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**DRUGS OF ABUSE AND ALCOHOL CONSUMPTION AMONG DIFFERENT GROUPS
OF POPULATION ON THE GREEK ISLAND OF LESVOS THROUGH SEWAGE-BASED
EPIDEMIOLOGY**

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Abstract

The occurrence of 22 drugs of abuse, their metabolites, and the alcohol metabolite ethyl sulphate was investigated in raw sewage samples collected during the non-touristic season from three sewage treatment plants (STPs), which serve different sizes and types of population in the Greek island of Lesbos. Using the sewage-based epidemiology approach, the consumption of these substances was estimated. Five target analytes, cocaine (COC), benzoylecgonine (BE), 3,4-methylenedioxymethamphetamine (MDMA), 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) and ethyl sulphate (EtS) were detected at concentrations above their limit of quantification, whereas the rest eighteen target compounds were not detected. The most often detectable compound was found to be THC-COOH. It was identified almost at all sampling days with concentrations ranging between < 20 and 90 ng L^{-1} , followed by EtS (range $< 1700 - 12,243 \text{ ng L}^{-1}$). COC, BE, and MDMA were present only in the STP that serves Mytilene (the main city of the island), at mean concentrations of 3.9 ng L^{-1} for COC (95% CI: 1.7-6.1), 9.4 ng L^{-1} for BE (95% CI: -1.6-23) and 3.2 ng L^{-1} for MDMA (95% CI: 1.2-5.1). Back-calculations to an amount of used substance indicated more intense use of drugs among city population than rural and University population with average values of 9.5 and 1.2 mg day^{-1} per 1000 inhabitants for COC and MDMA, respectively, and 2.8 g day^{-1} per 1000 inhabitants for tetrahydrocannabinol (THC), the active ingredient of cannabis. Alcohol consumption was observed to be higher in the city population ($5.4 \text{ mL pure alcohol per day per inhabitant}$) than in the rural population ($3.4 \text{ mL pure alcohol per day per inhabitant}$), but the difference was not statistically significant. Consumption of THC differed significantly among the three STPs.

Keywords: psychoactive compounds, alcohol consumption, sewage-based epidemiology, biomarker analysis, liquid chromatography-mass spectrometry

1. Introduction

The use of psychoactive substances, for e.g. cocaine or cannabis, has occurred since ancient times (Sullivan and Hagen, 2002) and was generally related with religious rites and medicinal therapies at that time. Since then the manner of their use has evolved significantly and they are increasingly consumed annually: some 243 million people of the world population aged 15-64 used an illicit drug in 2012 (UNODC, 2014) and this number increased by 3 million in 2013 (UNODC, 2015). In Europe, according to the last annual report of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2015a), 11.7% (14.6 million), 1.9% (2.3 million) and 1.4% (1.8 million) of young people aged between 15 - 34 used cannabis, cocaine or ecstasy during the last year, respectively. In Greece, 10.8% of the people in this age group have smoked cannabis at least once in their life, and 1.0% and 0.6% have consumed cocaine and ecstasy, respectively (EMCDDA 2012).

Alcohol (or ethanol) is a well-known and legal psychoactive substance with dependence-producing properties and according to the World Health Organization (WHO), globally, individuals older than 15 years old consume on average 6.2 L of pure alcohol per year, which is translated into 13.5 g of pure alcohol per day (WHO, 2014). In Greece the consumption of alcohol is almost twice as high as the global average. According to the same source, the total per capita consumption in 2010 in Greece for people > 15 years old was between 10.0 and 12.4 L of pure alcohol (WHO, 2014).

Soon after consumption, drugs of abuse and alcohol are metabolised to some extent and are finally excreted from the human body through mainly urine and faeces and end up in the sewer system. Based on their concentrations in raw sewage, important conclusions can be deduced regarding the use of these compounds. Up to now, thanks to sewage-based epidemiology (SBE), estimation of consumption of drugs of abuse and alcohol have been accomplished in several European and overseas countries such as Italy (Zuccato et al., 2008; Mari et al., 2009), Croatia (Terzic et al., 2010), France (Karolak et al., 2010), Spain (Postigo et al., 2010; Mastroianni et al.,

2014), Belgium (van Nuijs et al. 2011), Norway (Reid et al., 2011), Australia (Lai et al. 2013a), Greece (Ort et al. 2014), UK (Baker et al. 2014) United States (Subedi and Kannan 2014), Canada (Yargeau et al. 2014) and China (Khan et al., 2014). In these studies, data originate mainly from sewage samples collected at sewage treatment plants (STPs) which serve a population >70,000 inhabitants.

