Application of wastewater-based epidemiology to investigate stimulant drug, alcohol and tobacco use in Lithuanian communities

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HIGHLIGHTS

- Illicit drug, alcohol and tobacco use in Lithuania was assessed through wastewater-based epidemiology.
- Methamphetamine and MDMA were the most abundant substances used.
- State-of-the-art analytical techniques revealed a dumping event of amphetamine.
- Measured alcohol use was almost twice the European average.
- Some discrepancies with other data sets on nicotine use were observed, potentially suggesting illegal trade.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

WBE was applied to evaluate illicit drug (i.e. amphetamine, cocaine, MDMA and methamphetamine), alcohol and tobacco use in three Lithuanian cities in 2018 and 2019. Considerable concentrations of methamphetamine and MDMA were found in the three locations, suggesting a specific Lithuanian consumption pattern. Yet, unexpected high concentrations of amphetamine (>4 µg/L) were detected in two samples of Kaunas in 2018. Through the use of chiral analysis and non-target and suspect drug precursor compound screening, these extreme values were confirmed to be the result of direct disposal of amphetamine in the sewers. Furthermore, substantial alcohol use was measured in the three investigated catchment populations of Lithuania with almost 4 standard drinks/day/inhabitant aged 15+ on average in 2019. For tobacco, an average of 5.6 cigarettes/day/inhabitant aged 15 + in 2019 was reported with large discrepancies between WBE figures and sales data, potentially highlighting illegal trade of tobacco products.

1. Introduction

The population health status in Lithuania has improved for the past decade, but still remains much lower compared to most European countries (Organisation for Economic Co-operation and Development, 2018;

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Organisation for Economic Co-operation and Development, n.d.; OECD/European Observatory on Health Systems and Policies, 2017). Even though the life expectancy in Lithuania is on the rise, it still remains the lowest in the European Union (EU) (Organisation for Economic Co-operation and Development, n.d.; OECD/European Observatory on Health Systems and Policies, 2017). The health status and the related gender gap (with a life expectancy 10 years higher for women) are closely linked to behavioural risk factors (e.g. alcohol consumption, tobacco smoking, illicit drug use, low physical activity, diet) which attribute to almost 40% of the overall burden of disease in Lithuania (OECD/European Observatory on Health Systems and Policies, 2017).

Data from the World Health Organization (WHO) suggests that the per capita alcohol consumption (APC) in the Baltic States is 1.5 times higher than the EU average, with Lithuania situated among the heaviest-drinking countries worldwide (OECD/European Observatory on Health Systems and Policies, 2017; World Health Organization, 2019). As a result, many of the leading causes of death in Lithuania (e.g. cardiovascular diseases, liver diseases, accidental poisoning and road traffic accidents) are associated with high alcohol use and the alcohol-related mortality rate is estimated to be much higher compared to the EU average (OECD/European Observatory on Health Systems and Policies, 2017; World Health Organization, 2019; Higienos Institutosveikatos Informacijos Centras, 2019). Smoking is also an important health issue in Lithuania; in fact, 20% of the Lithuanian population smoked daily in 2014 (Organisation for Economic Co-operation and Development, n.d.) despite the strengthened tobacco regulations during the past decade (OECD/European Observatory on Health Systems and Policies, 2017). Even though the number of adults smoking tobacco in Lithuania has dropped sharply to below EU average, 33.9% of men still remain daily smokers (OECD/European Observatory on Health Systems and Policies, 2017).

General population survey (GPS) data shows that approximately 11.5% of Lithuanian adults used an illicit drug in their lifetime, with drug use being highest among young adults aged 15–34 years (*Drugs tobacco and alcohol control departement Litunia*, 2017). Additionally, methylenedioxymethamphetamine (MDMA) was used most frequently as a stimulant in 2016 based on survey data, followed by other amphetamines (i.e. amphetamine and methamphetamine) and cocaine (*Drugs tobacco and alcohol control departement Litunia*, 2017). Furthermore, Lithuania's death rates from drug overdoses are twice the EU average (The Lithuania Tribune, 2016; European Monitoring Centre for Drugs and Drug Addiction, 2019a). While the production of stimulants is considered to be limited, methamphetamine proves to be the most common illicit drug produced in Lithuania, as reflected by its domestic use and police reports on methamphetamine laboratories detections in the past. Lithuania is considered to be a transit country for illicit drug trafficking between Eastern and Western European and Scandinavian countries (European Monitoring Centre for Drugs and Drug Addiction, 2019b).

Even though a lot of valuable epidemiological information in Lithuania is already available at this moment, there are still some knowledge gaps, especially regarding illegal substance use. Current figures on the consumption of legal and illegal substances are mainly obtained through sales data and GPS, which are inherently linked to some limitations and challenges. A main limitation of the GPS is the infrequency of data reporting (i.e. not on a yearly basis) and reporting and concealment bias regarding substance use experiences. A limitation associated with sales data is that the locality between where these substances are sold and the consumption occurs might not be the same and countries with low taxation rates are often associated with high sales figures. As such, alcohol and cigarettes could be imported from neighbouring countries in order to avoid growing taxation rates in Lithuania. Additionally, data on substances produced, distributed and sold outside the formal channels under governmental control (i.e. home-made and illegal production, illegal trafficking, internet sales, …) are not included in official sales statistics. According to the WHO

up to 8% of total per capita alcohol consumption in Lithuania is not recorded in official sales statistics (World Health Organization, 2019). Additionally, smuggling of tobacco products from Belarus and supply of tobacco heating products from Ukraine is a well-known public health concern in Lithuania (Transcrime – Research Centre on Transnational Crime, 2016; Lietuvos Respublikos Muitinės, 2019; Ministry of Financies, n.d.), however the extent needs further exploration. In this light, new complementary data sources are needed to provide actual information on intra-country differences and trends in the amounts of substances consumed.

