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The first wastewater-based epidemiology study for alcohol monitoring in Cyprus: Temporal and spatial consumption trends from a one-year study

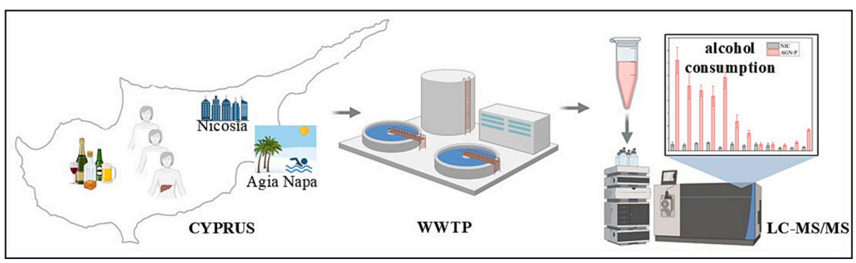
Magda Psichoudaki [a], Dimitrios Tzelios [b], Maria Savvidou [c], Christos Mina [c], Costas Michael [a], Despo Fatta-Kassinos [a,d,*]

[a] Nireas-International Water Research Centre, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus
[b] Paralimni and Agia Napa Sewage Treatment Plant, 5330 Agia Napa, Cyprus
[c] Cyprus National Addictions Authority, 2027 Nicosia, Cyprus
[d] Department of Civil and Environmental Engineering, School of Engineering, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus

HIGHLIGHTS

- First application of WBE for systematic monitoring of alcohol consumption in Cyprus
- Variations of alcohol consumption between the capital and coastal areas
- Alcohol consumption was significantly higher in the coastal areas during the summer.
- Significant correlation of alcohol consumption with certain illicit stimulants

GRAPHICAL ABSTRACT



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ABSTRACT

Recent studies conducted globally have identified a rising trend in alcohol consumption during the last years, generating a great concern, due to the negative consequences on human health and the broader effects on society. Monitoring of ethyl sulphate (EtS) by applying wastewater epidemiology (WBE) has been proven to be an effective technique for the assessment of alcohol use within a community. This work reports the results of a WBE-based study, conducted for the first time in the Republic of Cyprus, on alcohol consumption, throughout a one-year systematic monitoring. Daily influent wastewater samples from two wastewater treatment plants (WWTPs), one serving part of the capital city and one a coastal touristic area, were collected every three days for one year, and EtS was determined by means of liquid chromatography tandem mass spectrometry. The analysis revealed a relatively stable mean monthly alcohol consumption in the capital area, with daily consumption ranging from 0.31 to 10.60 mL/day/inh. In the coastal area, significant variations in alcohol consumption were observed, with tourist activity during the summer months being associated with nearly a tenfold increase in alcohol use compared to the winter months. Furthermore, four weekly campaigns were conducted for the determination of five stimulant illicit drugs. The results indicated a statistically significant association between alcohol and some stimulants, namely methamphetamine, MDMA, and ketamine. While this association does not imply direct causation or co-consumption, it highlights potential patterns of concurrent presence in wastewater.

* Corresponding author at: Nireas-International Water Research Centre, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus.
E-mail address: dfatta@ucy.ac.cy (D. Fatta-Kassinos).

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1. Introduction

Alcohol consumption is a prevalent social and cultural phenomenon globally, often associated with recreation and celebration activities. However, its widespread use presents significant public health challenges. According to the World Health Organization (WHO), alcohol and drugs consumption is a leading risk factor for premature mortality and disability worldwide, contributing to over 3 million deaths annually and representing 5.3 % of all global deaths, while 7 % of the global population over 15 years old suffer from an alcohol related disorder (*WHO Global Status Report on Alcohol and Health and Treatment of Substance Use Disorders*, 2024). Alcohol abuse is regarded as a pervasive public health concern globally, that significantly influences various facets of societal health, economic productivity, and overall quality of life. Excessive alcohol use is associated with a multitude of adverse health outcomes, including liver cirrhosis, cardiovascular diseases, cancers, as well as mental health disorders, such as depression and anxiety. Furthermore, excessive alcohol consumption is linked to social and economic issues, including accidents, violent behaviors and decreased productivity, among others (Goel et al., 2018; Gronbaek, 2009; Rehm et al., 2010; Room et al., 2005).

Apart from its consumption alone, alcohol is often combined with other licit or illicit substances, a behavior which can exacerbate the overall negative health effects. For instance, cocaine is generally excreted from the human liver as benzoylecgonine, however, when the use of cocaine is combined with alcohol, the human body excretes a different metabolite, cocaethylene, which has been found to be more toxic than benzoylecgonine; in relation to that, there is a higher risk of sudden death with the combination of alcohol and cocaine, compared to cocaine use alone (Harris et al., 2003; Herbst et al., 2011; Teheran et al., 2019).