In Greece, consumption of drugs of abuse has only been estimated for the city of Athens (Ort et al., 2014) where almost 40% of the total Greek population live, while no data exists for smaller cities. Literature reveals that SBE data on consumption of drugs of abuse is focused mainly on population of bigger cities in the countries studied, whereas much less information is available for people living in less populated areas (Banta-Green et al., 2009; Nefau et al., 2013; Damien et al., 2014; Östman et al. 2014). As a consequence, it is not known a) whether drugs of abuse are used or not in less populated areas, b) which drugs of abuse are preferably consumed in such areas, and c) if different populations regarding the place of living (city, village) and their profile (students, general population) present different habits in drug use. Therefore, it becomes evident that there is a need to collect information from more cities with different demographics within a country. Concerning alcohol consumption using SBE, available data is limited, since up to now there are only reports for Spain, Italy (Mastroianni et al., 2014; Rodríguez-Álvarez et al., 2014; Rodríguez-Álvarez et al., 2015) and Norway (Reid et al., 2011).

Based on the above observations, the objectives of the present study were to i) evaluate the presence of drugs of abuse and their metabolites, as well as ethyl sulphate (EtS) (a phase-II alcohol metabolite) in raw sewage from a Greek province and ii) estimate the consumption of drugs of abuse and alcohol among different population groups [an urban population, a rural population and University population (including both students and staff)]. To achieve these goals, raw 24-h composite sewage samples were collected during the non-touristic season from three different STPs in Lesvos Island. STP-A and STP-B were sampled for a week, whereas in STP-C sampling lasted five consecutive weekdays. Samples were analysed for EtS, drugs of abuse and their metabolites

belonging to several classes of drugs (Table S1). Furthermore, back-calculations were applied to detectable concentrations to assess the use of drugs of abuse and alcohol in the studied populations.

2. Material and methods

2.1. Reagents and materials

LC-grade methanol (MeOH) and acetonitrile (ACN), ammonium hydroxide (NH₄OH), hydrochloric acid (HCl), ammonium acetate (and acetic acid (CH₃COOH) were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained by purifying demineralized water in an Elga LabWater Purelab Flex system (Veolia Water Solutions & Technologies Belgium, Tienen, Belgium). Oasis HLB and MCX SPE cartridges (both 60 mg, 3 mL) were acquired from Waters (New Bedford, MA, USA). Analytical standards of ethyl sulphate sodium salt (EtS), cocaine (COC), benzoylecgonine (BE), ecgonine methyl ester (EME), amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA), methadone (MTD), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), 6-monoacetylmorphine (6-MAM), methoxetamine (MXE), butylone, ethylone, methylone, methiopropamine (MPA), 4-methoxymethamphetamine (PMMA), 4-methoxyamphetamine (PMA), mephedrone (MEPH), methylenedioxypyrovalerone (MDPV), ketamine (KET), dehydronorketamine (DHNK), norketamine (NK), 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), and their deuterated analogues EtS-D₅, COC-D₃, BE-D₃, EME-D₃, AMP-D₈, METH-D₈, MDMA-D₅, MTD-dD₉, EDDP-D₃ and 6-MAM-D₃, butylone-D₃, ethylone-D₅, methylone-D₃, MEPH-D₃, MDPV-D₈, NK-D₄, KET-D₄, THC-COOH-D₃ (purity >98%) were purchased either from Cerilliant (Round Rock TX, USA) at concentrations of 1 mg mL⁻¹ or 100 µg mL⁻¹ in MeOH (or acetonitrile), or from Athena Enzyme Systems (Baltimore MD, USA) or Toronto Research Chemicals (North York, Canada) in neat powder. Appropriate dilutions and working mixtures were prepared in MeOH (van Nuijs et al., 2009b; van Nuijs et al., 2014; Kinyua et al., 2015).

