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Wastewater-based monitoring of illicit drugs in Cyprus by UPLC-MS/MS: The impact of the COVID-19 pandemic

M. Psichoudaki^a, T. Mina^{a,b}, M. Savvidou^c, C. Mina^c, C. Michael^a, D. Fatta-Kassinos^{a,b,*}

^a Nireas-International Water Research Centre, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus

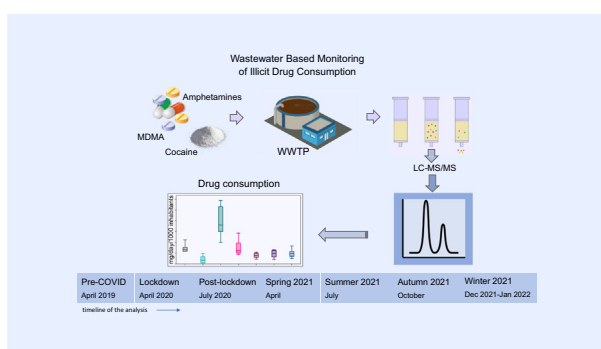
^b Department of Civil and Environmental Engineering, School of Engineering, University of Cyprus, P.O. Box 20537, 1678, Nicosia, Cyprus

^c Cyprus National Addictions Authority, 2027 Nicosia, Cyprus

HIGHLIGHTS

- Amphetamines use remained unchanged during lockdown and decreased post lockdown.
- Cocaine and MDMA decreased during lockdown but increased during post-lockdown.
- Methamphetamine, MDMA and cocaine use was higher during the weekends of 2021.
- Useful information for decision making obtained via wastewater-based epidemiology.

GRAPHICAL ABSTRACT



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ABSTRACT

The outbreak and spread of COVID-19 impacted through various ways the lives of millions of humans globally. In this work, wastewater-based epidemiology (WBE) was applied to investigate the effect of the actions taken by the Republic of Cyprus to confine COVID-19 on the use of illicit stimulant drugs. Daily influent samples were collected from the six main wastewater treatment plants (WWTPs) of the country i) before lockdown (3–9 April 2019), ii) during lockdown (21–27 April 2020), iii) during the post-lockdown period (14–20 July 2020), and, iv) during each season of the following year (20–26 April 2021, 19–25 July 2021, 11–17 October 2021, 25 December 2021–2 January 2022), and analyzed for amphetamine, methamphetamine, MDMA and cocaine. In most areas, amphetamine and methamphetamine use was not affected during the confinement period, but as availability of the substances decreased with time, a drop in their use was observed when most restriction measures were eased (up to 9- and 22-fold decrease, respectively). The limitations on social interactions and events during the quarantine period seem to have led to the reduction of MDMA and cocaine and driven a sharp decrease of their use in most areas studied (up to 11 and 6 times lower, respectively). However, the re-opening of activities led to a pronounced consumption increase, reaching maximum daily values of 800 and 2691 mg/1000 inhabitants/day, respectively. In 2021, drug use was re-established to lower levels. The examination of weekly patterns during this year revealed higher weekend use of methamphetamine, MDMA and cocaine. Our results suggest that both the implementation and the easing of COVID-19 related measures affected the availability and the use of drugs. This study also provides the first insight on the consumption of illicit drugs in the Republic of Cyprus during pre-, post- and pandemic times and demonstrates the importance of WBE.

* 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).

1. Introduction

On the 11th of March 2020, the World Health Organization (WHO), after a series of increasing human infections worldwide, announced the outbreak of a pandemic due to the spread of the novel virus SARS-CoV-2, which causes the respiratory COVID-19 disease (Cucinotta and Vanelli, 2020). The fast spread of the virus, along with the severity of the related symptoms, led >100 countries to impose strict measures, in an attempt to restrain its spread, by applying various types of restrictions on the mobility, socialization and everyday activities of citizens. These measures impacted societies in various ways and affected both social and economic aspects of life (Alygizakis et al., 2021b; Reinstadler et al., 2021a).

The already well-established wastewater-based epidemiology (WBE) approach has been widely applied after the COVID-19 outbreak, both for the detection of the SARS-CoV-2 viral RNA, which can provide information on the prevalence of the virus in a community, as well as for the detection and measurement of a series of chemical tracers, to investigate changes on consumption habits among the citizens (Alygizakis et al., 2021b; Nason et al., 2021). After the first confirmation of the appearance of viral RNA presence in human stool, research groups from Europe, USA and Australia successfully established a methodology to associate the concentration of SARS-CoV-2 viral RNA in untreated wastewater with the actual population infected by the virus (Ahmed et al., 2020; Medema et al., 2020).

In March 2021, the European Commission released a recommendation, inviting the European countries on a common strategy for the establishment of systematic surveillance systems for the monitoring of SARS-CoV-2 and its variants in wastewaters, in a harmonized procedure (Commission Recommendation EU 2021/472). The EC Recommendation suggested that the outcomes of WBE should be exploited for prevention, early warning purposes and monitoring trends and would allow for a better evaluation of the efficiency of the measures taken from authorities to restrain the spread of the pandemic.

WBE represents an analytical approach that can provide qualitative and quantitative information regarding, among others, eating, drinking, smoking and drug consuming habits of a population. This tool is based on the fact that chemical compounds consumed by a population, are excreted from the human body and end up, usually in trace levels, in the sewage system, and consequently they can be detected in the wastewater influents, either unchanged, or as their metabolites (Been et al., 2016; Daglioglu et al., 2019; Daughton, 2001; Devault et al., 2017). Thus, WBE enables near real-time monitoring of the consumption habits of a population by the investigation of the composition of the influent wastewater that arrives at the wastewater treatment plant (WWTP) of an area. Therefore, the monitoring of the composition of sewage influents can reveal the presence of various indicators, as well as their consumption profiles, supporting the development of regional or national policies particularly, in relation to health issues.

Illicit drug use is an activity that cannot be easily unveiled to its actual extent through the usual questionnaires and other similar methods used in epidemiology studies, owing to the fact that drug use is mostly illegal, and because of this, biased answers could be obtained. Additionally, in many cases drug users are often unaware of the actual drug or a mixture of substances they are consuming, making questionnaire-based studies less reliable (Ort et al., 2014). On the other hand, WBE can provide a more realistic approach and reveal the drug use problem in a community to its real extent, enabling the estimation of the consumption of illicit drugs by measuring the drugs or their metabolites in the influent wastewater that arrives at the WWTP which serves the community.

The low concentrations of trace compounds, such as the illicit drugs, (in the µg/L or even ng/L range) in the complex matrix of wastewater requires a highly sensitive method for their determination and quantification. Ultra-Performance Liquid Chromatography/tandem Mass Spectrometry (UPLC-MS/MS) can allow for the development of a rapid, sensitive and selective multi-residue method for the simultaneous measurement of a class of compounds, including a series of illicit drugs (Thomaidis et al., 2016; Zuccato and Castiglioni, 2009).