The significant implications from alcohol use underscore the importance of accurate and comprehensive understanding of the patterns and determinants of alcohol consumption, as well as the development of effective public health interventions and targeted policies aimed at controlling the alcohol-related harm. Traditional methods for assessing alcohol consumption, such as self-reported population surveys, interviews and sales statistics, often face substantial challenges. These methods are prone to biases, including stockpiling, underreporting, social desirability related bias, inaccurate recall, and are not able to capture unregulated, illegally imported or home-produced alcohol effectively. An emerging approach to overcome these limitations is wastewater-based epidemiology (WBE), which provides an objective and comprehensive measure of community-wide substance use. Since Reid et al. (2011) published the first application of WBE for alcohol consumption monitoring, this methodology was used in several countries in Europe (Baz-Lomba et al., 2016; Boogaerts et al., 2016; Brandeburova et al., 2020; Gatidou et al., 2016; Rodriguez-Alvarez et al., 2014; Salgueiro-González et al., 2021; van Wel et al., 2016), USA (Chen et al., 2019; Driver et al., 2022; Driver et al., 2020), China (Ryu et al., 2016; Yao et al., 2024; Zheng et al., 2022), and Australia (Bade et al., 2021a; Bade et al., 2021b; Lai et al., 2018; Zheng et al., 2020).

Once alcohol is ingested, its major fraction is rapidly converted to acetaldehyde in the liver and consequently to acetic acid, with aldehyde dehydrogenase involved in both oxidation processes. A small fraction of the ingested alcohol is excreted unchanged and another, smaller fraction, undergoes conjugation reactions which lead to the formation of ethyl glucuronide (EtG) and ethyl sulphate (EtS), which are finally excreted in the urine (Helander and Beck, 2004). Both metabolites are present in detectable amounts in the influent wastewater and have been used as biomarkers for the determination of alcohol consumption; however more comprehensive research suggests the selection of EtS, as it has been proven to be a stable metabolite of ethanol, under variable conditions, rendering it a reliable biomarker for alcohol intake, compared to EtG (Reid et al., 2011).

This study employs WBE to investigate alcohol consumption in two distinct urban environments of the eastern Mediterranean, in the Republic of Cyprus: The capital of the country, Nicosia (NIC), and a smaller, touristic area of Cyprus, the Agia Napa-Paralimni area (AGN—P). The metropolitan city, characterized by a relatively large and diverse population, presents a unique opportunity to study alcohol consumption in a complex urban setting. In contrast, the smaller area, which experiences seasonal fluctuations in population due to tourism, offers insights into how alcohol use may be affected by transient populations and tourism-related activities. By analyzing and contrasting these two environments, this study seeks to contribute to a better understanding of urban alcohol consumption dynamics and provide valuable data for public health authorities and policymakers. By comparing EtS in wastewater and alcohol consumption levels from these two locations, we aimed to elucidate differences in alcohol consumption patterns that may be influenced by factors such as population density, tourism, and socio-economic conditions.

The hypothesis we wanted to test in this study is whether seasonal variations in alcohol consumption, driven by tourism activity, can be detected through wastewater surveillance, and whether these fluctuations in wastewater alcohol levels provide a reliable indicator of alcohol consumption patterns in a touristic island country like Cyprus. In touristic areas, wastewater alcohol consumption is often closely tied to the seasonal influx of tourists, leading to significant fluctuations in consumption levels (Lai et al., 2012; Senta et al., 2023). During peak tourism seasons, typically in the summer months, alcohol consumption can surge drastically.

This study provides therefore the first systematic assessment of alcohol consumption in the Republic of Cyprus using wastewater-based epidemiology, offering valuable insights into temporal and spatial consumption patterns. The findings underscore the potential of wastewater analysis as a real-time, population-level monitoring tool to inform public health strategies, assess seasonal and regional variations, and support evidence-based policymaking. By identifying fluctuations linked to tourism and social behaviors, this work contributes to a broader understanding of substance use dynamics and the need for targeted public health interventions. Simultaneously, the study investigates the presence of stimulant illicit drugs in the same areas, shedding light on their coexistence with alcohol consumption. This analysis aims to uncover temporal and spatial patterns of substance use across the studied communities, providing a deeper understanding of consumption behaviors and informing potential public health strategies.

2. Materials and methods

2.1. Chemicals and reagents

EtS and isotopically labeled EtS-d5 were purchased from Cerilliant (Round Rock, TX, USA); amphetamine, methamphetamine, benzoylecgonine, ketamine and MDMA analytical standards, and amphetamine-d5, methamphetamine-d5, benzoylecgonine-d3, ketamine-d4 and MDMA-d5 were obtained from LGC Standards (Luckenwalde, Germany) all in the form of 1 mg/mL methanol solutions. Oasis MCX cartridges were purchased from Waters Corporation (Milford, MA, USA). LC-MS grade methanol and LC-MS grade formic acid (98 %) used for mobile phase were supplied by Merck (Darmstadt, Germany). Hydrochloric acid (35 %) and ammonia solution (25 %) were purchased from Merck (Steinheim, Germany). Ultrapure water used for mobile phase was obtained from a Milli-Q system (Millipore).