Over the past decade, wastewater-based epidemiology (WBE) has become a reliable and complementary approach to monitor and back-estimate illicit drug use in populations (van Nuijs et al., 2011; Lai et al., 2013; Banta-Green et al., 2009; Zuccato et al., 2008; González-Mariño et al., 2020). Within this methodology, human metabolic excretion products are collected, pooled and transported in the wastewater system, providing valuable information on the amount and type of substances used by defined population groups (Zuccato et al., 2008). Concentrations of biomarkers in wastewater are multiplied with daily wastewater flow rates and divided by the population served by the wastewater treatment plant (WWTP) to obtain population-normalized mass loads (mg/day/1000 inhabitants). This allows comparison of results across different locations and at different time points. Proof-of-concept studies have been performed to show that WBE is further suitable to assess community health. For example, WBE was applied to monitor alcohol, tobacco and pharmaceutical use (Boogaerts et al., 2016; Lai et al., 2018a; Lai et al., 2018b; Lai et al., 2017; Zhang et al., 2019; Tscharke et al., 2016; Gao et al., 2020; Mackie et al., 2019; Mastroianni et al., 2017), to measure endogenous substances of disease or health and to estimate the exposure to emerging contaminants, such as pesticides or flame retardants (Been et al., 2017; Daughton, 2018). WBE can thus provide complementary epidemiological information and fill in some of the current knowledge gaps regarding the public health status in Lithuania. WBE can be used to continuously and in near real-time monitor the evolution of lifestyle-related indicators in different communities (Gracia-Lor et al., 2017). This unique feature makes WBE an excellent early-warning information system able to capture quickly developing changes in substance use. This characteristic provides policy-makers crucial information to allow timely decision-making and evaluating the effectiveness of new guidelines and national and local initiatives. By monitoring at a high spatial resolution, WBE offers a possibility to assess intra-country differences in the consumption of illicit drugs alcohol and tobacco (Banta-Green et al., 2009; Boogaerts et al., 2016; Lai et al., 2018b). Furthermore, WBE could be particularly useful to deliver information on illegal consumption and trade of substances. However, WBE is not able to provide any information on individual consumption patterns and socio-demographics of the user.

This manuscript reports on the investigation of substance use (i.e. stimulants, alcohol and tobacco) in Lithuania with a focus on intra-country differences and temporal variations by a WBE approach. The obtained data can be used to gather more evidence on the lifestyle-related health status of the Lithuanian population but also to put this in a broader European perspective.

2. Materials and methods

2.1. Reagents and materials

The analytes of interest (purity ≥ 99%) and their deuterated analogues used as internal standards (IS) were obtained from Cerilliant (Round Rock, Texas, USA), Toronto Research Chemicals (Toronto, ON, Canada) and Athena Enzyme Systems (Baltimore MD, USA). Reference standards and deuterated reference standards were of analytical grade and purchased as neat powder or as solutions of 1 mg/mL or 100 g/mL in methanol (MeOH) or acetonitrile (AcN). Dilutions and working mixtures with concentrations ranging between 0.05 and 100 μg/mL were

prepared in MeOH. Analytical grade hydrochloric acid (HCl), ammonium hydroxide (NH$_4$OH), formic acid (HCOOH), acetic acid (CH$_3$COOH) and ammonium acetate and LC grade AcN and MeOH were purchased from Merck (Darmstadt, Germany). Milli-Q ultrapure water was obtained by purifying demineralised water in an Elga LabWater Purelab Flex system (Veolia Water Solutions & Technologies Belgium, Tienen, BE). Oasis MCX (60 mg, 3 mL) solid-phase extraction (SPE) cartridges were purchased from Waters (New Bedford, Massachusetts, USA). A Supelco Visiprep SPE Vacuum Manifold 24-port model with a self-cleaning dry vacuum system Welch 2023 was used for the loading of the sample on the cartridge and the drying of the cartridges. Safe-lock tubes (1.5 mL and 2 mL) were obtained from Eppendorf (Rotselaar, Belgium) and centrifugal filters (0.20 μm and 0.45 μm) containing modified nylon were acquired from VWR (Leuven, Belgium).

2.2. Sampling and analysis

2.2.1. Sampling

Influent wastewater (IWW) samples were collected from three Lithuanian cities (Fig. 1) (i.e. Kaunas, Klaipeda and Vilnius), covering approximately 35% of the Lithuanian population (European Commision - Eurydice, 2020). 24-h composite IWW samples were collected time-proportionally in 2018 and 2019 for at least seven consecutive days (see Table 1). Aliquots of 500 mL were immediately frozen after collection and stored at −20 °C until analysis to prevent degradation of biomarkers (van Nuijs et al., 2012). No special events were reported within the sampling period in the different locations. pH of the IWW samples ranged between 7.4 and 8.5 and temperature between 13 and 15 °C. Average residence time was in all locations less than 24 h.

Table 1
Summary of sampling locations and periods.

Sampling location	Sampling period	Inhabitants served by wastewater treatment plant	% of Lithuanian population served by the wastewater treatment plant	Age distribution (%)
Vilnius	12/04/2018–19/04/2018 11/09/2019–18/09/2019	536,631	19.2	0–14: 17.1 15–24: 8.3 25–44: 34.1 45–64: 24.2 65+: 16.3
Kaunas	11/04/2018–18/04/2018 27/03/2019–2/04/2019	288,363	10.5	0–14: 15.1 15–24: 9.5 25–44: 28.1 45–64: 26.4 65+: 20.9
Klaipeda	10/04/2018–16/04/2018 27/03/2019–02/04/2019	164,038	5.9	0–14: 16.6 15–24: 8.8 25–44: 27.7 45–64: 27.2 65+: 19.7

2.2.2. Sample preparation and instrumental analysis

Sample preparation was done accordingly to previously validated bioanalytical methods. Performance criteria of these bioanalytical methods (i.e. accuracy, precision, …) met the criteria for method validation provided by the European Medicines Agency (EMA) guidelines. Either direct injection or solid-phase extraction (SPE) were employed based on the biomarker concentrations in IWW. A detailed description

Fig. 1. Geographical map of Lithuania.
Adapted from: (Centers for Disease Control and Prevention, 2020).

of the well-established bioanalytical methods is given in the Supplementary Information (S1, Tables S1 and S2). Extraction of benzoylecgonine (BE), amphetamine (AMP), methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA) in IWW was done according to van Nuijs et al. (2009). Sample preparation for the analysis of ethyl sulphate (EtS) and cotinine (COT)/hydroxycotinine (COT-OH) in IWW was performed according to a validated method by Boogaerts et al. and Lai et al., respectively (Boogaerts et al., 2016; Lai et al., 2017). Multi-level calibration curves with final concentrations ranging between 1 and 3000 ng/L were constructed for each analyte with different working standard solutions and a fixed amount of deuterated analogue in methanol. For analyte confirmation, the quantifier/qualifier ratio must not differ more than ±15% and the relative retention time must not differ more than 2.5%. Quality control was performed by in-house QA/QC measures and for illicit drugs through participation in an inter-laboratory exercise provided by Sewage Analysis Core group Europe (SCORE) (van Nuijs et al., 2018).