2.2. Sewage sampling

Using an autosampler, which was adjusted to collect 6 mL min^{-1} , 24-h time-proportional composite raw sewage samples were collected from three STPs which serve different populations (Table S2): STP-A serves the capital of the island, Mytilene (population served: 26,000), STP-B serves two small villages (population served: 1,250) and STP-C serves the University Campus (population served: 1,600) which does not cover dormitories but only offices, laboratories, lecture rooms and a student restaurant. As a result, during the weekdays only students who attend lectures or lab activities plus the staff of the University (academic and administrative) visit the Campus. It is worth mentioning that in STP-C most of the people belong to the 20-39 age group (40%), whereas in STP-A and STP-B less than 30% of the population is in the range of 20-39 years old (Fig. S1). Sampling was conducted between the 10th of February and 10th of March 2015 to avoid contribution of touristic activities. Each site was sampled for seven consecutive days, except for STP-C where sampling lasted five consecutive weekdays as no significant amount of sewage was expected to be produced during the weekend. Sewage flow rates in STP-A, B and C were measured by the STPs. Samples were directly filtered after collection through a $0.7 \text{ }\mu\text{m}$ glass fiber filter (GF/A, Millipore) to remove solid particles and stored at $-20 \text{ }^{\circ}\text{C}$ until analysis.

2.3. Sample preparation

Sample clean-up and concentration was accomplished by solid-phase extraction (SPE). For COC, BE, EME, AMP, MDMA, METH, 6-MAM, MTD, EDDP and some of the new psychoactive substances (MXE, butylone, ethylone, methylone, PMMA, PMA), Oasis MCX cartridges were used according to analytical methods developed from van Nuijs et al. (2009b) and Kinyua et al. (2015). For the remaining target drugs of abuse and metabolites (MEPH, MDPV, KET, DHNK, NK, and THC-COOH), Oasis HLB cartridges were used for extraction based on an earlier developed and validated analytical method (van Nuijs et al., 2014).

Samples analysis for the determination of EtS was performed using a method which was adapted from a method already developed in the Mario Negri Institute after personal communication. Specifically, 1 mL of raw sewage was transferred to a 2 mL Eppendorf tube and centrifuged for 5 min at 8000 rpm. Afterwards, an aliquot of 190 μL was transferred to a 1.5 mL Eppendorf tube, spiked with 10 μL of internal standard ($1\text{ ng } \mu\text{L}^{-1}$) and vortexed for 1 min. Next the sample was transferred to a centrifugal filter (0.2 μm), centrifuged for 5 min at 8000 rpm, transferred to a vial for injection.

2.4. LC-MS/MS analysis

Analyses for drugs of abuse and metabolites were carried out using an Agilent 1260 High Pressure LC (HPLC) system comprising a degasser, a binary high-pressure gradient pump, a thermostated LC column compartment and an autosampler module. Separation of the target compounds was achieved using appropriate chromatographic columns [Phenomenex Luna HILIC (150 mm $\times$ 3 mm, 5 μm) and Phenomenex Gemini C18 (50 mm $\times$ 2 mm, 3 μm)]. The mobile phases were composed of (A) ammonium acetate 5 mM in milli-Q water and (B) ACN and suitable gradient elution programs were used. The LC system was coupled to an Agilent 6410 triple quadrupole mass spectrometer (MS/MS) equipped with an electrospray ionisation interface operating in both positive and negative mode. More details regarding the validated LC-MS/MS analysis of drugs of abuse and their metabolites can be found in van Nuijs et al., (2009b, 2014) and Kinyua et al. (2015).