2.2. Sampling strategy

EtS was monitored in two areas of the Republic of Cyprus: NIC, the capital of the country and a smaller, coastal area, AGN—P, for a total sampling period of one year (12 months). Sampling was conducted every three days at the inlet of the two WWTPs over the course of one year. The sampling began on May 10, 2023, and continued until April

30, 2024. Each sampling started at 9:00 am, with samplers collecting volume-proportional 24-h composite influent wastewater samples. For both WWTPs a common sampling protocol and schedule was applied, ensuring that samples were collected on the same days from both sites throughout the entire sampling period. In total, 242 individual samples were collected and analyzed for EtS from both sites (121 samples from each site). After collection, all samples were acidified on-site and stored at −20 °C until being transported to the laboratory once a month for analysis.

The levels of the stimulant drugs (amphetamine, methamphetamine, MDMA, cocaine and ketamine) were also periodically analyzed in the inflow wastewater, during the one-year of alcohol monitoring period: Four weekly sampling campaigns took place for the determination of the abovementioned stimulants, during summer (17–23 July 2023), autumn (9–15 October 2023), winter (25 December 2023 to 1 January 2024) and spring (8–14 April 2024), for seven consecutive sampling days each time, except for the winter sampling (8 days, in order to include both Christmas and New Years day). It is noted that during these specific periods, the alcohol sampling schedule was differentiated and instead of collecting one sample every three days, consecutive daily samples were collected, in order to determine both alcohol and drugs in the exact same samples. Additional samples were also collected during the drug monitoring months, in order to analyze a minimum number of ten samples for EtS, each month. In total, 29 samples from each site were collected for the stimulant drugs determination.

2.3. Sample preparation

2.3.1. EtS analysis

For the estimation of alcohol consumption, EtS was determined as follows: A sample aliquot of roughly 1.2 mL from each sample was transferred into a 1.5 mL microcentrifuge tube and centrifuged for 5 min at 8000 rpm, at a temperature of 4 °C. The supernatant was then collected and filtered through a 0.22 μm pore PTFE filter and 1 mL of the filtered sample was directly transferred to chromatography glass vials, where it was spiked with 5 μL of 10 μL/mL EtS-d5 solution and analyzed using a Liquid Chromatography tandem Mass Spectrometry system. Each daily sample was analyzed in duplicate.

2.3.2. Stimulant drugs analysis

The sample preparation step for the determination of the stimulant illicit drugs is described in detail elsewhere (Psichoudaki et al., 2023). In brief, after successive filtration through 2.7, 1 and 0.45 μm pore size glass fiber filters, all samples were divided into aliquots of 100 mL (duplicates for each sample). Each sample aliquot was spiked with a mixture of internal standards, containing 200 ppb of amphetamine-d5, methamphetamine-d5, MDMA-d5, bezoylecgonine-d3, and ketamine-d4, which was prepared each time, prior to analysis. A matrix matched set of calibration solutions was also prepared in the range of 2 to 100 ppb. The samples were percolated through OASIS-MCX (60 mg, 3 cc) cartridges under gravity, the analytes were eluted consecutively with methanol and 2 % ammonia solution in methanol and after solvent evaporation, the samples were finally reconstituted to a total volume of 1 mL.

2.4. LC-MS/MS analysis

All analyses were carried out using an ACQUITY TQD LC-MS/MS system (Waters Corp., Milford, MA, USA). The triple quadrupole detector was equipped with an electrospray ionization source, operating in negative ionization mode for EtS and positive ionization mode for stimulant drugs analysis.

2.4.1. EtS chromatographic analysis

The chromatographic separation was performed using a high-strength silica, C18 column (Waters Acquity HSS T3, 1.8 μm, 1.0 × 100 mm) with a guard column of the same packing material and particle size, and gradient elution at a flow rate of 0.25 mL/min. The column temperature was maintained at 40 °C. Mobile phase A consisted of 0.1 % formic acid in ultrapure water and mobile phase B was methanol. The eluent gradient was 0 min 10 % B, 2 min 20 % B, 2.1 min 90 % B, 3 min 99 % B, 4 min 1 % B, 6 min 1 % B. The injection volume was 10 μL. The ESI source and desolvation temperatures were set at 150 and 500 °C, respectively. Calibration curves were constructed from calibration standards prepared in filtered and centrifuged wastewater matrix, at nine concentrations (range 1–100 μg L^{-1}), with a coefficient of determination of $R^2 > 0.99$. The limit of detection (LOD) and quantification (LOQ) for EtS was 0.1 and 1 μg L^{-1}, respectively. For quantification, multiple reaction monitoring (MRM) was performed for EtS at *m/z* 124.8 > 96.9 and 124.8 > 80.0 and m/z 129.8 > 98.0 was monitored for EtS-d5.

2.4.2. Stimulant drugs chromatographic analysis

The optimized chromatographic and MS conditions applied for the determination of the stimulant drugs, as well as the quantification and confirmation MRM transitions, are fully described in Psichoudaki et al. (2023). Briefly, the chromatographic column used was a Bridged-Ethyl Hybrid column (Waters BEH C18, 1.7 μm, 2.1 × 50 mm) with the corresponding pre-column and mobile phase composed of 0.1 % formic acid in H_2O (A) and methanol (B). The sample injection volume was 10 μL and the total analysis time was 6 min. All chromatographic data acquisition and evaluation was performed with MassLynx V4.2 (Waters Corporation, Milford, MA, USA).