To the authors knowledge, up to now, there is no scientific literature on the actual drug use in the Republic of Cyprus. In this work, we investigate whether the implementation and easing of restriction measures in the Republic of Cyprus, due to COVID-19, impacted the illicit stimulant drugs use in the country, applying the WBE approach. The presence, the changes in their concentrations and the consumption of four illicit stimulant drugs, i.e., amphetamine, methamphetamine, methylenedioxymethamphetamine (MDMA) or ecstasy and cocaine, using as tracer its biomarker, benzoylecgonine, were investigated through their determination in influent wastewater samples taken from six WWTPs, by means of UPLC-MS/MS. Sampling campaigns took place before, during the lockdown period, during the period that followed the lockdown, when most restriction measures were eased and finally during four different periods of the following year. These campaigns allow the examination of the extent of the influence of the pandemic and the implemented measures on the stimulant drugs use in this long-term monitoring programme. Seasonal and weekly variations of these drugs are also discussed.

2. Materials and methods

2.1. Chemicals and reagents

Benzoylecgonine, amphetamine, methamphetamine and MDMA, as well as the deuterated compounds that were used as internal standards, MDMA-d5 (0.1 mg/mL), methamphetamine-d5, amphetamine-d5 and benzoylecgonine-d3, were supplied by Cerilliant (Round Rock, TX, USA) in the form of 1 mg/mL solution in methanol or acetonitrile. Working solutions were prepared in methanol. LC-MS grade methanol, LC-MS grade formic acid (98 %) and hydrochloric acid (35 %) were purchased from Merck (Darmstadt, Germany). Ammonium hydroxide (25 %) was acquired from Sigma-Aldrich (Steinheim, Germany), while the ultrapure water used was from a Milli-Q system (Millipore). Nitrogen (99.9995 % purity) for sample drying was supplied by Linde Ltd. (Cyprus). Oasis MCX SPE cartridges (60 mg, 3 cc) were purchased from Waters Corporation (Milford, MA, USA).

2.2. Sampling campaigns, sampling sites, and sample collection

In the Republic of Cyprus, the first COVID-19 patients were confirmed on March 9th 2020. When the detected positive cases of SARS-CoV-2 started increasing in the country (Fig. 1), and after the announcement of a series of escalating restriction measures for the prevention of the spread of COVID-19, a strict lockdown was implemented on the 24th of March 2020: Schools, universities, entertainment areas, construction sites and retail services were closed and access to public places was prohibited. From March 15th the borders were closed and after the 31st of March only in special circumstances, e.g., repatriation, flights were allowed to reach the island. The mobility of the citizens was also restricted and limited to accessing food and other essential supplies, while moving from one district to another was only allowed for very specific reasons. The strict lockdown ended on April 30th, but significant restrictions remained thereafter, including night curfew and limitations on the number of people present in various settings. The first flights started arriving to the Republic of Cyprus after the 1st of June (Quattrocchi et al., 2020).

Sampling campaigns took place one year before the lockdown (3–9 April 2019, sampling period marked as SP1), during the lockdown (21–27 April 2020, marked as SP2), after the lockdown, (post-lockdown period, 14–20 July 2020, marked as SP3) and the following spring (20–26 April 2021, marked as SP4), summer (19–25 July 2021, marked as SP5), autumn (11–17 October 2021, marked as SP6) and winter (25 December 2021–2 January 2022, marked as SP7). All spring campaigns were performed during non-event periods, in compliance with the yearly drug monitoring for the SCORE network (Sewage analysis CORE group Europe). Each sampling campaign lasted seven days, except the last one which lasted nine days, to include both Christmas and New Year's weekends.

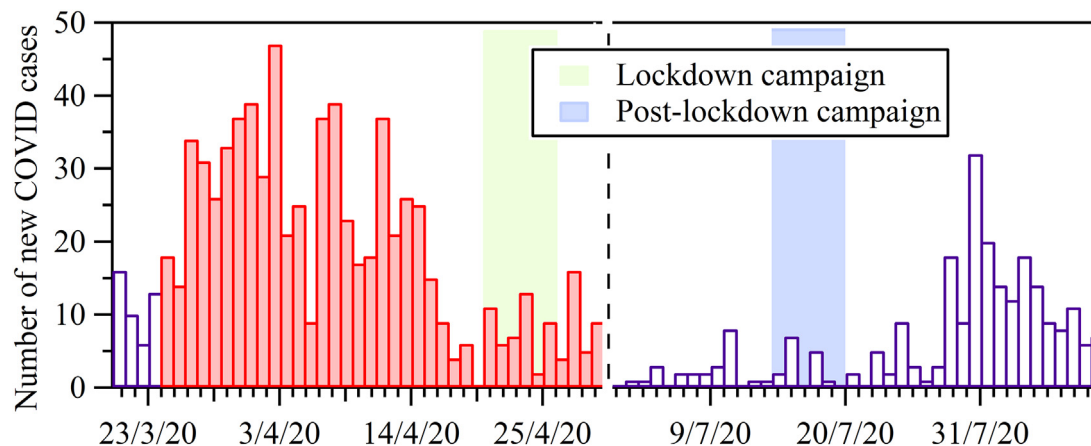


Fig. 1. Number of new COVID-19 cases per day recorded in the Republic of Cyprus, during the two sampling periods of 2020. Shaded bars indicate the number of cases during the strict lockdown period. The lockdown and post-lockdown sampling campaigns are also shown.

During all campaigns, samples were collected from the entrance of the six largest WWTPs of the Republic of Cyprus, serving around 50 % of the population of the country. In specific, samples were collected from: Two WWTPs that serve parts of the capital city; one that serves the southern and southwestern part of Nicosia (area Nicosia I) and the other the eastern and southeastern parts of Nicosia (area Nicosia II); three WWTPs serve the municipalities of Limassol, Larnaca and Paphos respectively, all including also catchments from smaller neighbouring municipalities and one WWTP serves the Agia Napa and Paralimni municipalities (Agia Napa area).

The exact location of the WWTPs and the average population served during all sampling campaigns are shown in Fig. 2.

The collection of the influent 24-hour composite samples was starting at 8:00 am and ending at 8:00 am of the following day. Amber glass bottles of 1 L were used for the sample collection. The bottles were pre-rinsed with de-ionized and ultra-pure water consecutively and dried. Right after the collection, all samples were acidified onsite with HCl at pH = 2.5, to ensure the stability of the analytes, and stored at -20°C until analysis. The time between sampling and analysis was always less than a week. Each sample was analyzed in triplicate.

2.3. Analytical method

2.3.1. Sample pre-treatment and solid phase extraction

All samples were successively filtered through 2.7 and 1.0 μm glass fiber filters and 0.45 μm pore size PVDF filter, to remove suspended solids. Samples of 100 mL were preconcentrated by applying off-line SPE, using a Whatman vacuum system. Before sample percolation through the OASIS-MCX cartridges, all samples were spiked with 100 μL of a 200 $\mu\text{g/L}$ solution containing a mixture of isotopically labeled compounds (MDMA-d5, methamphetamine-d5, amphetamine-d5, benzoylecgonine-d3) for internal standard-based analyte concentration measurement.

