3 mL of a 2% ammonia solution in methanol. The eluates were dried using a vacuum rotary dryer (EZ-2-Plus Genevac, SP Scientific Industries, PA, USA) and added to 200 μl of water with 0.1% of acid formic, centrifuged at 1000 rpm for 10 min at 20 °C and transferred into glass vials to be analyzed.

Analysis of drug target residues

The eluates, except for THC-COOH, were analyzed by liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) using a TSQ Quantiva triple-stage quadrupole mass spectrometer system interfaced to UHPLC Ultimate 3000 (Thermo Fisher Scientific Inc., Waltham, USA). For THC-COOH detection the same instrumentation was used with addiction of TurboFlow™ online sample preparation technology. The recoveries, repeatability, instrumental limits of detection (LODs), and limits of quantification (LOQs) for the entire method were calculated in wastewater samples

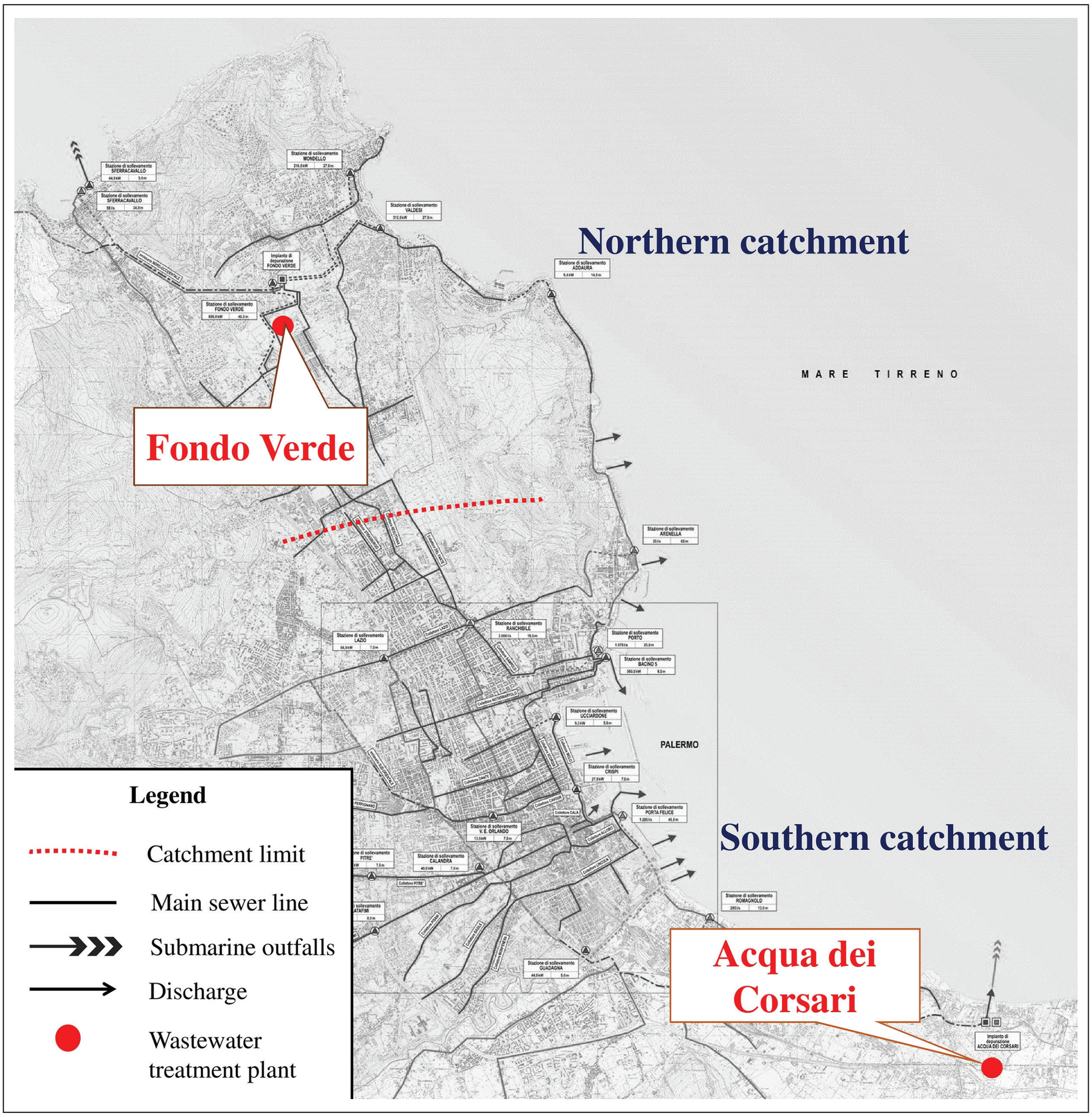

Figure 1
Schematic overview of the two Palermo full-scale wastewater treatment plants (WWTPs), catchment limit, main sewer line and submarine outfalls location.

as described by Castiglioni *et al.* [12] (data not shown). LOQs for drug target residues were 3.0 ng/L for BEG, 10.0 ng/L for cocaine, 5.0 ng/L for amphetamines and 10.0 ng/L for THC-COOH.