2.5. Quality assurance/quality control

For all analytes, the analytical methods were validated successfully for linearity, LOD, LOQ, recovery and repeatability. For the quantification of both EtS and stimulants, calibration curves were constructed using a set of 8 calibration standards in wastewater matrix, ranging from 2 to 100 μg L^{-1}, for both classes of analytes. Both EtS and stimulants analytical methods showed good linearity in this concentration range, with a minimum coefficient of determination of $R^2 = 0.990$ for all analytes. LOD and LOQ were determined as 3 and 10 signal/noise ratio, respectively. The estimated recovery of stimulants was in the range 85–110 %. The repeatability of the analytical methods was determined by spiking sets of 5 wastewater samples at different concentrations, with relative standard deviation (RSD) lower than 3.5 %. MRM transitions, MS conditions and LOQs for all analytes are reported in Table S1.

2.6. Calculation of alcohol and drugs consumption

EtS mass loads were calculated in mg/day/inh by multiplying the measured concentrations of EtS (ng/L) with the daily flow rate of the influents (L/day) and divided by the equivalent population. The population-normalized daily mass loads were multiplied by 3047, a correction factor which accounts for the excretion rate (assuming 0.012 % excretion) of alcohol to EtS and the molar mass ratio of alcohol to EtS, to convert daily metabolite mass loads to alcohol consumption (Daglioglu et al., 2020; Lopez-Garcia et al., 2020; Rodriguez-Alvarez et al., 2015; Thai et al., 2021). Finally, the mass loads were then divided by ethanol's density (0.789 g/mL), in order to express the result in mL/day per inhabitant. Similarly, for the estimation of stimulants consumption, daily mass loads were calculated as above. Then correction factors of 2.77, 2.44, 4.4, 3.59 and 3.3 were used to convert mass loads to daily consumption for amphetamine, methamphetamine, MDMA, benzoylecgonine and ketamine respectively (Castiglioni et al., 2013; Estevez-Danta et al., 2022; Gracia-Lor et al., 2016; van Nuijs et al., 2011). Benzoylegonine's mass loads were used to obtain cocaine's consumption (Bijlsma et al., 2013; van Nuijs et al., 2009).

Due to the outdated census data for the cities of the Republic of Cyprus (2021) but also because of the highly fluctuating population of the sampling sites during the sampling period of one year, especially for AGN—P, due to the significant touristic activity of the area, the equivalent population (PE) for each site was estimated, based on the influent BOD values, that were determined daily at both treatment plants. The estimated PE was then used for the calculation of both alcohol and stimulants daily consumption values. A BOD increase of 60 g/day per person was assumed (Bauer et al., 2002; van Nuijs et al., 2011). It should be noted that BOD-based PE calculation has some limitations, related to the variability of the BOD produced per person, but also to non-direct human contributions. Thus, this estimation can introduce uncertainties to the final calculated alcohol and stimulants consumption, as BOD can deviate from the abovementioned value, due to household activity fluctuations, as well as potential industrial or agricultural discharges (Hou et al., 2021; Rico et al., 2017).

2.7. Statistical analysis

Statistical analysis was performed with Wavemetrics Igor Pro, version 6.3.7.2 and Origin Pro 2024. One-way ANOVA test with Bonferroni posthoc analysis was performed to investigate statistically significant differences between different sites or different sampling periods. Shapiro-Wilk test was applied to examine normality of the data. Spearman correlation was performed for non-normally distributed datasets. A p-value lower than 0.05 indicated significant differences between two sets of values, with a confidence level of 95 %.

3. Results and discussion

3.1. Identification of trends

During the one year of alcohol consumption monitoring, EtS was detected and quantified in all samples collected, with concentrations above the analytical method's LOQ at both sampling locations (frequency of detection: 100 %). The lowest consumption levels observed for alcohol were 0.26 and 0.49 mL/day/inh, whereas the highest were 8.92 and 51.18 mL/day/inh, at NIC and AGN—P, respectively. These values were calculated as described in section 2.5, and thus, represent the total population served by the treatment plant of each area. Using the 2021 census data, from which the total percentage of the population above 15 years of age was calculated, it was estimated that daily consumption at NIC ranged from 0.31 to 10.60 mL/day/inh (aged 15+) and for AGN-P from 0.58 to 60.85 mL/day/inh (aged 15+). For the following analysis, all consumption values refer to population aged 15 years old and above.

Fig. 1 represents the time series of population-normalized daily alcohol consumption during the monitoring period, at the two locations under study. The figure reveals significant differences of the levels of alcohol consumed at the two communities, mainly from the beginning of the sampling period, in May, until October 2023. During this period, the daily alcohol consumption at NIC ranged from 0.49 to 10.61 mL/day/inh, while at AGN-P from 4.88 to 60.85 mL/day/inh, being significantly higher than that of NIC ($P < 0.001$). After October, alcohol use at AGN-P dropped abruptly, to levels comparable to those observed at NIC, with values below 10 mL/day/inh for most of the sampling days. The mean yearly consumption was estimated to be 2.14 mL/day/inh at NIC and considerably higher, 14.71 mL/day/inh at AGN area.