Sample analysis for EtS was performed using a similar LC-MS/MS (Agilent 6460) system with the electrospray interface operating in negative ionisation mode. The column which was used was an Atlantis T3 (2.1 mm x 150 mm, 3 μm) obtained by Waters (Milford, MA, USA). During analysis, column and precolumn temperatures were set at 20 $^{\circ}\text{C}$ and the flow rate at 0.18 mL min $^{-1}$. An injection volume of 4 μL was used. The mobile phase consisted of (A) milli-Q water with 0.1% CH₃COOH and (B) ACN. The gradient elution was performed as follows: 2% B at 0 min, 15% B at

10 min, increase to 95% B in 1 min, hold for 1 min and decrease to 2% in 1 min. Source parameters were as follows: gas temperature 250 °C, gas flow 12 L min⁻¹, nebulizer 35 psi, capillary voltage 2750 V. The optimized mass spectrometer compound dependent parameters for both EtS and EtS-D₅ are given in Table S3.

2.5 Estimation of consumption

Rates of consumption were estimated from biomarker levels in sewage samples (Zuccato et al. 2008; Castiglioni et al., 2013; Rodríguez-Álvarez et al. 2015). Initially, biomarker loads (in g/day) were calculated by multiplying the concentrations of the target analytes measured in raw sewage samples and the daily flow rates recorded in the STPs. Dividing these mass loads with the population served by each STP (based on census data given by the Hellenic Statistical Authority in the present study for STPs A and B and the mean value of population, which was measured every day, for STP-C) results in population-normalized mass loads of the biomarkers (in mgday⁻¹ per 1000 inhabitants). These values are in a final stage transformed into an actual consumed substance per day per 1000 inhabitants taking into account the percentage of target substance excreted as biomarker or drug target residue (DTR) in urine and the parent drug-to-DTR molar mass ratio. The DTRs used in the present study are presented in Table S4. The above calculation procedure can be summarized in the following equation (Eq. 1):

$$\begin{aligned} & \text{Consumption (mg day}^{-1}\text{1000 inhabitants}^{-1}\text{)} \\ &= \frac{[\text{Concentration of analyte (ng L}^{-1}\text{)] * sewage flow rate (L d}^{-1}\text{) / } 10^6 \text{] * correction factor}}{\frac{\text{population served (inhabitants)}}{1000}} \end{aligned} \quad (\text{Eq. 1})$$

For every compound the correction factor was calculated according to Eq.2:

$$\text{Correction factor} = \frac{\frac{\text{molar mass ratio of parent compound}}{\text{DTR}}}{\% \text{ of parent compound excreted as DTR in urine}} \quad (\text{Eq. 2})$$

2.6. Statistical analysis

Statistical analysis was performed with Prism5 for Windows. The Kolmogorov-Smirnov test was used to determine whether the consumption for each compound had a normal distribution among the three STPs. Consumption of THC showed a normal distribution ($p\text{-value} > 0.10$) and thus one-way ANOVA was used with Tukey–Kramer’s post-hoc test for significant differences among groups. As for alcohol consumption, results in the two STPs (where EtS was detected) indicated no normal distribution ($p\text{-value} = 0.0044$) and for this reason the non-parametric Mann Whitney t-test was used to evaluate any statistical differences between the sampling sites.

3. Results and discussion

3.1 Occurrence of target compounds in sewage samples

Overall, five out of 23 target analytes were measured at concentrations above the limit of quantification (LOQ) in all sewage samples, indicating a lower number of consumed drugs in a Greek province compared to the capital (Athens) (Ort et al., 2014). THC-COOH was the most often detectable compound since it was quantifiable in 84% of the samples (STP-A: 37%, STP-B: 37%, STP-C: 11%), followed by EtS which was detected in 74% of the samples (STP-A: 37%, STP-B: 37%) at concentrations higher than the LOQ (Fig. S2). Finally, COC, BE and MDMA were detected at percentages between 26 and 32%. The other 18 investigated compounds could not be quantified in any of the samples indicating negligible use of these compounds. Perhaps one of the reasons for the non-quantification of many drugs was the time of sample collection since February is a non-touristic season for Lesvos Island.

Among the three different investigated STPs, as it was expected only STP-A was found to be “positive” for COC, its major metabolite BE, and MDMA (Table 1). The mean ratio of COC/BE (Table S5) in the samples, when both compounds were detected, was calculated to be 0.40 and is in accordance with the value of 0.38 reported by van Nuijs (2009c) in Belgium and 0.39 reported by

Mari et al. (2009) in Italy. It should be noted that the COC/BE ratio of 1.46 on Wednesday (12th of February) could be identified as an outlier and thus it was not taken into account. Lai et al. (2013b) also reported high ratios of COC/BE which were possibly attributed to direct dumping of cocaine, perhaps due to a raid by the Police and/or hand washing after use rather than human metabolism.