2.2.3. Confirmation of a direct dumping event by enantiomeric analysis and non-target and suspect screening

Enantiomeric analysis and non-target and suspect screening were only considered when a potential dumping event was suspected based on daily mass load fluctuations. This was only the case for the sampling week in 2018 in Kaunas, where two extremely high values for AMP (1000-fold higher than baseline loads) were detected. For the other locations and years we observed daily variations in population-normalized mass loads that could be attributed to human consumption; no indications for dumpings could be found. Other biomarkers were not considered for enantiomeric analysis and non-target and suspect screening for the same reason. Additionally, this should only be considered when the parent compound is used as human biomarker.

The chiral signatures of AMP in influent wastewater were verified with chiral liquid chromatography coupled to tandem mass spectrometry to distinguish between consumption and direct disposal of AMP. Chiral analysis was undertaken according to the methodology described elsewhere (also in the Supplementary information) (Castrignano et al., 2016; Castrignano et al., 2018).

The enantiomeric fraction (EF) was calculated using the following Eq. (1):

$$EF = \frac{S(+)}{(S(+) + R(-))} \tag{1}$$

where

$$S(+) = \frac{\text{peak area of analyte } S(+)-\text{enantiomer}}{\text{peak areas of internal standard } S(+)-\text{enantiomer}}$$

$$R(-) = \frac{\text{peak area of analyte } R(-)-\text{enantiomer}}{\text{peak areas of internal standard } R(-)-\text{enantiomer}}$$

EF equals 0.5 in the case of a racemate, while 1 or 0 in the case of the enantiopure compound.

Non-target and suspect screening of drug precursors was previously described in Emke et al. (2018). An in-house suspect list containing relevant compounds (i.e., (pre-)precursors, intermediates, impurities, by-products) was compiled from available literature for both MDMA and AMP. The latter contained 197 and 70 compounds for MDMA and AMP, respectively. Day 7 and 8 of Kaunas from 2018 were considered to be from non-consumption origin. Hence the ratio between the consumption and non-consumption group was determined. A minimum peak intensity of 500,000 counts was used for peak picking, together with a log-fold 2 change greater than 4 and a group ratio greater than 20. A mass tolerance of 3 ppm and retention time shift of 1 min were used for feature detection. Library searches were conducted against *mzCloud* (HighChem Ltd., Slovakia), *mzVault* (Thermo Fisher Scientific Inc., USA) (with the *mzVault May 2018* library), and *Chemspider* (Royal Society of Chemistry, USA) (with *EAWAG biocatalysis/biodegradation*, *EPA DSSTox*, *EPA toxcast*, *Drugbank*, *ACToR*, and *FDA UNII – NLM* databases), while suspect screening was performed using the abovementioned in-house list.

2.2.4. Back-calculations and data analysis

Measured concentrations of all biomarkers (expressed in ng/L) were multiplied by daily wastewater flow rates (L/day) recorded in the WWTPs and divided by the population sizes to obtain population-normalized mass loads (mg/day/1000 inhabitants). These can be considered as a proxy for consumption of the parent compound. The normalization for population enables the comparison of consumption patterns across different locations and different time points. For the illicit drugs, back-calculations to population-normalized mass loads were performed with no further back-calculations to doses. For alcohol and tobacco, mass loads were further transformed into per capita daily standard doses to allow a brief comparison between WBE data and official sales figures.

In order to transform standard doses of alcohol and tobacco, excretion rates and molar mass ratios were taken into account (Boogaerts et al., 2016; Lai et al., 2017; Gao et al., 2020; Mackie et al., 2019; van Wel et al., 2016). A conversion factor of 3049 was applied to transform population-normalized mass loads of EtS in per capita loads of alcohol. In order to obtain standard alcohol doses, daily per capita loads of alcohol were divided by the alcohol content in a standard alcohol drink (9.86 g), as illustrated by Eq. (2).

$$\text{Daily per capita standard alcohol doses} = \frac{g \ EtS \ per \ day \ per \ capita \times 3049}{9.86 \ g} \tag{2}$$

In this study, population-normalized mass loads of nicotine were derived from COT and COT-OH. A correction factor of 3.13 and 2.31 were used respectively to derive population-normalized mass loads of nicotine, as illustrated by Eq. (3). Population-normalized, COT and COT-OH derived estimates of nicotine were averaged and divided by the average amount of nicotine per cigarette to obtain daily cigarettes consumption per capita, as reflected by Eq. (4).

$$\text{mg nicotine per day per capita}$$
$$= \frac{(mg \ COT \ per \ day \ per \ capita*3.13) + (mg \ COT-OH \ per \ day \ per \ capita*2.31)}{2} \tag{3}$$

$$\text{Daily per capita standard cigarette doses}$$
$$= \frac{mg \ nicotine \ per \ day \ per \ capita}{0.9 \ mg} \tag{4}$$

To compare WBE figures with the other Lithuanian data sources only inhabitants aged 15+ were included (i.e. 84.9% of Lithuanian population), which resulted in standard doses per capita aged 15+ (European Commision - Eurydice, 2020). Results from the different locations were combined to estimate an annual national amount of consumed substances. Weighted averages were used for this calculation in order to take the proportions of the populations covered by the different catchments into account. Furthermore, the WBE estimates reported in this study were compared with WBE results from other European countries collected in a similar manner (Boogaerts et al., 2016; Lai et al., 2017; Gao et al., 2020; van Wel et al., 2016; Gonzalez-Marino et al., 2020; Reid et al., 2011; Rodríguez-Álvarez et al., 2014; Lopes et al., 2014; Castiglioni et al., 2015; Baz-Lomba et al., 2016). In order to compare results with these figures, the population was not normalized for age 15+.