Before sample percolation, the SPE cartridges were conditioned with 6 mL of methanol, followed by 3 mL of ultrapure water and 3 mL of acidified water (pH = 2.5). Next, 100 mL of each sample was percolated through the cartridges by gravity. A volume of 10 mL of ultrapure water was used to rinse the sample containers and was also percolated through the cartridges, to improve the recovery of analytes. Then the cartridges were washed with 5 mL of 2 % v/v aqueous ammonia solution and dried

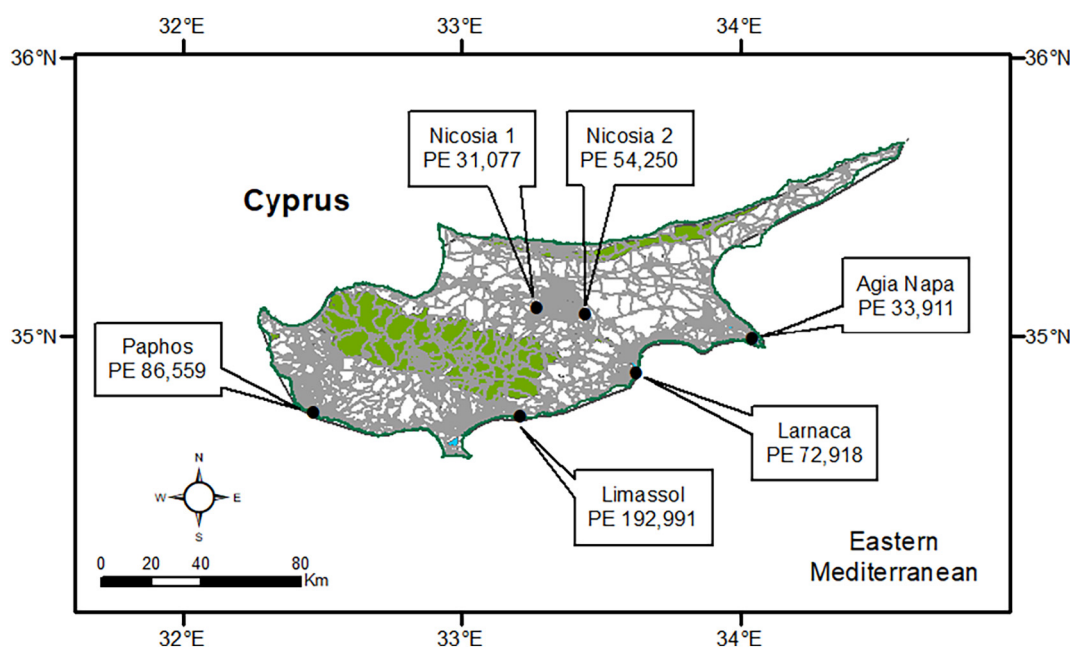


Fig. 2. The exact location of the six WWTPs sampling sites during the seven sampling campaigns. The average population equivalent (PE) for each WWTP is also presented.

under vacuum for 20 min. Elution of the analytes was performed with 8 mL of 2 % v/v ammonia solution in methanol. At all stages the flow rate was 1 mL/min. The collected eluent was then evaporated to dryness under a nitrogen stream at 35 °C and reconstituted with 1 mL of H₂O-methanol (90:10 v/v) solution. The analytical procedure is described in detail elsewhere (Gros et al., 2006; Hapeshi et al., 2015).

2.3.2. UPLC-MS/MS analysis

Chromatographic separation and quantification of the analytes were performed using an ACQUITY TQD UPLC-MS/MS system (Waters Corp. USA). A triple quadrupole mass spectrometer coupled with an electrospray ionization (ESI) source was used. All compounds were analyzed in positive ionization mode (ESI +) and a minimum of two transitions in multiple reaction monitoring (MRM) mode were scanned. The chromatographic separation was achieved on a Waters ACQUITY UPLC BEH C18, 1.7 µm column, with gradient elution, at 40 °C. Eluent A was 0.1 % v/v formic acid in water, while eluent B was methanol. The eluent gradient was 0 min 10 % B, 3 min 90 % B, 3.5 min 90 % B, 3.6 min 10 % B and 6 min 10 % B, with a mobile flow rate of 0.3 mL/min. The injection volume was 10 µL.

The MS parameters were adjusted as follows: ESI source and desolvation temperatures were 120 and 500 °C respectively, the capillary voltage was set at 3.50 kV, and the cone nitrogen and desolvation gas flows were 60 and 1000 L/h respectively. The quantification of each compound was achieved based on the most abundant daughter ion (Q transition) while the second transition and their ion ratios were used for confirmation of the identification. For all drugs, optimization of collision energies and cone voltages was performed. Retention times (R_t), optimal fragments and the conditions used, are shown in Table S1 (Supplementary Information, SI).

2.3.3. Method validation

The protocol for the sample analysis and method validation was based on the SCORE “Protocol of Action for monitoring illicit drugs in wastewater”, a European network of collaboration in the field of WBE, supported by the European Monitoring Centre for Drug and Drug Addiction (EMCDDA), which ensures a common analytical approach and the validity of obtained results in wastewater analysis (https://www.emcdda.europa.eu/drugs-library/common-protocol-action-monitoring-illicit-drugs-wastewater_en).

To ensure minimization of matrix effects on the quantification of the drugs by the UPLC-MS/MS, matrix-matched standard calibration curves were prepared prior to each run. The linearity of the calibration curves in influent wastewater matrix was excellent, with coefficient of determination R² > 0.9900, for all drugs. The analytical method was validated in terms of Limit of Quantification (LOQ), Limit of Detection (LOD), linearity, recovery and instrument and method repeatability. Instrument and method repeatability were controlled by analyzing each sample three times. Details on the validation procedure can be found elsewhere (Boleda et al., 2007; Hapeshi et al., 2015). The LOQ for each compound is shown in Table S1. In cases when daily concentration values were below LOQ, these values were substituted with LOQ/2, for consumption calculation purposes (González-Mariño et al., 2020). The validity of the results is also monitored by the successful participation in the yearly SCORE interlaboratory study.

2.4. Back-calculation of population drug use

As mentioned above, for the estimation of amphetamine, methamphetamine and MDMA the measured concentrations of the parent drug residues were used for the estimation of the consumption, due to their high stability in wastewater and the fact that the parent compounds are excreted from the human body unaltered, to a significant extent. Cocaine consumption was calculated based on the concentration of benzoylecgonine, its main human metabolite that is produced in the liver and excreted in urine (Devault et al., 2017; van Nuijs et al., 2011).

The back-calculation of drug consumption is based on the concentration of each drug (parent/metabolite), the influent wastewater flow rate, and

the population equivalent, served by each WWTP. Thus, the consumption is expressed in terms of mg per day per 1000 inhabitants from Eq. (1) (Castiglioni et al., 2014; van Nuijs et al., 2011).

$$\text{Drug Consumption (mg/day/1000 inhabitants)} = \frac{C \times FR \times CF}{PE} \quad (1)$$

where,

C is the concentration of each drug (ng/L), FR is the flow rate per day (m³/day), CF is a correction factor, unique for each drug and PE the population equivalent.