Back calculation

To achieve the amount of drug consumed by the population starting from the concentration found in wastewater, we used the method suggested by Zuccato *et al.* [16], modified by Gracia-Lor *et al.* [27]. Briefly, drugs load (g/day) at each sampling site – calculated from the drug concentration in wastewater (ng/L) and water flow rate (m^3/sec) – were used to estimate the loads of parent compound, multiplying by a factor of correction. This takes into account the metabolite/parent compound molar mass ratio and the average molar fraction of a parent compound dose that is excreted as metabolite. Drugs loads were then related to the local population equivalents (i.e. the number of people served by a WWTP). The estimated consumption (g per day per 1000 people) at each site was referred to the general population. To compare our estimates of collective drug consumption with others studies that mainly refer to drug use prevalence, we had to translate total consumed amounts of parent drugs into the corresponding number of doses. The average content of pure active drug in a typical dose taken by the most common route was assumed-to be approximately 100 mg for intranasal cocaine and 125 mg for smoked THC (based on high potency cannabis: 14% THC in hashish/marijuana [28].

Statistical analysis

Data analysis (counts, percentages, means) was performed with Excel software (Microsoft Office Excel 2016 for Apple™). The level of significance was calculated by two sample t test with unequal variance performed with the STATA MP statistical software package v14.1 for Apple™ (StataCorp).

RESULTS

A total of 32 samples of untreated wastewater were analyzed during the study period. Samples were taken every month from March to November 2015, with the exception of May and August, at the following intervals: March (n = 2), April (n = 2), June (n = 3) July (n = 4), September (n = 2), October (n = 2) and November (n = 1). In *Table 1* mean values for month of sampling for each drug are showed. Since concentrations of BEG and THC-COOH obtained from the two WWTPs were very similar, the mean of concentrations of each drug in each WWTP was considered representative of the whole urban area of Palermo. In particular, we obtained mean values of BEG of 194.94 ng/L in WWTP1 and 215.96 ng/L in WWTP2, corresponding in a cocaine use of 0.17 g/day/1000 people for urban areas served by WWTP1 and 0.15 g/day/1000 for those served by WWTP2. The BEG average concentration in both Palermo WWTPs was of 205.69 ng/L (standard deviation = 60.82). We have estimated a mean value of cocaine consumption in Palermo of 0.16 g/day/1000 people equivalent to 1.62 doses/1000 people. Cocaine was quantifiable in the influents of the two WWTP samples, as unmodified cocaine, with a concentration of 58 75 ng/L. Also, for THC-COOH we used a mean of drug concentration in wastewater, since values in the two WWTPs were similar (55.98 ng/L, 3.09 g/day/1000 people for WWTP1 and 62.12 ng/L, 2.76 g/day/1000 people for WWTP2). THC-COOH was detected in both the Palermo WWTPs with a mean concentration of 59.05 ng/L (standard deviation = 16.30), corresponding to a cannabinoids consumption of 2.92 g/day/1000 people equivalent to 36.52 doses/1000 people. The amphetamines concentration for all samples was always below the limit of quantification (LOQ). Significant differences in BEG recovery in WWTP1 were documented in relation to the different months of survey. In particular, we detected a BEG concentration of 154.33 ng/L (standard deviation = 55.49), corresponding to 0.14 g/day/1000 people and 1.38 doses/1000 people during summer holidays (from the second week of June to the first week of September, coinciding with the closure of schools) as compared to 235.55 ng/L (standard deviation = 30.70), corresponding to 0.21 g/day/1000 people and 2.12 doses/1000 people during the other months of survey (p = 0.004). The same differences were not observed for THC-COOH.