Fig. 2 represents the monthly mean alcohol consumption at the two monitored cities throughout the whole monitoring period. For NIC, one-way ANOVA revealed no statistically significant differences among mean consumption values during different months of the year. Contrarily, at AGN-P area large variations were observed between warmer and colder months: Alcohol consumption was significantly higher, at a 99 % confidence level, during the months May to September, compared to the months November to March ($p < 0.001$); consumption during October was lower than that during September ($p < 0.001$), July ($p < 0.05$), June ($p < 0.01$) and May ($p < 0.01$), while the use during April was lower compared to May ($p < 0.001$), June($p < 0.001$), July ($p < 0.001$), August ($p < 0.01$) and September ($p < 0.001$). The differences observed among the two locations are also represented by the proportion of alcohol consumed from May to September compared to that consumed from October to April: At NIC, 48.3 % of alcohol consumed throughout the whole year is consumed during the first period (May to

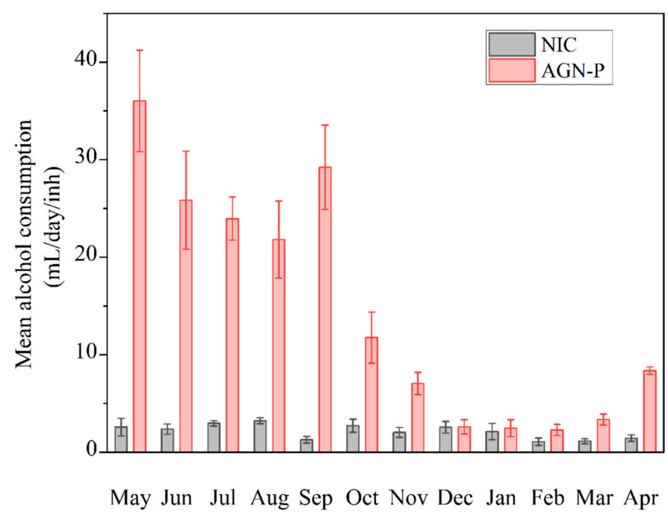

Fig. 2. Mean monthly alcohol consumption in NIC and AGN-P throughout the year (May 2023 – April 2024).

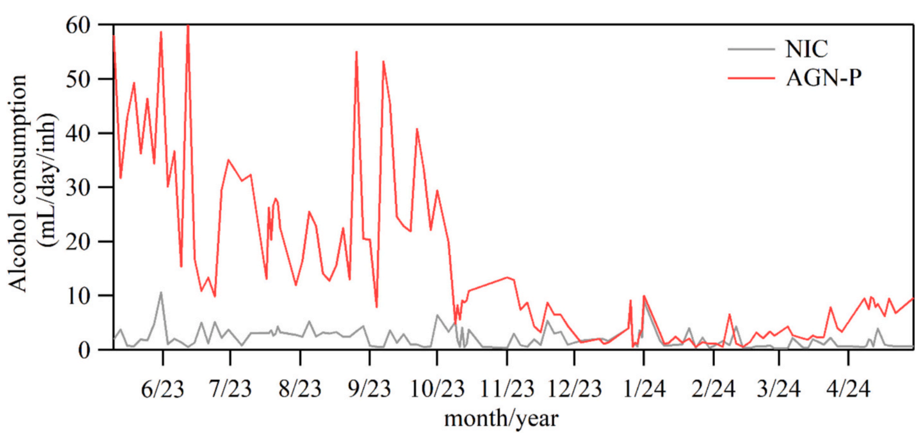

Fig. 1. Time series of alcohol consumption in NIC and AGN-P during the one-year monitoring period.

September) and 51.7 % during the second (November to March) while at AGN-P 78.4 % is consumed during the first period and 21.6 % during the second. When comparing winter and summer consumption findings, again at NIC no significant differences were found; conversely, at AGN-P the mean consumption during the summer is almost ten times higher than that of the winter (Fig. 3).

The above-mentioned spatial differences of alcohol consumption patterns can primarily be attributed to the divergent demographic and population characteristics of the two areas. NIC is an urbanized area with a relatively stable population and consistent activity levels throughout the year. In contrast, AGN-P is a considerably smaller area, where population dynamics are significantly influenced by seasonal fluctuations. As a popular holiday destination, particularly among younger visitors during the warmer months of the year, the active population at AGN-P undergoes substantial changes during these months, often doubling compared to the non-touristic season.