The average flow rate and average population of each WWTP are shown in Table S2. The CF value for each drug depends on the molar mass ratio of parent drug/metabolite and the excretion rate of the drug or metabolite. For amphetamine, methamphetamine and MDMA, the Mw ratio is 1, as the calculation of their consumption is based on the measurement of the same compounds, whereas for cocaine is 1.05 (Mw cocaine/Mw benzoylecgonine). An excretion rate of 29 % of benzoylecgonine from cocaine consumption was assumed, while the excretion rates adopted for amphetamine, methamphetamine and MDMA were 36 %, 41 % and 22.5 %, respectively, according to González-Mariño et al., 2020. Thus, the CFs for amphetamine, methamphetamine, MDMA and benzoylecgonine used for the back-calculation of the drugs consumption were 2.77, 2.44, 4.40 and 3.59 respectively (Castiglioni et al., 2013; Estévez-danta et al., 2022; González-Mariño et al., 2020; Gracia-Lor et al., 2016; van Nuijs et al., 2011). The population equivalent was estimated using the BOD measurements of the samples, assuming 60 g/day BOD per person (Bauer et al., 2002).

2.5. Statistics

Statistical analysis was performed with Wavemetrics Igor Pro, version 6.3.7.2 and Origin Pro 2021. One-way ANOVA with Bonferroni post hoc analyses was used to investigate statistically significant differences between different sites or different sampling periods. A p-value lower than 0.05 indicates significant differences between two sets of values, with a confidence level of 95 %.

3. Results and discussion

3.1. Amphetamine

According to most recent EMCDDA annual reports, it is estimated that almost one third of adults (aged 15–64) in the European Union have used illicit drugs at least once in their lifetime. Studies between 2015 and 2020 in 25 countries suggest that 1.4 million young adults used amphetamines (amphetamine or methamphetamine) during the last year (EMCDDA 2019, 2021a). Fig. 3 illustrates the estimated consumption of amphetamine obtained during the seven campaigns at the six areas of the Republic of Cyprus under study. Based on relevant literature and in particular the study by Deng et al. (2020), the ratio between the concentration of amphetamine and methamphetamine was calculated and this was found to be higher than 0.1 in most cases. According to this study, when the ratio is above 0.1 (4–10 % transformation of methamphetamine to amphetamine), the calculated amphetamine concentration is attributed to amphetamine's use, rather than the metabolism of methamphetamine (Deng et al., 2020).

Amphetamine was detected in quantifiable levels in 92 % of all samples analyzed, in 95 % of those collected during the lockdown (April 2020) period and in 71 % of the samples taken during the post-lockdown (July 2020) period. The implementation of the lockdown did not show a significant effect on the use of amphetamine, as in most areas it was at a comparable level to that before lockdown and to that of the following year's measurements, except in Larnaca, where a sharp decrease in the consumption of amphetamine was observed during the lockdown, compared to the consumption one year before (April 2019). In general, during the lockdown, similar and relatively low levels of amphetamine consumption

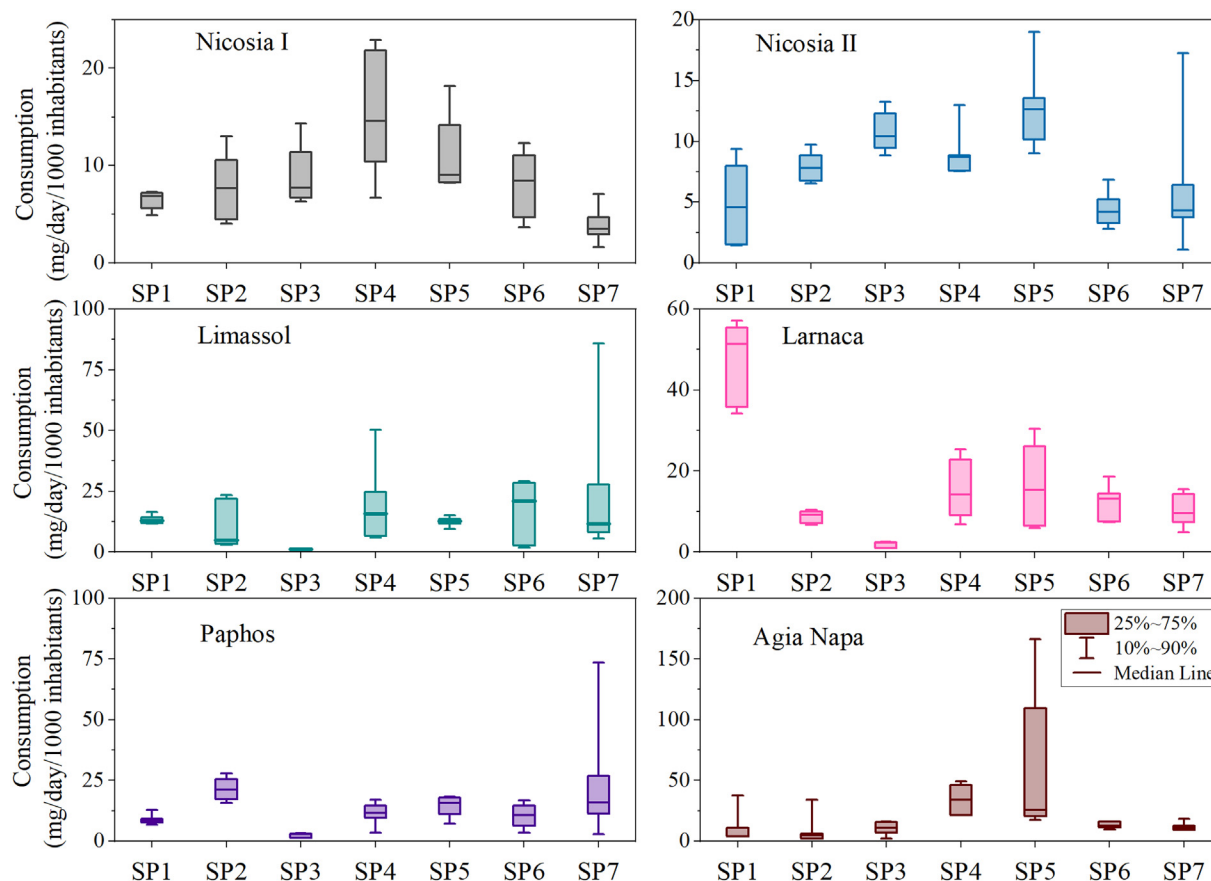


Fig. 3. Amphetamine consumption variation at the six sampling sites during all sampling periods. The boxes represent median (horizontal line), 25th and 75th percentiles (bounds), and whiskers extend to the 10 % and the 90 % values.

were observed at all areas of the Republic of Cyprus, except for the case of Paphos, where the consumption was significantly higher ($P < 0.05$) and almost double, compared with the other areas. In the period that followed the lockdown (post-lockdown campaign), a statistically significant, sharp decrease of the amphetamine use was observed at half of the sampling sites (Larnaca, Paphos, Limassol, $P < 0.05$). However, for the other areas, no significant change on the consumption was observed with the easing of the restriction measures. This mixed picture could indicate a limited availability of the drug during the post-lockdown period at parts of the country, affecting specific areas and leading to the observed decrease of the drug's use. In the same period, illegal stockpiling probably had covered the needs of the users in Nicosia I, Nicosia II and Agia Napa, where availability of the drug was observed. Additionally, according to the EMCDDA 2020 report, local drug markets have been significantly affected by the implemented restrictions on citizens movement and assembly, with users reporting higher prices of illicit drugs during this period (EMCDDA, 2020). Such an implication could have impacted the access and the use of specific drugs among different cities with different social or demographic characteristics, in various ways. According to the same report, these alterations appeared to have a short-term impact on the drug markets, as relaxation of measures allowed the markets to return to a pre-COVID situation. In general, the findings on the consumption levels of amphetamine determined at all areas of the Republic of Cyprus are consistent with other WBE-based estimations (Been et al., 2021; EMCDDA 2020, 2021b; Estévez-danta et al., 2022), according to which, relatively low levels of amphetamine consumption are traditionally observed in the cities of southern Europe.