DISCUSSION

Our study evaluated across different months of the year the potential difference in drug consumption in the Palermo city general population served from the two urban WWTPs. The comparison showed no differences in drug consumption, except for cocaine in WWTP1, although the two systems serve different populations (45 000 for WWP1 and 330 000 for WWTP2). We used pooled estimates of the WWTPs to compare our data with those obtained from other studies conducted in Italian cities (> 350 000 people) and in Palermo in recent years [17, 18]. We estimated a prevalence of cocaine use in Palermo of 0.19 g/day/1,000 people, lower than those recorded in other recent Italian studies (0.43 in 2010, 0.26 in 2012 and 0.29 in 2014) [17]. Prevalence in cannabis use in Palermo in 2015 was 2.85 g/day/1000 people, in line with those of South Italy (2.74 in 2010, 1.45 in 2012 and 3.66 in 2014) [17]. It was quite difficult to compare data on the amphetamines consumption because they were below the limit of quantification of method. However, it is evident that even for this class of molecules the consumption in Palermo is lower than other Italian cities. Although use of amphetamines is lower in all Italian cities, especially when compared with other European cities as London (80 mg/day/1000 people) [16] and Berlin (115.8 mg/day/1000 people) [29], we have never detected these classes of drugs in wastewater, at least at concentrations above the limit of quantification. This could be explained by the different use of these drugs in relation to recreational activities. The use of amphetamines,

Table 1
Average drugs concentration and standard deviation (SD) of the influent wastewater of the two WWTPs and drug consumption in the urban area of Palermo

Date	Cocaine (SD) ng/l	BEG (SD) ng/l	Cocaine (g/day/1000 people)	Cocaine (doses/1000 people)	THC-COOH (SD) ng/l	Cannabinoids (g/day/1000 people)	Cannabinoids (doses/1000 people)
March	94.15 (24.55)	204.10 (27.71)	0.18	1.8	58.40 (29.55)	2.59	32.37
April	101.32 (42.13)	282.90 (25.41)	0.25	2.5	38.15 (3.04)	1.68	21.00
June	38.45 (18.14)	177.87 (63.89)	0.13	1.3	51.39 (19.06)	2.52	31.50
July	39.32 (12.45)	160.69 (39.91)	0.12	1.2	59.76 (19.83)	3.10	38.75
September	60.14 (17.80)	330.19 (104.52)	0.25	2.5	59.11 (6.97)	3.00	37.50
October	116.09 (40.21)	218.36 (47.37)	0.19	1.9	51.56 (29.33)	2.55	31.87
November	81.43 (49.56)	254.04 (50.26)	0.21	2.1	62.75(0.64)	3.22	40.25
Total (Mean)	75.82 (30.58)	232.59 (60.13)	0.19	1.90	54.44 (8.34)	2.85	35.62

ecstasy and similar molecules, in fact, could be related more closely to the frequencies of nightclub parties or electronic dance music parties generally held as "raves" [30-33], poorly present in the Palermo area. As reported from several authors, amphetamines use was found to be predominant in Germany while cocaine in Switzerland, confirming the north to south gradient of stimulants use in Europe (i.e. amphetamines being the main stimulant in northern countries, while cocaine is more widespread in the south of Europe) [1, 13]. The results obtained during the summer holidays (period June-first week of September) as compared to those obtained during the other months of the year, showed a significant lower use of cocaine during this period of the year in people served by WWTP1 (p = 0.004). Cocaine use tends to be lower in summer in the same Palermo areas, while cannabinoids use resulted almost constant in the different seasons of the survey period. We hypothesize that the decrease in cocaine use could be attributable to the closure of schools, so many people move to summer residences, out of town, decreasing the number of inhabitants in that residential area of the city. However, this does not find confirmation in decreased flow of wastewater at the WTPs, probably due to an increase of water consumption during the summer period. As reported by other Authors [34, 18], the choice of the sampling period is very important and have to include both winter and summer months to get a more accurate drug consumption estimate in the general population.

CONCLUSIONS

Our findings are in line with those of other studies investigating how illicit drug loads vary in small catchments more than in large ones [18, 35-37]. Although we tried to identify significant differences in the consumption of drugs in different areas of Palermo, we didn't detect them except during the summer period in the city area served by WWTP1. However, we speculate that is possible to obtain more particular data investigating by different district of the city, maintaining the aggregated data to avoid identification of particular group within the community [30, 38, 39] and finding a correlation with other information as income per capita, employment and level of education. This information could be relevant to identify the appropriate contrast strategies and actions, as well as effective health education policies. Further studies are needed to explore these epidemiological aspects. Despite the problems and the limitations described in other studies [38, 40], WBE can be considered an additional tool to the available classic indirect indicators of drugs consumption and abuse to support institutions in order to understand and monitoring drug addiction and effectiveness of health education policies for drug prevention [40, 41].

Authors' contribution statement

CMM and GV planned and designed the study with contributions from AC, FT, WM. CMM, GV, AC and DP organized the collection of the wastewater samples which were analyzed by FDG and DP. CMM and GV performed data analysis and interpreted the results with contribution from all co-authors. CMM, with contribution from FT and WM, drafted the manuscript, which was critically revised by all co-authors. All authors are aware of the content, and accept responsibility, for the manuscript.

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Conflict of interests statement

Authors declares also that no conflict of interest exist that could inappropriately bias conduct and finding of the study and that no grant support was used for this study.

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