The metabolite of THC, THC-COOH, was detected in all examined STPs. Its concentrations ranged between 16.9 and 90 ng L⁻¹ (Table 1) and these values are one or two orders of magnitude higher than those measured for COC, BE and MDMA.

Regarding EtS the mean concentrations were 5283 (95% CI: 1968-8597) and 4023 (95% CI: 2318-5728) ng L⁻¹ for STP-A and STP-B, respectively but <LOQ in STP-C (Table 1). So far, no data is available in Greece regarding the occurrence of EtS in sewage, while limited data is available for Italy, Spain and Norway. Much higher concentrations of EtS compared to drugs of abuse were not surprising since alcohol legally is consumed by adults according to the Greek legislation.

3.2. Estimation of drugs of abuse consumption

Overall results permitted the estimation of consumption for COC, MDMA, THC and alcohol. COC consumption in STP-A was found to be between 0.5 and 33 mg day⁻¹ per 1000 inhabitants with a mean value of 9.5 mg day⁻¹ per 1000 inhabitants (95% CI: -1.43-20.4) (Fig. 1). According to EMCDDA, a typical dose of COC is 100-200 mg (EMCDDA, 2015b). Using the lower limit of 100 mg, the above calculated cocaine consumption corresponds to a range from 0.005 to 0.33 doses day⁻¹ per 1000 inhabitants and a mean value of 0.13 doses day⁻¹ per 1000 inhabitants (95% CI: -0.0138-0.201). Of course, since the dose amount is highly variable, the above data are only estimations. Over weekends, COC consumption was estimated to be higher than the consumption during weekdays (Fig. 1). This observation agrees with the fact that COC has a recreational character and that a higher consumption is expected during weekends (Damien et al., 2014).

According to data reported in the Appendix S3 of Ort et al. (2014), mean BE mass load in Athens was 59 mg day^{-1} per 1000 inhabitants in 2013. Modifying this value with the correction factor of 3.59 (Table S4), the consumption of COC in Athens is estimated to be $213 \text{ mg}^{-1} \text{ day}^{-1}$ per 1000 inhabitants. This value is much higher than data observed in STP-A, indicating the lower use of the compound in the Greek province than in Athens. On the contrary, present results were found to be comparable with other cities like Budweis (Czech Republic), Turku (Finland), Piestany (Slovakia) and Umeå (Sweden). The served population in the above cities in 2013 varied from 30000 (Piestany) to 275000 inhabitants and the estimated consumptions after correcting BE mass loads given in Appendix S3 of Ort et al. (2014) with the correction factor of 3.59, were in the range of 5 (Turku) to 18 (Umeå) mg day^{-1} per 1000. Moreover, results were closer to the lower range of concentrations (23 mg day^{-1} per 1000 inhabitants) reported by Nefau et al. (2013) in a study which was conducted in France at STPs serving less than 36,000 inhabitants.

Compared with other studies, consumption of COC in STP-A was found to be much lower than the estimated values worldwide. Zuccato et al. (2005, 2008) reported a mean cocaine consumption from 440 to 910 mg day^{-1} per 1000 inhabitants in Italy, while in South Wales and London estimated consumption of COC was on average 600 and 1200 mg day^{-1} per 1000 inhabitants, respectively (Kasprzyk-Hordern et al., 2009; Zuccato et al., 2008). In Spain, similar ranges were estimated by Postigo et al. (2010), while Subedi and Kannan (2014) estimated COC consumption as high as 1380 mg day^{-1} per 1000 inhabitants in Albany Area, NY state, USA.