The data collected during the 2021 sampling campaigns allowed for the examination of possible seasonal changes of amphetamine use in the country, as one campaign took place during each season of the year. During this year, no impact of the pandemic on drug use was expected, as no restriction

measures were applied. One Way ANOVA was used to reveal statistically significant differences between the estimated consumption during each season. No statistical differences were found among the amphetamine consumption levels during the sampling periods of 2021 at half the areas studied (Limassol, Larnaca and Paphos). On the other hand, the consumption during the summer campaign was found to be higher compared to that in the autumn and winter in both Agia Napa and Nicosia II, which could have been a result of the higher touristic activity during this period. In Nicosia I, amphetamine consumption was found to be significantly higher during spring, compared to that in autumn and winter. The fact that the winter measurements were conducted during the Christmas and New Year's period did not show any significant impact on the amphetamine's seasonal consumption variation. The results may suggest that the availability of the drug at the local drug market, together with other factors (e.g., touristic activity, illegal stockpiling, drug purity, etc.), might have been the most significant factors affecting the amphetamine use, rather than the season of the year.

3.2. Methamphetamine

In Europe, methamphetamine is regarded as a stimulant drug that is both imported and locally produced and its use was traditionally limited at central European countries, mainly Slovakia and Czech Republic (EMCDDA, 2019, 2020, 2021b); however, our findings indicate that there is a significant consumption of this drug in the Republic of Cyprus, during the monitoring periods of this study. According to the Republic of Cyprus Police reports, amounts of methamphetamine and methamphetamine chemical precursors have been seized the last years in the country, suggesting that a local production may exist (<https://in-cyprus.philenews.com/news/local/new-large-scale-methamphetamine-production-laboratory-busted-in-limassol-three-arrested/>).

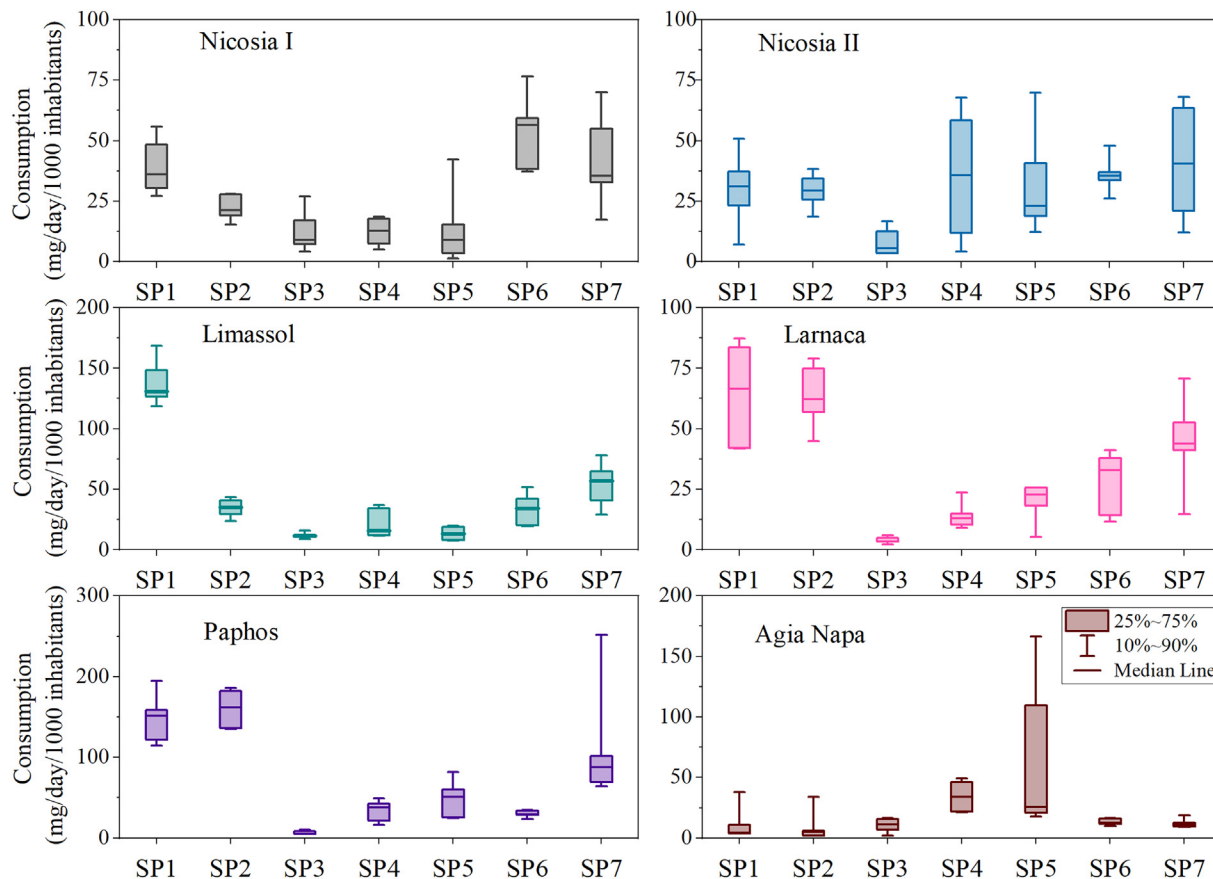


Fig. 4. Methamphetamine consumption variation at the six sampling sites during all sampling periods. The boxes represent median (horizontal line), 25th and 75th percentiles (bounds), and whiskers extend to the 10 % and the 90 % values.

Fig. 4 illustrates the findings regarding methamphetamine consumption during the monitoring periods of 2019 to 2021. On national level, the highest use was observed during the spring of 2019 in Limassol and Paphos (where daily consumption reached almost 200 mg/day/1000 inhabitants) and also in Paphos even during the lockdown period. Comparing methamphetamine consumption during the spring of 2019 and the spring of 2020, it is shown that the implementation of the restriction measures did not have any significant impact on its use, at the majority of the areas studied (Nicosia II, Larnaca, Paphos, Agia Napa). However, a significant decrease of its consumption was observed in Nicosia I and Limassol ($P < 0.05$). Regardless of this difference among the areas, the use of methamphetamine dropped sharply during the post-lockdown period at all the areas studied. The most pronounced decrease was observed in Paphos and Limassol, where consumption dropped 22-fold and almost 17-fold, respectively. This drop of methamphetamine use during the post-lockdown period could be related to the reduction of the availability of this drug in the country and/or difficulties in its distribution. Possible lack of availability of methamphetamine's precursors could also have led to the decrease or ceasing of local production of this stimulant, affecting its purity and the extent of its use.