Statistical analysis was performed with Prism version 8.3.1. (GraphPad Software, California, USA). A Shapiro-Wilk normality test was applied to test if data was distributed normally in order to test if parametric tests were appropriate or not. Variations between years and locations were assessed by applying a parametric One-way ANOVA test, followed by a Dunn's post hoc test or the non-parametric equivalent depending on the normal distribution ($\alpha = 0.05$).

3. Results and discussion

3.1. Illicit drug consumption

3.1.1. Intra-country differences in illicit consumption

The population-normalized mass loads of illicit drugs in the three Lithuanian catchments are shown in Fig. 2.

Daily variations in population-normalized mass loads of all biomarkers are given in Table S4 and Fig. S1. For COC and MDMA, no significant spatial differences were found in the consumption patterns in both years, as illustrated by Fig. 2B and C. In 2018 no significant spatial differences were found in the consumption of AMP in the investigated catchment areas (Fig. 2A). However, the use of AMP was in 2019 significantly higher in Kaunas compared to Klaipeda. In addition, METH consumption was higher in Kaunas and Vilnius compared to Klaipeda in both years (Fig. 2D), which was in line with GPS data (*Drugs tobacco and alcohol control departement Lituania*, 2017). It should be noted that AMP found in the sewer could also originate from METH consumption since AMP is a human metabolic excretion product. However, this fraction is limited since METH is only excreted in urine as AMP to a minor extent.

We hypothesize that the higher use of AMP and METH in Kaunas and Vilnius is due to the closer proximity of these cities to production sites and drug trafficking routes compared to Klaipeda. Additionally, it should also be noted that tourism is considerably higher in Kaunas and Vilnius compared to Klaipeda, which could potentially contribute to differences in the consumption patterns of METH.

3.1.2. Inter-country differences in illicit drug consumption

Population-normalized mass loads for the stimulants were compared with the results from other European WBE studies. MDMA consumption in Lithuania proved to be similar to Central Europe and Scandinavian countries, but was lower compared to Western European countries, as illustrated by Fig. 3. MDMA is mainly consumed by young adults which could explain the present population-normalized loads of MDMA in the investigated urbanized areas (European Monitoring Centre for Drugs and Drug Addiction, 2019a; European Monitoring Centre for Drugs and Drug Addiction, 2019c). Lower consumption rates in Lithuania in contrast to Western Europe could be the result of the higher availability of MDMA in Western Europe (i.e. high abundance of illegal production sites of MDMA in Belgium and the Netherlands), as reported in the EU Drug Markets Report from the EMCDDA and Europol (EMCDDA and EUROPOL, 2019a).

WBE shows that COC, in contrast to Western-European countries, proved to be a less frequently used illicit drug in Lithuania (Fig. 3) (Gonzalez-Marino et al., 2020; European Monitoring Centre for Drugs and Drug Addiction, 2019c). Population-normalized mass loads of BE were considerably higher compared to Finland. The presence of COC could be due to the economic growth in Lithuania which resulted in cocaine entering the illicit drug market. Western-European countries, however, remain the primary import countries for wholesale cocaine trafficking with the Ports of Antwerp and Rotterdam as the most important transit ports which is reflected by the higher availability in Western Europe compared to Central and Eastern Europe (EMCDDA, 2018).

AMP consumption was lower compared to Western-European countries and Scandinavian countries and similar to Central Europe (Fig. 3) (Gonzalez-Marino et al., 2020; European Monitoring Centre for Drugs and Drug Addiction, 2019c). Although population-normalized mass loads of AMP were rather low in a European perspective, unexpected high mass loads of AMP were found in two wastewater samples from Kaunas from 2018 (Fig. 4). The observed load on day 7 was 1000-fold higher compared to the rest of the week. These extreme values were suspected to be the result of direct disposal of AMP in the wastewater system. While illicit drug production in Lithuania is considered limited, a few amphetamines production sites have been dismantled in recent years which could explain its domestic use (European Monitoring Centre for Drugs and Drug Addiction, 2019a). In addition, consumption of amphetamines is high due to Lithuania's position as part of a trafficking route. It has been reported by the EMCDDA that significant production
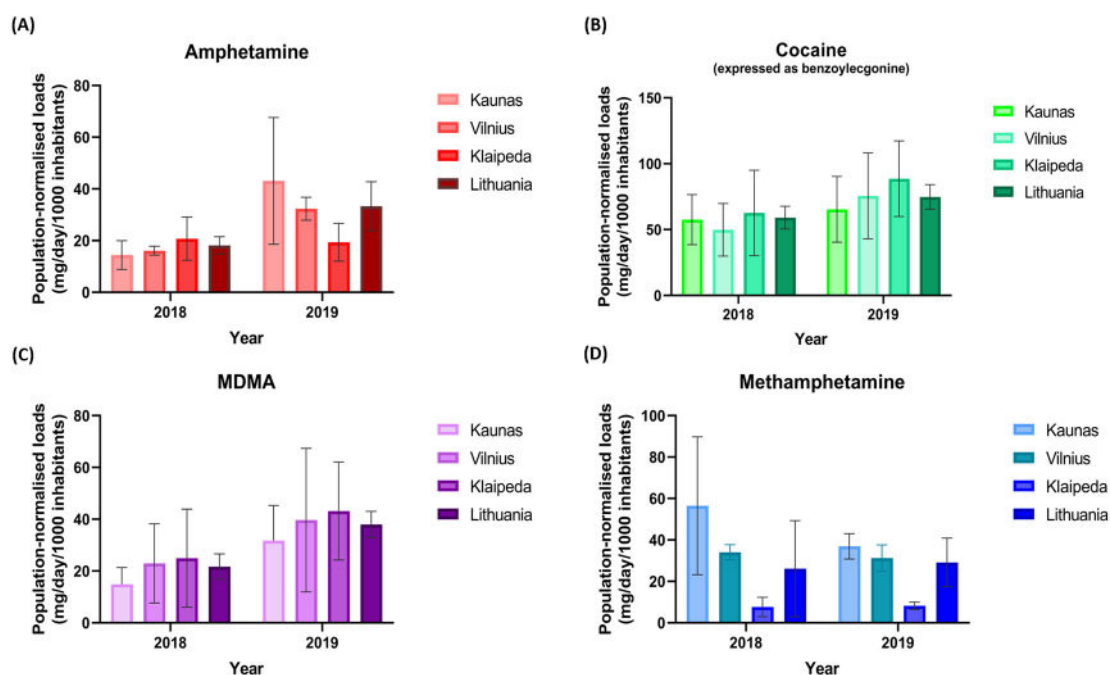