Consumption of MDMA (or ecstasy), in STP-A (Fig. 1) varied from 0.2 to 2.2 mg day^{-1} per 1000 inhabitants with a mean value of $1.2 \text{ mg}^{-1} \text{ day}^{-1}$ per 1000 inhabitants (95% CI: 0.52-1.85). As reported in Appendix S3 of Ort et al. (2014) the mean mass load of MDMA in Athens was found to be 2 mg day^{-1} per 1000 inhabitants in 2013. Using the correction factor of 1.5 (Table S4) the resulting consumption of MDMA in Athens is estimated to be $3 \text{ mg}^{-1} \text{ day}^{-1}$ per 1000 inhabitants and is comparable with the MDMA use estimated in the present study. Compared with other countries, results are comparable with Nicosia (Cyprus). Based on data reported in Appendix S3 from Ort et

al. (2014) the estimated consumption of MDMA in Nicosia is 1.5 mg day^{-1} per 1000 inhabitants for MDMA. Regarding other studies, present data was found to be comparable with the lower levels reported by Zuccato et al. (2008) and Andrés-Costa et al. (2014). Zuccato et al. (2008) estimated MDMA consumption varied between 5 (London and Milan) and 12 (Lugano) mg day^{-1} per 1000 inhabitants⁻¹, while Andrés-Costa et al. (2014) reported values in the range of 2.9 to 21.9 mg day^{-1} per 1000 inhabitants in Spain.

MDMA consumption seems to be lower (Fig. 1) compared with COC. Perhaps this fact is due to the lower prevalence of MDMA use in Greece. According to recent official estimations (EMCDDA, 2015a) among the adult Greek population aged 15–64 years, the lifetime prevalence of MDMA use is almost half (0.4%) the lifetime prevalence of COC (0.7%).

Cannabis consumption in Lesvos Island varied from 0.05 to 3.4 g day^{-1} per 1000 inhabitants with a mean value of 1.5 g day^{-1} per 1000 inhabitants (95% CI: 0.92-2.0) (Table S6). Regarding the three different sampling sites, the highest mean value was observed in STP-A followed by STP-B and STP-C with 2.8 (95% CI: 2.4-3.1), 1.2 (95% CI: 0.83-1.5) and 0.1 (95% CI: 0.013-0.19) g day^{-1} per 1000 inhabitants, respectively. Since the University Campus does not provide student housing, both students and staff are thus only present at the campus in the daytime from Monday to Friday, and this fact could be an explanation for the lower consumption of cannabis observed in STP-C.

Assuming that an average ‘reefer’ cigarette contains around 200 mg of herbal cannabis or cannabis resin (EMCDDA, 2015c), the above mean consumptions correspond to about 14, 6 and <1 doses day^{-1} per 1000 inhabitants for the three STPs, respectively. Comparing the estimated consumptions with those found for other cities, the obtained values (except from those estimated for STP-C) were in accordance with data reported in the literature concerning mainly southern countries. Terzic et al. (2010) estimated consumptions of cannabis as high as 3.7 g day^{-1} 1000 inhabitants⁻¹ in Zagreb, while Zuccato et al. (2008) a value of 3.7 g day^{-1} 1000 inhabitants⁻¹ in Milan. Similarly, Andrés-Costa et al. (2014) in two STPs close to Valencia city reported values between 1.6 and 10.5 g day^{-1} 1000 inhabitants⁻¹. On the other hand, compared to Athens the results of the present study were

found to be significantly lower. According to data reported in Appendix S3 of Ort et al (2014) the calculated mass load of THC-COOH in Athens were 104 mg day^{-1} per 1000 inhabitants which corresponds to a consumption of 15.8 g day^{-1} per 1000 inhabitants of THC.

Regarding daily variations, results are shown in Fig.2. Although no clear trend was observed, slightly higher use of THC in STP-A was obtained on Saturday probably due to the existence of more recreational places (cafes, pubs, etc.) in the main city of the island. Furthermore, quite high consumption was also observed on Monday which could be linked to the metabolism of THC which reaches maximum urinary concentrations of THC-COOH 6-10 hrs after smoking of cannabis, the relatively long period of excretion as well as with the time needed for sewage to be transferred via the sewerage system to the STP (Brenneisen et al. 2010). The highest consumption in STP-B was observed during the week and specifically on Thursday. It should be noted that the sampling week in STP-B coincided with the week of the Greek “Carnival” where several events took place throughout the entire week and perhaps this could explain the different pattern of use observed.