The measurements of the following year revealed a small increasing trend of methamphetamine's use during the last months of the year at most of the areas studied. Higher consumption during autumn and winter was observed at the majority of the areas under study. Apart from Nicosia II and Agia Napa, where a stable profile of the consumption of the drug was observed throughout the year, at all the other areas the highest methamphetamine use was found during the winter period. The significant difference that was observed for methamphetamine use during the winter ($P < 0.05$) is mainly attributed to the higher consumption during the week-ends of Christmas and New Year's Eve and New Year's Day (Fig. S1).

3.3. MDMA consumption

MDMA is one of the stimulant drugs that is mainly used in nightlife settings, such as clubs and bars and is regarded as a party drug. In the European territory it is produced both for local and global markets. Almost 2 % of young adults (15–34) have used MDMA at least once, according to a survey in 26 countries (EMCDDA, 2021a).

The variation of MDMA consumption during all campaigns is presented in Fig. 5. During the lockdown, at four out of six areas, its consumption decreased significantly ($P < 0.05$), compared to the previous spring measurements. The most pronounced decreases were found in Larnaca and Agia Napa, where the decrease was >11- and 9-fold respectively, followed by Nicosia I and Limassol areas (almost 6-fold and 5-fold, respectively). On the other hand, at Nicosia II and Paphos areas, the MDMA use was not significantly affected by the lockdown, and the consumption at these areas during both periods of spring of 2019 and lockdown were rather low, compared with the other sites. For Agia Napa, the measured MDMA concentrations during the lockdown sampling were all below the LOQ, hence the consumption was estimated based on concentrations equal to LOQ/2.

As shown in Fig. 5, MDMA use changed drastically at most areas during the post-lockdown period. A notable increase of the consumption was observed at four out of six areas (Nicosia I, Nicosia II, Limassol and Agia Napa, statistically significant at the last three) during this period, compared with that of the lockdown campaign. It should be noted that at four sites (Agia Napa, Nicosia I, Nicosia II and Paphos) the consumption levels observed during the post-lockdown period were the highest of the whole monitoring period of this study. Remarkably high daily MDMA consumption values were also recorded: At Nicosia I area, the highest daily consumption observed exceeded the value of 800 mg/day/1000 inhabitants, while the second highest daily MDMA consumption was reported at

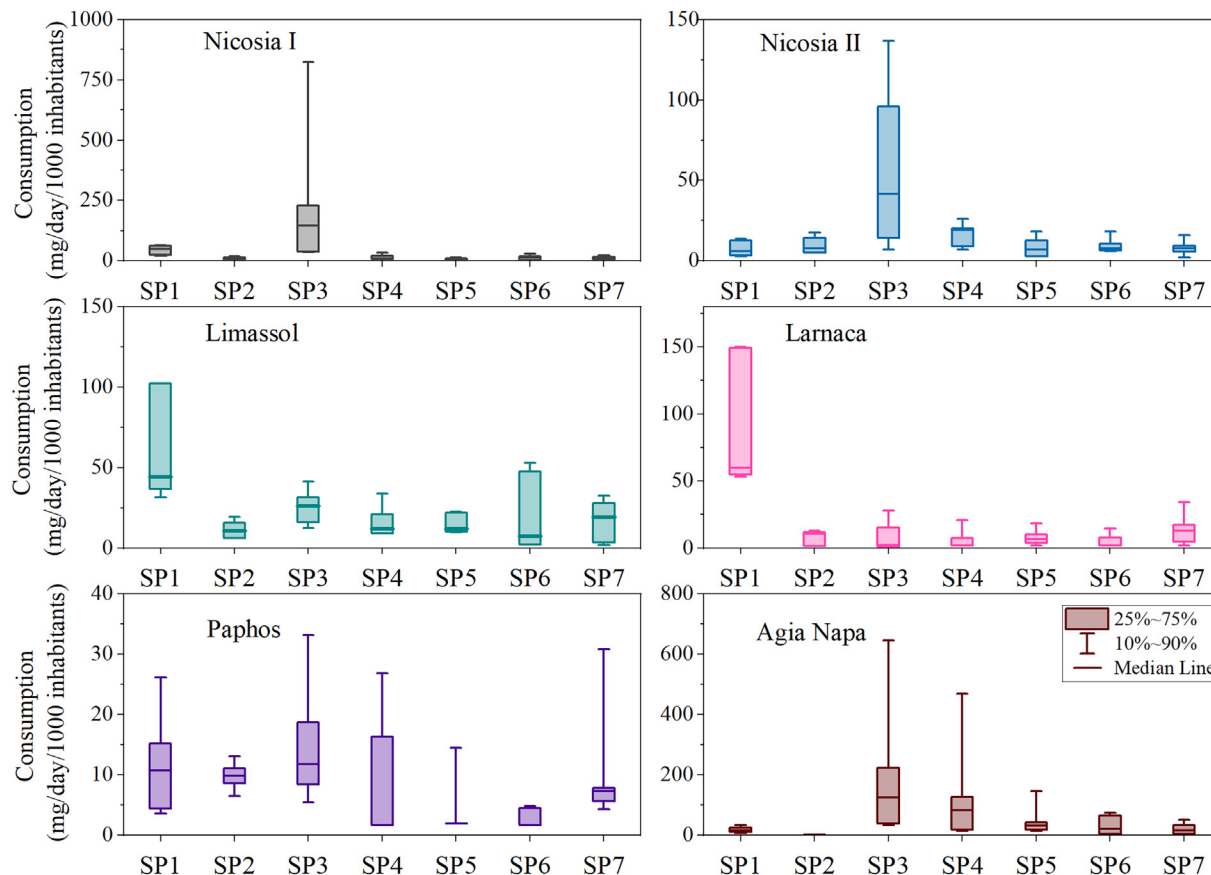


Fig. 5. MDMA consumption variation at the six sampling sites during all sampling periods. The boxes represent median (horizontal line), 25th and 75th percentiles (bounds), and whiskers extend to the 10 % and the 90 % values.

the most touristic area of the country, Agia Napa (645 mg/day/1000 inhabitants). The observed decrease of MDMA use during the lockdown is attributed to the shutdown of entertainment industry, the cancellation of various events and social gatherings and to difficulties related with the distribution of drugs during this period. The easing of transportation limitations and the reopening of entertainment activities during the summer of 2020 facilitated both the distribution and the use of the drug and hence the observed increase of its consumption during the post-lockdown sampling. Based on the determined concentrations, the following year (2021) MDMA use seems to have been stabilized in the whole country and no seasonal differences were detected at all the areas under study. In general, the consumption levels during this year were lower, compared to those during the post-lockdown period.

3.4. Cocaine consumption

Due to the relatively low stability of cocaine in sewage samples, its consumption was estimated based on the concentrations of its major metabolite, benzoylecgonine, from Eq. 1 and with 3.59 as the correction factor (McCall et al., 2016). Benzoylecgonine was detected in all daily samples collected from all the six WWTPs. Cocaine is the second most commonly used illicit drug in Europe; in this work, it was found to be the most commonly used illicit stimulant drug in the Republic of Cyprus, as well.