Fig. 2. Intra-country differences in stimulant consumption. The error bars in each location represent the daily variations in population-normalized mass loads. For Fig. 2B population-normalized mass loads were expressed for benzoylecgonine. Weighted averages were used to obtain national annual amounts of consumed stimulants.
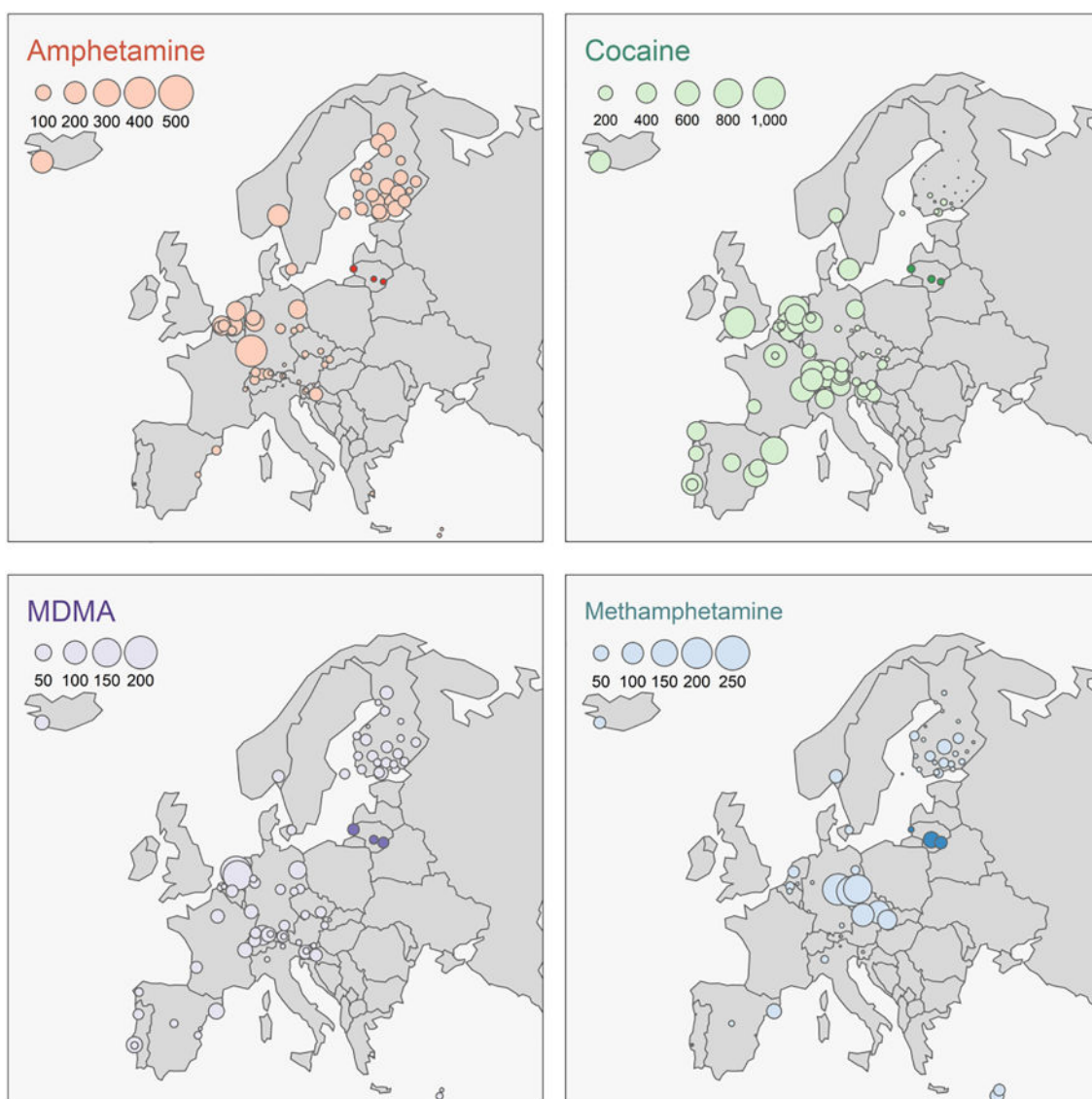
Fig. 3. Population-normalized mass loads for AMP, BE, MDMA and METH (expressed as mg/day/1000 inhabitants) from 2018. Mass loads from other countries were derived from the SCORE 2018 monitoring (Gonzalez-Marino et al., 2020). The deep coloured bubbles represent the Lithuanian cities.

and trafficking of amphetamines takes places in North-East European countries including Lithuania, Estonia, Latvia and Poland (EUROPOL and EMCDDA, 2011). Amphetamines available in Norway, Finland and Sweden are almost exclusively produced from benzylmethylketone (BMK) by means of a reductive amination, the so called Leuckart route and trafficked as racemate from the Baltic States. Non-target and suspect screening of these two wastewater samples indicated the presence of Leuckart-specific intermediates (e.g. N-formylamphetamine) and impurities (e.g. 4-benzylpyrimidine, N,N-di-(b-phenylisopropyl)formamide, N,N-di-(b-phenylisopropyl)amine), which are formed when BMK is being used as a precursor for AMP synthesis through the Leuckart reductive amination route (Emke et al., 2018). A list of all possible structures correlating with the high peak load of amphetamine found in these samples related to AMP production was given in Table 2. Chiral profiling of AMP revealed that AMP found in the IWW samples from Kaunas was racemic on the days following the suspected dumping event (Fig. 4). Castrignano et al. revealed that consumption of racemic AMP favours the excretion of the R-(-)-enantiomer (EF < 0.5) (Castrignano et al., 2018). This further corroborates direct disposal of

racemic (unconsumed) AMP in the wastewater system, possibly by criminal organizations prior to a police raid.