The above observations are supported by the findings from the examination of the relationship between the recorded ambient temperatures during the sampling period and the estimated monthly alcohol consumption (temperature data source: Meteostat, NOAA). The linear regression plots (Fig. S1) between the monthly mean temperatures against the monthly mean consumption provided R-square coefficients of determination of 0.2745 for NIC, and 0.7009 for AGN—P, respectively, indicating a weak correlation for the case of NIC and a strong correlation of alcohol consumption at the AGN-P area with temperature. This correlation with the ambient conditions should not be considered as a driving cause for alcohol consumption, instead it is further linked to the presence of a significant number of visitors in the area, mainly during the warmer months, which explains to a good extent the increased alcohol consumption during these months in the touristic area of AGN—P. Fig. S2 represents the linear regression plots between the mean monthly alcohol consumption in the two areas under study versus the total number of visitors at each distinct per month, during the twelve months of this study. The coefficient of determination of 0.675 for AGN-P indicates a significant correlation between these two parameters, and based on the above, the large monthly variation of alcohol consumption at this location can be attributed to the yearly variation of the touristic activity in the area. In contrast, the corresponding coefficient for NIC was found to be 0.030, demonstrating no correlation between the two parameters. The lack of correlation between alcohol use and total arrivals for the case of NIC could be explained by the relatively small number of tourists visiting the capital each month. The rationale behind the association of increased alcohol use from visitors is that excessive alcohol use during holidays has been previously observed in many studies, and can be attributed to a combination of cultural, social and psychological reasons. Holidays are typically associated with celebrations, gatherings, and festive traditions, where alcohol is often a central element of socializing. People may also use holidays as an opportunity to relax, leading to more permissive attitudes towards drinking. This convergence of celebratory norms, emotional stress and persuasive marketing contributes to the observed increase of alcohol use during the holiday season. (Carlisle and Ritchie, 2020; Hesse et al., 2008; Kim et al., 2017; Syed et al., 2023).

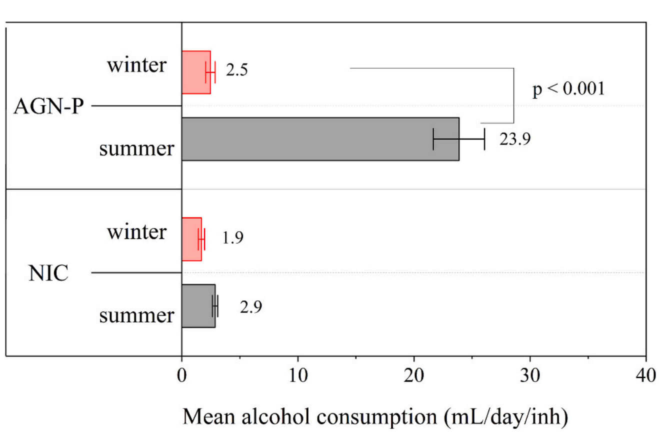

Fig. 3. Comparison of mean alcohol consumption during winter and summer at the two sampling locations.

3.2. *Weekly profiles of alcohol consumption*

The divergence in the patterns of alcohol use at the two monitored locations are also demonstrated by the weekly profiles of the consumption. Weekly profiles, calculated based on mean daily consumption values, were estimated for the winter and summer months, in order to assess and evaluate the discrepancies between the different alcohol consumption motives during the touristic and non touristic periods of the year.

Fig. 4 represents the average daily alcohol consumption at NIC during winter (a) and summer months (b) and at AGN—P, the corresponding periods (c, d). According to these graphs, alcohol use at NIC peaks the days around the weekend during the winter season (Saturday, Sunday, Monday). A typical weekday-weekend pattern is also observed during the summer season, with the highest mean consumption values being found on Saturday and Sunday. A rather similar pattern is also observed during spring and autumn at NIC (Fig. S3), while high consumption was also found on Wednesday during spring, however related with higher variation of the dataset during that day. At AGN-P a relatively stable weekly consumption profile was observed during the winter, with slightly higher mean daily consumption from Friday to Sunday. During the summer, no weekday-weekend consumption pattern was observed, which could be attributed to the relentless touristic activity in this area during this period. Spring and autumn showed mixed patterns, with absence of weekday-weekend differences during spring and higher alcohol consumption from Friday to Sunday during autumn. These periods though are partially affected by the touristic activity of the area, as shown by the monthly averages (Fig. 2).

Deviations from the weekday-weekend patterns, such as the high mean consumption detected on Monday during autumn and winter at NIC, could be partially attributed to late Sunday night consumption, as EtS is excreted to detectable levels for up to 24 h after alcohol consumption, although it reaches its maximum concentration in urine before 24 h. Other explanations for such deviations from the expected patterns could be the delay due to time necessary for the sewage to be transferred to the treatment plant, which is dependent on the treatment plant location and daily influent flow rates, the formation and the proportion of other metabolites, e.g. ethyl glucuronide, or the co-consumption of other licit or illicit substances, which would lead to the formation of different metabolites (Alsayed et al., 2022; Gao et al., 2018; Halter et al., 2008; Rodriguez-Alvarez et al., 2015; Ryu et al., 2016; Wurst et al., 2006; Zheng et al., 2019).

3.3. *Comparison with other studies in the Mediterranean region*

The levels and the range of alcohol consumption observed at the two cities monitored in this study were compared to those reported from other studies focusing on the Mediterranean region. The work of Andres-Costa et al. (2016) estimated the alcohol consumption during a festive and non-festive period in three locations of Valencia, Spain. During the normal, non-festive period, alcohol consumption ranged from 1.1 to 18.31 mL/day/inh among the three locations tested, while during the festive days the corresponding range was from 4.4 to 52.7 mL/day/inh. The values observed during the non-festive period are higher than those observed at NIC, but the festive period estimates were comparable to the yearly range of alcohol consumption at AGN—P.