The implementation of the lockdown severely impacted the cocaine consumption in the country. Its daily consumption varied from 25 to 385 mg/1000 inhabitants/day during the lockdown, with an average value of 213 mg/1000 inhabitants/day, less than half of the 2019 average. Variations of cocaine consumption among all studied areas during all sampling campaigns are presented in Fig. 6. For five out of six areas, i.e., Nicosia I, Nicosia II, Limassol, Paphos and Agia Napa, a statistically significant, pronounced decrease of cocaine consumption was observed

during the 2020 lockdown period, compared with the previous year ($P < 0.05$), with the strongest decrease observed at Limassol (6-fold). Interestingly, at the city of Larnaca, cocaine use increased significantly, by more than double, compared with the consumption in 2019. Similar decreasing trends were also observed in other countries during COVID-19 restrictions, however, such findings were not consistent everywhere, as cocaine use during lockdowns presented a mixed behaviour, highly dependent on the location of the study (Alygizakis et al., 2021a; Bade et al., 2021; Been et al., 2021; Reinstadler et al., 2021b). This heterogeneity in drug use trends suggest a complexity of the society responses to the various stressors related to COVID-19 implications. Surveys suggest that the most important factors leading to decreased use of the typically recreational drugs, cocaine and MDMA, are the lack of occasions for use in social/recreational settings and the impediments to socialization (Bendau et al., 2022).

During the post-lockdown period of 2020, cocaine consumption showed a remarkable increase at most areas of the Republic of Cyprus (Nicosia I, Nicosia II, Limassol, Paphos and Agia Napa), compared with the lockdown period. An almost 10-fold increase of the consumption was observed at Agia Napa, 9-fold at Nicosia I, 4-fold at Nicosia II, while the consumption at Limassol and Paphos was around 2.5 times of that during the lockdown period. Daily consumption values during the post-lockdown period ranged from 109 to 2691 mg/1000 inhabitants/day with an overall average of 949 mg/1000 inhabitants/day among all sites (data not shown). At most areas, cocaine use was highest during the post-lockdown campaign, compared to all the other monitoring periods. Only Larnaca city again presented a different behaviour, where cocaine, but also MDMA consumption levels remained similar during the lockdown and the post-lockdown periods. The different effect of the lockdown implementation and relaxation of restriction measures at this area could be attributed to various reasons, including the availability of cocaine and other stimulants, the purity of the drugs, which would reflect on the levels of the mass loads detected

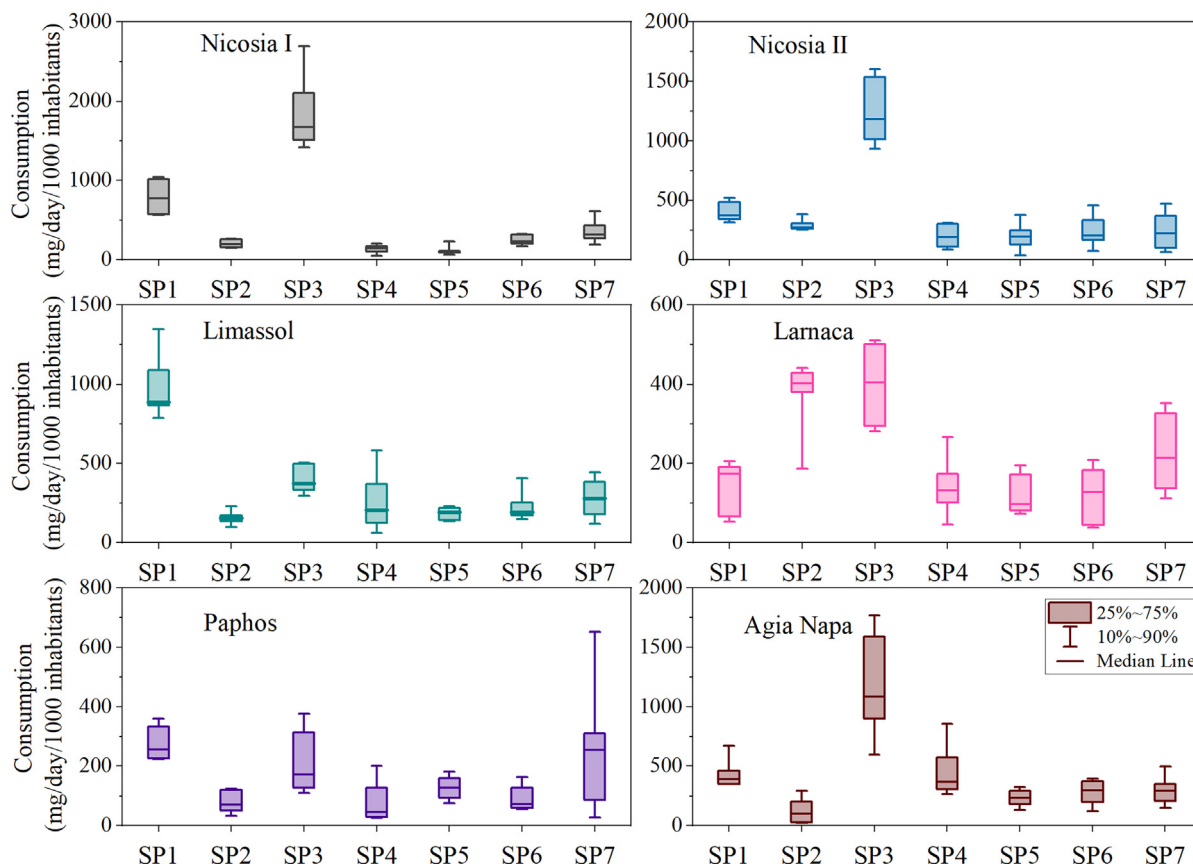


Fig. 6. Cocaine consumption variation at the six sampling sites during all sampling periods. The boxes represent median (horizontal line), 25th and 75th percentiles (bounds), and whiskers extend to the 10 % and the 90 % values.

and/or temporary changes of the drug preferences of the users due to social or economic factors. The observed trend of high cocaine use after the relaxation of COVID-19 related restriction measures in the Republic of Cyprus is supported by other WBE-based studies, depicting an increase of cocaine and other stimulants during post-lockdown periods (Bade et al., 2021; Repec and Vuci, 2022). The results also show a stabilization of cocaine during the following year, with no significant seasonal variations. Only in Paphos, statistically significant higher cocaine use was observed during the winter, which is mainly attributed to the high consumption during New Year's Eve and New Year's Day (Fig. S1).

3.5. Weekly patterns of stimulants use

The daily consumption data from all six areas under study from spring, summer, autumn and winter of 2021 campaigns was grouped, in order to investigate the weekly patterns of the drugs use. These periods were selected as they are not affected by implications related to the pandemic and reflect the most recent and “normal” drug use patterns in the country. For each drug, data points from all sampling sites were combined, to reflect the weekly patterns of stimulants use in the Republic of Cyprus. Fig. 7 represents the variation of the consumption of each drug, depending on the day of the week.