It is also important to note that these aberrant loads of AMP were excluded from the spatio-temporal comparison. However, depending on the distance between the dumping event and the WWTP, it might take several days for the dumped AMP to reach the inlet of the WWTP. For this reason, we should be careful with interpreting the population-normalized mass loads observed within this sampling period. A better approach would be to analyze a different 'normal' week (i.e. without special events) for the spatio-temporal analysis, however, no IWW samples are available anymore for this retrospective analysis.

Although the METH market in Europe is relatively low on a global scale, substantial loads of this stimulant were measured in all locations sampled in Lithuania. This is different to the consumption patterns observed in most Western-European countries and similar to Central Europe albeit in a lesser proportion. This might also be attributed to the considerable domestic production of METH and trafficking in the North-Eastern parts of Europe (EMCDDA and EUROPOL, 2019b).
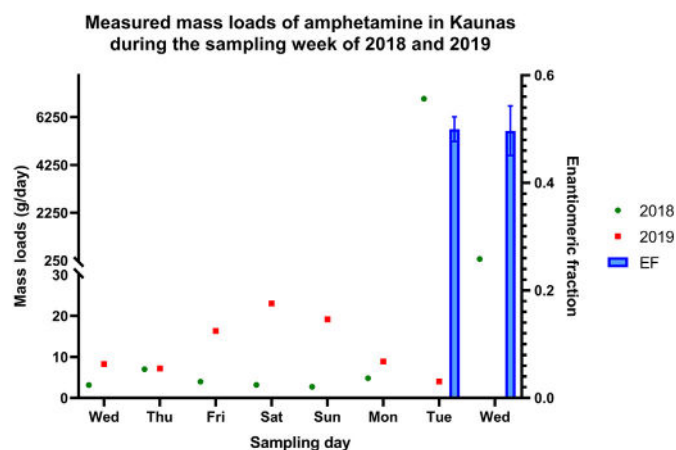
Fig. 4. Weekly variations in amphetamine loads (g/day) in Kaunas in 2018 and 2019. Mass loads of amphetamine during 2018 and 2019 in Kaunas are shown on the left Y-axis. Enantiomeric fractions (EF) for the influent wastewater samples of 2018 are displayed on the right Y-axis. For the first six days of Kaunas in 2018 measured concentrations of (R)- and (S)-amphetamine were below the LLOQ during chiral analysis and therefore no EFs could be obtained for these days. Amphetamine measured on day 7 and day 8 was racemic.

3.1.3. Temporal changes in illicit drug consumption

The use of COC was stable between 2018 and 2019. AMP consumption increased significantly in 2019 in Kaunas and Vilnius. The use of MDMA also statistically increased in 2019 in Kaunas and Klaipeda, while the use of METH in Kaunas statistically decreased in 2019. Additionally, weekly variations (Fig. S1) were also observed in the consumption of the different stimulants with the highest population-normalized mass loads retrieved during the weekends.

In Lithuania seized amounts of cocaine, amphetamine and MDMA increased respectively from 13.8 kg to 16.1 kg, 6.5 kg to 23.6 kg and 17.4 to 282 kg between 2018 and 2019, while the seized amounts of METH decreased drastically from 36.9 kg to 4.4 kg. Extrapolated national population-normalized loads of BE, AMP and MDMA also increased slightly between 2018 and 2019, however, not to the same extent compared to seizure data. In contrast to seized amounts of METH, extrapolated national population-normalized mass loads of METH remained stable between 2018 and 2019 (Drug Tobacco and Alcohol Control Department, 2019). It has to be noted that seizure data and WBE data do not reflect the same aspect of the illicit drug status (i.e. law enforcement vs actual consumption) and it highlights the need of combining complementary data sources to obtain a more complete picture on the illicit drug situation.

3.2. Alcohol consumption

3.2.1. Intra- and inter country differences in alcohol consumption

Daily alcohol consumption ranged between 1.7 and 7.4 standard drinks/day/capita aged 15+ in 2018 and 2019 among the different catchments. Spatial differences were observed in 2018 with Vilnius having a consumption per capita 30% higher than Kaunas. In 2019, alcohol consumption in Vilnius and Klaipeda was higher compared to Kaunas. Alcohol use in Vilnius and Klaipeda did not differ significantly. In Vilnius the age group of inhabitants between 25 and 44 years old is also considerably larger (34% of the population of Vilnius) compared to Kaunas (28% of the population of Kaunas) and Klaipeda (28% of the population of Klaipeda). According to the GPS, the highest prevalence of alcohol use is among the 25–44 years old age group. Another possible explanation could be related to the availability of alcohol in Klaipeda and Vilnius compared to Kaunas. The density of licences issued for alcohol sales is higher in Klaipeda and Vilnius compared to Kaunas.

Alcohol consumption among the different Lithuanian cities reported in this study still remains significantly higher in comparison with the European average reported by the WHO. This is further evidenced by other European WBE studies in which alcohol consumption was monitored through the measurement of EtS in wastewater (Boogaerts et al., 2016; Gao et al., 2020; Reid et al., 2011; Rodríguez-Álvarez et al., 2014; Baz-Lomba et al., 2016). It should be noted that all these WBE studies reported amounts consumed between 2012 and 2015 and temporal changes in alcohol use may therefore complicate the comparison with these measurements. While spatial differences between our study and the other WBE studies are obvious, the overall order of magnitude is in good agreement.

3.2.2. Temporal changes in alcohol consumption

No significant temporal differences in consumption patterns between 2018 and 2019 were observed for Kaunas and Vilnius. In Klaipeda, alcohol consumption statistically increased with 53% in 2019. Alcohol use in Lithuania still remains problematic despite the new alcohol regulations (i.e. restricting sales times, banning alcohol advertising and increasing the legal age for alcohol consumption to 20 year) that went into effect in January 2018 (OECD/European Observatory on Health Systems and Policies, 2017). These regulations were deemed necessary in response to the persistently high consumption rates after introducing policy changes in 2007–2008, which restricted advertising and sales and increased taxes.