In Antalya, Turkey, with samples collected from March to December the average alcohol consumption was found to be 25.9 mL/day/inh, which is significantly higher than the yearly average consumption of 2.1

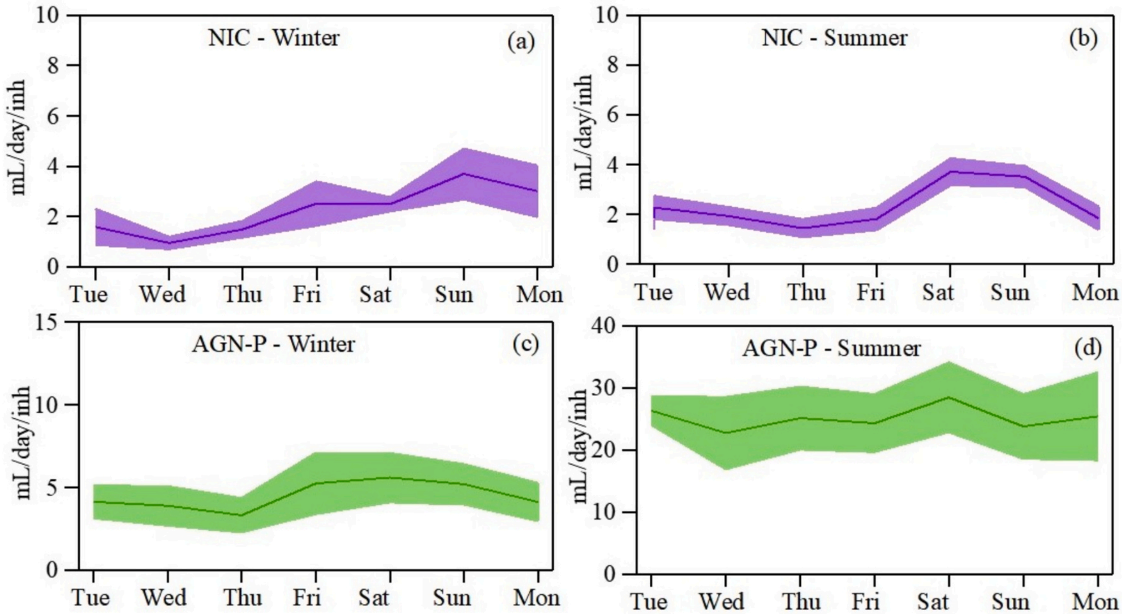

Fig. 4. Weekly profiles of alcohol consumption in NIC during winter (a), summer (b) and AGN-P during winter (c) and summer (d). The central line in each graph indicates the daily mean consumption, while the shaded areas represent one standard error above and below the mean value.

mL/day/inh estimated for NIC and 14.7 mL/day/inh for AGN-P (Kuloglu Genc et al., 2021). Gatidou et al. (2016) also analyzed samples collected from the island of Lesvos, Greece, during a non-touristic period and found that the consumption ranged from 1.7 to 11.2 mL/day/inh, with averages of 5.4 and 3.4 mL/day/inh at the two locations studied. These observations fall within a similar range to the data obtained from NIC, which is assumed to maintain a relatively constant population throughout the year. The corresponding yearly range and mean consumption estimated for AGN-P was significantly higher, but mainly due to the high concentrations measured during the warmer touristic months. Otherwise, during periods when touristic activity is less significant, the levels at AGN-P are also comparable to those measured at the island of Lesvos.

Salgueiro-González et al. (2021) reported a large variation of mean alcohol consumption in Italy, ranging from 3.8 to 22.9 mL/day/inh. This study, which estimated alcohol consumption in seventeen cities in total, also found that alcohol consumption was higher in smaller cities, compared to the larger ones, with differences being statistically significant. By comparing the winter mean values estimated at the studied areas of Cyprus, the same pattern can be observed. To the other end, the study of Boogaerts et al. (2016) who focused on the estimation of alcohol use in several cities in Belgium, revealed a contradictory trend, with higher consumption in more urbanized areas, compared to smaller cities. In general, the levels of alcohol consumption estimated during this one-year study, were relatively low at both sites, compared to other Mediterranean and European cities. The average yearly alcohol consumption at NIC corresponds to 0.2 drinks per day per person, while at AGN-P 1.2 drinks per day per person, assuming that one standard drink is 10 g or 12.5 mL of ethanol (Kalinowski and Humpreys, 2016).

3.4. Correlation of alcohol consumption with stimulant drugs use

A selected number of samples (7 consecutive samples, once every season of the year) collected from the two monitored locations were also analyzed for the presence and the concentrations of several stimulant drugs and metabolites. The levels and spatiotemporal variability of most of the stimulants discussed in this study have been previously studied for the case of the Republic of Cyprus, thus the scope of this section mainly focuses on the investigation of potential relationship of alcohol and stimulants consumption patterns (Psichoudaki et al., 2023).