All drugs, except amphetamine, showed weekly consumption cycles, with higher drug use during weekends, however Monday samples also showed higher consumption compared to the other weekdays. One way ANOVA comparisons showed that only for cocaine, Saturday and Sunday use was significantly higher compared to Tuesday, Wednesday and Thursday ($P < 0.05$). The use of methamphetamine, cocaine and MDMA is also high during Mondays, especially for MDMA, probably because of the excretion of drug or metabolite residues on Monday morning after late Sunday use. Differences among the weekend patterns of the drugs are

in general related to differences in excretion patterns, time between drug consumption and excretion of the parent/metabolite in urine, as well as differences in usage trends (van Nuijs et al., 2011). Regarding the use of amphetamine, no daily differences of its use could be detected, with the drug presenting a very stable weekly profile.

Similar profiles for cocaine and MDMA consumption during weekdays and weekends have been previously reported in other works, revealing weekly patterns of the use of these drugs (Irvine et al., 2011; Krizman-Matic et al., 2019). The weekly patterns of MDMA and cocaine confirm the recreational character of the use of these drugs, which has often been stressed in previous studies. However, in this work, methamphetamine was also found to be used more during the weekends, indicating its consumption for similar reasons.

These weekly trends were absent for cocaine and methamphetamine during the lockdown period and only MDMA was found to be used more during the weekends (Fig. S2). Less pronounced or lack of weekend consumption peaks during the lockdown were also reported in other WBE-based studies during similar lockdown periods. This observation is attributed to the absence of events and the impediments in socialization (Been et al., 2021).

4. Conclusions

This work investigated the illicit stimulant drugs use in the Republic of Cyprus during a pre-COVID period, during the quarantine period, right after it, and during the four seasons of the following year, by applying the WBE approach. The results point towards a heterogeneous effect of the pandemic on the use of stimulants and an overall short-term impact of the COVID-19 related restrictions. The little to no effect on the use of amphetamine and methamphetamine by the implementation of the lockdown is mainly attributed to stockpiling, which led to the observed decreasing trend during the post-

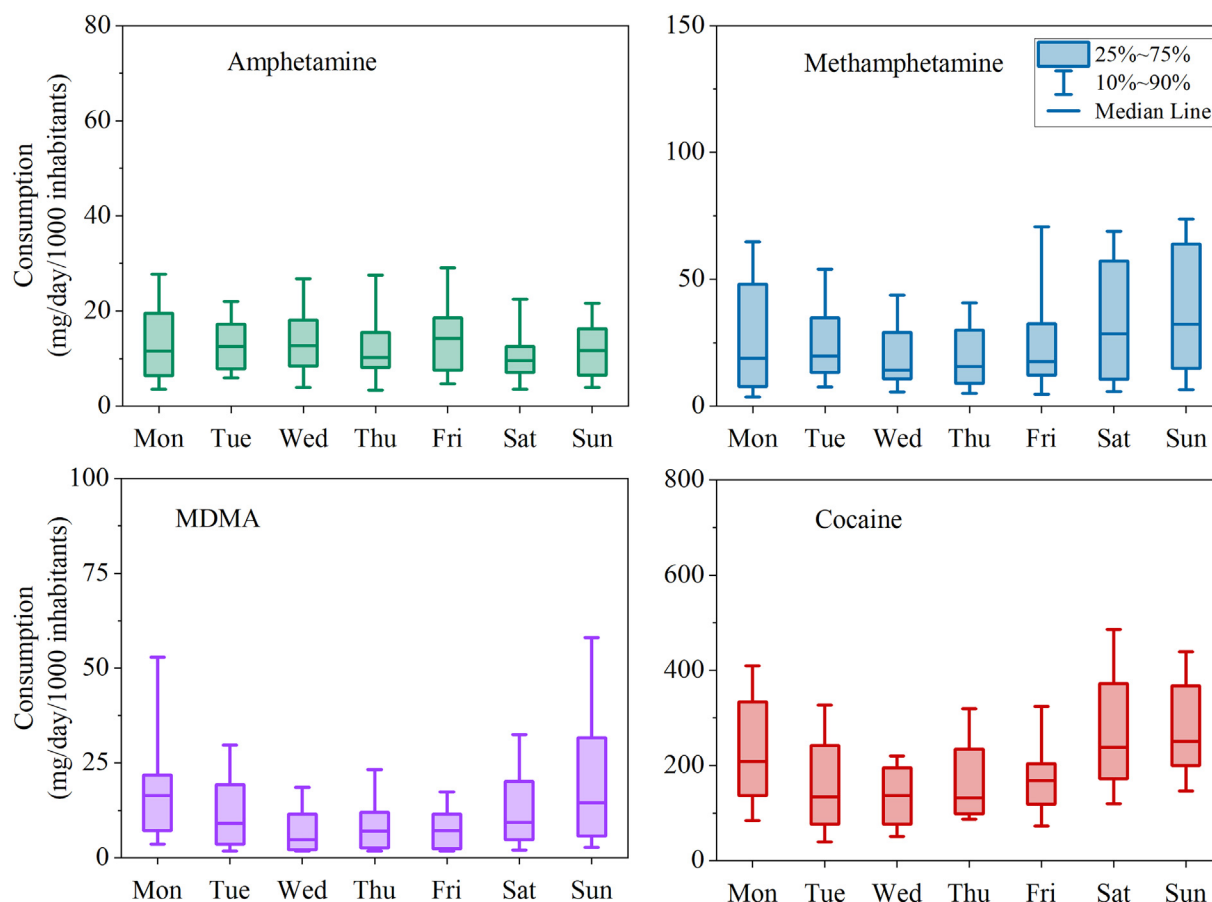


Fig. 7. Daily consumption of amphetamine, methamphetamine, MDMA and cocaine, estimated from all areas studied, during the 2021 campaigns (grouped data). The boxes represent median (horizontal line), 25th and 75th percentiles (bounds), and whiskers extend to the 10 % and the 90 % values.

lockdown period, probably due to a delayed shortage. On the other hand, the closure of entertainment industry and the cancellation of all events led to a significant drop of the use of other substances, typically related to recreational occasions i.e., MDMA and cocaine, which bounced back when most restrictions were lifted, reaching high consumption levels. The occurring differences support the high complexity of the impact of the pandemic on drug use, which is dependent on the substance and the location and cannot be attributed to a single factor. Our results also indicate that not only the confinement, but also the relaxation measures can drive alterations on drug use patterns. The study also revealed that methamphetamine use is evolving in the Republic of Cyprus, as the estimated consumption of this drug was significant at all areas, during most of the monitoring periods of this work. For cocaine, MDMA and methamphetamine, a recreational pattern of their use was also observed, confirming previous studies. The application of WBE demonstrates that this technique can provide real-time information on the illicit drugs use at local level, revealing their actual temporal and spatial trends, and stress the importance of the establishment of wastewater surveillance networks, which can address current threats and provide support to law enforcement and prevention interventions.

CRedit authorship contribution statement

M. Psichoudaki: Conceptualization, Methodology, Investigation, Validation, Visualization, Data curation, Writing – original draft. **T. Mina:** Investigation, Methodology. **M. Savvidou:** Conceptualization, Funding acquisition. **C. Mina:** Conceptualization, Funding acquisition. **C. Michael:** Methodology, Validation, Investigation, Writing – review & editing. **D. Fatta-Kassinis:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition.

Data availability

Data will be made available on request.

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.2022.158747>.

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