Statistical analysis indicated that THC consumption was significantly different between the three STPs (Fig. 4c, one-way ANOVA: $p < 0.0001$). Since the distribution of population among the different age groups is quite similar in STP-A and STP-B (Fig. S1), the above observation could be attributed to the differences in population type (city population in STP-A, semi-agricultural population in STP-B) that is served by the two STPs. Nefau et al. (2013) also observed lower prevalence of drug consumption in rural areas than in cities. Differences between STP-A or STP-B and STP-C were, probably, due to the absence of dormitories at the University Campus, which led to the inevitable loss of important biomarker pulses (e.g. morning toilet visit) in the sewage samples.

Compared to COC and MDMA, THC use was found to be more common. This preference for cannabis is in accordance with the official data reported by the EMCDDA (2015a) where the lifetime prevalence of cannabis use in Greece among the people aged 15-64 years is 8.9%. This value is 22 and 13 times greater than the reported prevalence percentages of MDMA and COC,

respectively. Furthermore, higher consumption of cannabis might also reflect easier sustaining supplies of this drug into the island, in contrast to COC and MDMA which must be “imported” into the island from external sources. Since Lesvos is an island, authorities can control the entry of drugs more efficiently and perhaps this lower availability is another reason for the lower use of COC and MDMA.

3.3. Estimation of alcohol consumption

Alcohol consumption in Lesvos varied from 1.7 to 11.2 mL day⁻¹ per inhabitant with an average value of 5.4 (95% CI: 1.9-8.9) and 3.4 (95% CI: 1.8-5.1) mL day⁻¹ per inhabitant in STP-A and STP-B, respectively (Table S6). According to the WHO (2014), alcohol consumption by type of alcoholic beverage is distributed in Greece as 28% beer, 47% wine, 24% spirits (distilled beverages) and 1% other (one or several other alcoholic beverages). Furthermore spirits, wine and beer contain on average 40, 12 and 4.5% of pure alcohol (percentage of total volume), respectively (Mastroianni et al., 2014). Taking into account the above percentages, the obtained overall mean value of 72 L day⁻¹ of pure alcohol (95% CI: 16-129) (for STP-A and STP-B) corresponds to the consumption of approximately 43 L of spirits, 448 L of beer or 282 L of wine per day in the investigated area.

Compared to the data reported by the “Global Information System on Alcohol and Health” of the WHO, the results of the present study were found to be lower. According to the WHO (2014), the annual per capita consumption of alcohol in Greece, considering the total population, was 13.3 L per adult in 2010 which is translated to 36.4 mL day⁻¹ inhabitant⁻¹. This reported consumption is about 7 and 11 times higher than the present values for STP-A and STP-B, calculated based on the SBE approach. Several reasons could explain these discrepancies such as the fact that in official reports consumption estimations are usually based on sales records and population interviews or the population of the whole country is taken into account and local differences are often not considered (Rodríguez-Álvarez et al., 2014). So far, few studies are available regarding alcohol consumption using SBE. Rodríguez-Álvarez et al. (2014) estimated average alcohol consumption as 16.3 mL

day⁻¹ per inhabitant in NW Spain. A value of 18 mL day⁻¹ per inhabitant has been reported by Reid et al. (2011) in Oslo and by Mastroianni et al. (2014) in Barcelona. More recently Rodríguez-Álvarez et al. (2015) estimated mean alcohol consumption in Santiago de Compostela and Milan as 13.6 and 5.1 mL day⁻¹ per inhabitant, respectively.

Regarding variation of alcohol consumption during the week, as can be seen in Fig. 3, higher values were calculated on Sunday, for both STPs, and these observations were similar to those made by other authors (Mastroianni et al., 2014; Rodríguez-Álvarez et al., 2014; 2015). Furthermore, a high consumption of alcohol in the region served by STP-A was observed on Tuesday (10.5 L of ethanol day⁻¹ per 1000 inhabitants), although there was no special festival or sports event on that day. The above results do not present any clear trend. Greece is part of a group of southern European countries where alcohol is considered an important part of their culture (Foster et al., 2007). As a result, the observed distribution in the present study could be considered as an evidence that Greeks do not have any special preference on the day of the week to drink in contrast to other countries. For instance, in the Nordic countries, higher consumption over the weekend is an expected pattern of use (Reid et al. 2011). Regarding statistical analysis, alcohol consumption did not differ statistically ($p=0.3176$) between STP-A and STP-B (Fig. 4d).