The Shapiro-Wilk test was conducted to determine whether the alcohol and drug consumption data follow a normal distribution, before applying an appropriate correlation test to compare them. Due to the relatively limited size of the data set, collected during different periods of the year, for all stimulants the test showed that the data did not fall to a normally distributed population; thus, Pearson correlation test was rejected, and Spearman test was performed, to estimate the extent and the significance of the correlation between alcohol and stimulant drugs consumption. Fig. S3 illustrates the correlation plots between alcohol and stimulant drugs consumption levels, for both sites.

As is observed for NIC, a significant correlation of alcohol use with methamphetamine and MDMA was identified ($p < 0.05$), while a weak correlation of alcohol with cocaine consumption (Spearman correlation coefficient $p = 0.2$) was also observed. At AGN—P, alcohol showed statistically significant correlations with MDMA, and with ketamine; the consumption of ketamine at AGN-P was higher than that at NIC, especially during the summer and autumn periods (Fig. S4). Again, a similar, weak correlation between alcohol and cocaine ($\rho = 0.2$), as in the case of NIC, was identified. It is also worth noticing the positive significant correlation of methamphetamine with cocaine, a finding observed at both cities. Similarly, MDMA also correlated significantly with ketamine at AGN-P (the levels of ketamine found at NIC were very low most of the sampling days).

To the authors knowledge, correlation studies between alcohol and stimulants use based on wastewater epidemiology are very scarce in the literature. The work of Mastroianni et al. (2014), also showed significant correlation of alcohol use levels with those of amphetamine, MDMA and cocaine, among others, in Barcelona, Spain. No correlation of alcohol with methamphetamine was found, while ketamine was not monitored in this work. Available data on alcohol and stimulants co-consumption is also very limited. Rodriguez-Alvarez et al. (2015) examined the co-consumption of cocaine and alcohol, by monitoring cocaethylene (COE), a metabolite excreted by the human body after the use of both substances, in Santiago de Compostela, Spain, and in Milan, Italy. The researchers found that cocaine was combined with alcohol mostly during the weekends. This work also implied discrepancies on the co-consumption behavior among the two cities. Similarly, da Silva et al. (2018) also found higher co-consumption during the weekends in the capital of Brazil, using the cocaethylene to benzoylecgonine ratio. As COE is excreted in very low amounts from the human body (less than 1 %), and its excretion is dependent on the amount of alcohol and cocaine consumed, as well as the route of administration of the latter, more research is necessary for the better understanding and evaluation of COE estimation (da Silva et al., 2018).

The findings of this study may imply that the combined use of alcohol with MDMA, ketamine and methamphetamine is common among users in Cyprus and to a much lower extent with cocaine, however, such correlations alone cannot be used to provide certainty for co-consumption. These correlations could also be partially attributed to previously reported findings, according to which, alcohol, as well as several illicit stimulants, may be consumed for recreational reasons under similar settings and occasions (e.g., nightlife, festivals, etc.), which could result to some correlation of the levels of alcohol and stimulants (Ferguson et al., 2023). Still, other questionnaire-based studies highlighted the complementary use of stimulants (amphetamine, methamphetamine, MDMA or cocaine) with alcohol, among stimulants users, due to several reasons, mainly related to the combined effect of these substances (McKetin et al., 2014). These observations underscore the necessity for extended biomarker metabolomics and drugs-alcohol interaction studies, in order to elucidate the human metabolic byproducts and discover specific biomarkers for stimulants and alcohol co-consumption, to enable the further understanding of alcohol and drug use behaviors and patterns. Further studies incorporating pharmacokinetic analyses could provide deeper insights into whether these substances are used simultaneously or simply occur under similar social or environmental contexts.

4. Conclusions

This work presents, for the first time, a systematic study of near-real time alcohol consumption measurements in two areas of the Republic of Cyprus, by exploiting the potentials of wastewater surveillance. The study determined the levels of daily alcohol consumption at NIC, and at one smaller and touristic community, the AGN-P area, for a total period of one year. Our findings suggest a relatively low and constant alcohol consumption throughout the year in the capital of the country, and a much more variable consumption at AGN-P, mainly related to the touristic activity of the area. Alcohol levels correlated well with those of several stimulants, suggesting either the combined use, or consumption of these under similar circumstances. Such correlations could help us identify and understand alcohol and drug consumption patterns and behaviors. Further pharmacokinetic research, focusing on the co-consumption of alcohol with different drugs, as well as with different amounts and proportions of alcohol and these drugs is necessary to identify and elucidate co-consumption biomarkers and excretion rates, but also to redefine existing ones. This way a more comprehensive understanding and more extensive utilization of wastewater-based epidemiology potential will become plausible. Despite the limitations of wastewater-based epidemiology, this methodology can be exploited as a powerful tool that can shed light on alcohol abuse patterns, informing clinical guidelines and public health policies.

CRediT authorship contribution statement

Magda Psichoudaki: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dimitrios Tzelios:** Methodology, Conceptualization. **Maria Savvidou:** Resources, Funding acquisition. **Christos Mina:** Resources, Funding acquisition. **Costas Michael:** Writing – review & editing, Methodology. **Despo Fatta-Kassinos:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180113.

Data availability

Data will be made available on request.

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