3.2.3. Comparison between WBE estimates and other data sources

Consumption estimates for alcohol based on WBE were compared with consumption rates acquired from the WHO which includes recorded and unrecorded alcohol per capita consumption (World Health Organization, 2019). Unrecorded alcohol consumption is regarded as alcohol consumption not accounted for in official taxation and sales statistics usually produced, distributed and sold outside formal governmental channels. However, the monitoring of unrecorded consumption continues to be challenging for national surveillance systems and is mostly based on self-reported survey data, which is subject to concealment and reporting bias. This could lead to underestimation of the illegal alcohol consumption per capita. Nation-wide estimates based on WBE data in 2018 and 2019 were in line with data from the WHO, as illustrated by

Table 2
Leuckart-specific intermediates and impurities found in the wastewater samples from Kaunas following the suspected dumping event.

Name	Formula	Molecular weight (Da)	Theoretical molecular weight (Da)	Δppm	Retention time (min)	Peak intensity	Ratio (dump)/ (no dump)	Log-2 fold change (dump)/ (no dump)
Amphetamine	C9 H13 N	135.1047	135.104251	−3.5	9.02	4.02E+07	120	6.9
N-ethylamphetamine	C11 H17 N	163.136	163.13555	−2.8	10.45	7.10E+06	106	6.7
N-formylamphetamine (formetorex)	C10 H13 N O	163.0996	163.0991656	−2.7	15.96	4.36E+08	1379	10.4
N-formylmethamphetamine	C11 H15 N O	177.1152	177.1148156	−2.2	16.26	6.01E+07	240	7.9
4-Benzylpyrimidine	C11 H10 N2	170.0843	170.0838499	−2.5	16.32	1.45E+07	181	7.5
N,N-di-(b-phenylisopropyl)amine	C18 H23 N	253.1829	253.1825013	−1.4	17.24	2.28E+08	633	9.3
1-oxo-1-phenyl-2-(β-phenylisopropylimino) propane	C17 H19 N O	253.1466	253.1461158	−1.8	20.63	1.49E+08	283	8.2
N,N-di-(b-phenylisopropyl)formamide	C19 H23 N O	281.1781	281.1774159	−2.4	23.34	5.60E+07	45	5.5

Fig. 5A. No significant differences were found between the WHO estimate and the weighted mean of each year. It should be noted that the latest numbers from the WHO were from 2016 while wastewater samples originated from 2018 and 2019 and that consumption rates obtained from WHO data might be different in 2016 compared to 2018 and 2019. However, more recent data was not available at the time of the investigation.

3.3. Tobacco consumption

3.3.1. Intra-country and intercountry differences in tobacco consumption

Daily cigarette consumption ranged between 3.5 and 10.6 cigarettes/day/capita aged 15+. In 2018, no significant differences were observed in the cigarette consumption between all measured locations. In 2019, however, tobacco use was significantly higher in Vilnius and Klaipeda compared to Kaunas. Currently, a specific explanation for this is yet to be found.

Population-normalized mass loads of nicotine were derived from COT/COT-OH and were used to compare smoking between European countries. Population-normalized mass loads of nicotine found in this study were in line with other European WBE studies, which further supports the applicability of the proposed methodology (Fig. 6B). It should be noted that only a limited number of WBE studies focussed on tobacco consumption in Europe and that most of the WBE studies focussing on tobacco are from Australia and China.

3.3.2. Temporal changes in tobacco consumption

Over the past few years, the tobacco regulation in Lithuania has become stricter with the introduction of indoor smoking bans and the prohibition of smoking in cars with children or pregnant women. In spite of these tighter regulations and governmental efforts to diminish smoking, a significant decrease in tobacco consumption in 2019 was only observed in Kaunas. Tobacco consumption in Vilnius and Klaipeda remained stable throughout the entire sampling period.

3.3.3. Comparison between WBE estimates and other data sources

Measured cigarette consumption based on WBE loads was significantly higher compared to cigarette consumption based on sales data (also includes self-made cigarettes) (Drope and Schluger, 2019). It should be noted that national estimations are only based on measurements in three locations and that different consumption rates might be observed in other areas. Another limitation is that the latest available estimates for Lithuania from the Tobacco Atlas were from 2016 and this might not be representative for the sampling period due to temporal variations. Another reason for the discrepancies between the national estimates based on WBE and the sales statistics could be related to the conversion of population-normalized mass loads of COT and COT-OH to the number of cigarettes, especially with regards to the back-calculation factors and the varying nicotine content in cigarettes.

Nicotine biomarkers found in wastewater could also be derived from human exposure to nicotine from other sources, such as tobacco heating products (e-cigarettes, water pipes, …), nicotine patches and gums, …, which could further lead to discrepancies between sales statistics and WBE estimates. Additionally, purchase and consumption locations might not be the same. Cigarettes may also be (illegally) imported from neighbouring countries (e.g. Belarus) to avoid increasing taxation rates in Lithuania (Lietuvos Respublikos Muitinės, 2019). Data on illegal trade of tobacco products is not covered by these sales figures which could lead to an underestimation of the actual consumption.

Finally, cigarette buds might end up in the sewer system and could contain unconsumed amounts of nicotine (especially runoff in combined sewers). It is also possible that these remains could be transformed into COT and COT-OH in presence of microbiota present in the sewer system (Banks et al., 2018; Gao et al., 2018).