4. Conclusions

In the present study, four out of 22 drugs of abuse and metabolites (COC, BE, MDMA and THC-COOH) were detected at concentrations above LOQs in raw sewage samples of a Greek island (Lesvos) during the non-touristic season. COC, BE and MDMA were observed only in the main city (STP-A) at mean concentrations of 3.9, 9.4 and 3.2 ng L⁻¹, respectively. THC-COOH was the most often detectable drug biomarker, measured in all sampling sites, indicating its wide usage regardless the profile of population (city, rural and university population). EtS was detected for the first time in sewage from Greece. Among the different STPs, all target compounds were higher in STP-A (city) and lower in STP-C (University Campus).

Estimation of consumptions indicated very low and low use of MDMA and COC, respectively but somewhat higher use of THC in Lesvos. Regarding alcohol consumption no significant difference was observed between city and rural population.

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Table 1. Concentrations (in ng L⁻¹) of drugs of abuse and metabolites as well as ethyl sulphate (ethanol metabolite) in raw sewage collected from different sewage treatment plants (STPs) in Lesvos Island. THC-COOH: 11-nor-9-carboxy-delta-9-tetrahydrocannabinol; COC: cocaine; BE: benzoylecgonine; MDMA: methylenedioxymethamphetamine; EtS: ethyl sulphate; SD: standard deviation.

Target compound	STP-A (city population)		STP-B (rural population)		STP-C (student population)	
	Mean (SD) (ng L ⁻¹)	Range (ng L ⁻¹)	Mean (SD) (ng L ⁻¹)	Range (ng L ⁻¹)	Mean (SD) (ng L ⁻¹)	Range (ng L ⁻¹)
THC-COOH^a	70.7 (13.4)	49.5-90.2	34.9 (9.4)	22.5-50.0	16.9 (10.3)	<LOQ-32.9
COC^b	3.9 (2.4)	<LOQ-7.5	<LOQ	<LOQ	<LOQ	<LOQ
BE^b	9.4 (13.4)	<LOQ-37.5	<LOQ	<LOQ	<LOQ	<LOQ
MDMA^b	3.2 (2.1)	<LOQ-6.2	<LOQ	<LOQ	<LOQ	<LOQ
EtS^c	5283 (3584)	2190-12,243	4023 (1844)	2316-7814	<LOQ	<LOQ

^a: LOQ = 20 ng L⁻¹ (van Nuijs et al., 2013)

^b: LOQ = 1 ng L⁻¹ (van Nuijs et al., 2009)

^c: LOQ = 1700 ng L⁻¹ (present study regarding EtS)

Fig. 1. Consumption of cocaine (COC) and methylenedioxymethamphetamine (MDMA) for STP-A (city population) in Lesvos Island during one week period.

Fig. 2. Daily variation of tetrahydrocannabinol (THC) consumption (g per day per 1000 inhabitants) estimated from raw sewage from different STPs of Lesvos Island. STP-A: city population, STP-B: rural population, STP-C: University population.

Fig. 3. Daily variation of ethanol consumption (L per day per 1000 inhabitants) estimated in raw sewage from different STPs of Lesvos Island. STP-A: city population, STP-B: rural population.

Fig. 4. Mean consumption of cocaine (COC), methylenedioxymethamphetamine (MDMA), tetrahydrocannabinol (THC) and alcohol in the three sewage treatment plants (STP-A: city population, STP-B: rural population, STP-C: University-population) of Lesvos Island. Same letters indicate no statistical differences at 95% confidence level; t-bars represent 95% confidence interval. For COC and MDMA in STP-B and STP-C as well as for ethanol in STP-C, LOQs values were used only for comparison since concentrations of the compounds in raw sewage were found to be <LOQs.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.