4. Study limitations

In general, WBE has some inherent limitations and uncertainties with regards to biomarker stability, real-time population, sampling frequency, chemical analysis and pharmacokinetic information on the different substances (mainly metabolism and excretion) (Castiglioni et al., 2013). In this particular study, time-proportional sampling was used for the collection of daily 24-h IWW samples. In contrast to flow-proportional sample collection, time-proportional sampling mode is not weighted properly for sewer flows. However, a high frequency (<20 min) was used to compile the daily IWW samples. Therefore, it is reasonable to assume that the applied sampling mode and frequency are suitable to accurately capture average biomarker concentrations over the 24-h period. Human biomarkers could potentially be transformed by biological and chemical processes that take place during the in-sewer transport from the place of excretion to the WWTP (Ramin et al., 2017). Multiple studies have proven the in-sewer stability of the analytes of interest in wastewater (for at least 24 h at pH 7.5 and 20 °C) (van Nuijs et al., 2012; Banks et al., 2018; Gao et al., 2019). However, EtS is found to degrade considerably in real rising main sewer and simulated rising main and gravity sewer conditions (Banks et al., 2018; Gao et al., 2019). The average residence time reported in this study was less than 24 h for all locations of interest. Additionally, in-sewer pH and temperature measured in the locations of interest were comparable to the studies above. For this reason, in-sewer (bio)transformation of illicit
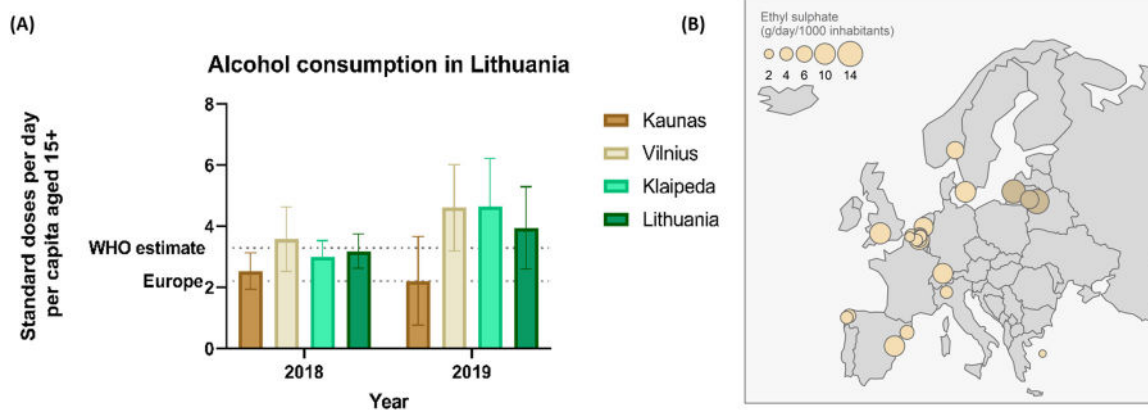

Fig. 5. (A) Alcohol consumption (expressed as doses/day/capita aged 15+) in Lithuania in 2018 and 2019. The Lithuania bar is the weighted mean of the three locations in that given year. The dotted lines are based on WHO estimates for Lithuania and Europe (World Health Organization, 2019). (B) Comparison of population-normalized mass loads of ethyl sulphate between this study and other European WBE research.
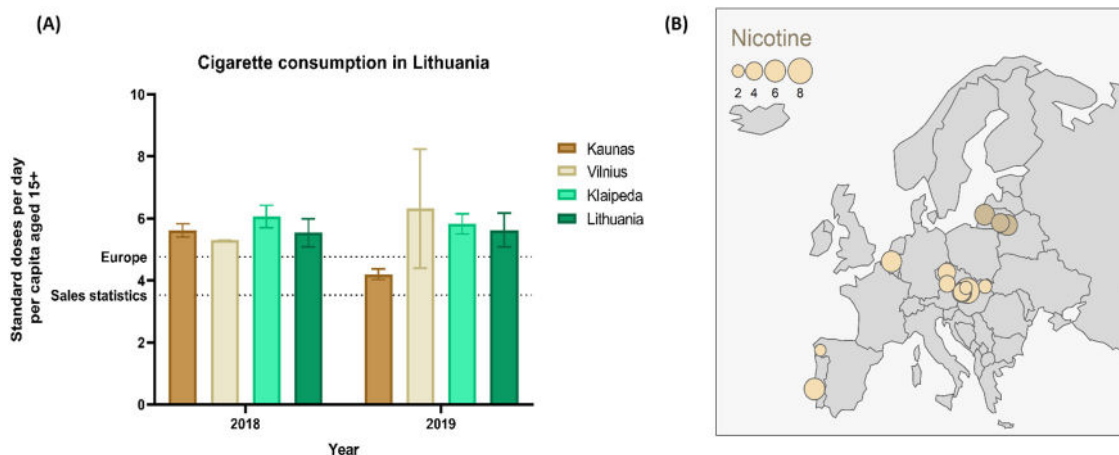
Fig. 6. (A) Cigarette consumption (expressed as doses/day/capita aged 15+) in Lithuania in 2018 and 2019. The Lithuania bar is the weighted mean of the three locations in that given year. The dotted lines are based on Tobacco Atlas estimates for Lithuania and Europe from 2016 (Drope and Schluger, 2019). The error bars represent the variation between daily population-normalized COT and COT-OH mass loads. (B) Comparison of population-normalized loads of nicotine between this study and other European WBE research.

drug and tobacco biomarkers can be considered negligible. However, the uncertainty of potential in-sewer degradation of EtS could contribute to discrepancies between WBE and other epidemiologic data.

In this WBE study, fixed population equivalents were used in order to obtain population-normalized mass loads while in- and efflux of people within the catchments (e.g. tourism, commuting, …) might be substantial. In the future, we should therefore assess the possibility of using dynamic population size proxies (e.g. online measurements of ammonium in wastewater, mobile phone data, …) to cope with fluctuating population dynamics within the different catchments (Gao et al., 2018; Been et al., 2014; Thomas et al., 2017).

Additionally, this study only investigated a snapshot of substance consumption during one 'normal' week of sampling per location and per year and subsequently extrapolated to national amounts. Consumption rates might be different in the sampling period compared to the rest of the year due to seasonal and weekly variations, which increases the overall uncertainty with regards to the between-year trends. However, the samples were collected in a 'normal' week with no particular events occurring which might bias the findings within this week. All monitored catchments also have more than 100,000 inhabitants, which might not be representative for less populated (rural) areas. While we cover 35.6% of the Lithuanian population, it cannot be ignored that socio-demographics of these municipal areas might also differ significantly from other parts of Lithuania.

In order to calculate standard doses of alcohol and daily cigarette consumption, excretion factors were derived from pharmacokinetic studies with a limited number of participants which might not reflect the average excretion profile in large populations. In order to acquire more accurate absolute estimates, refinement of these excretion factors should be considered (Thai et al., 2016). However, the primary goal of WBE is to investigate spatio-temporal trends in the consumption patterns of different substances and for this purpose population-normalized mass loads prove to be appropriate.

5. Conclusions

The results of this study illustrate the complementarity of WBE to evaluate substance use at the population level. While WBE has been widely adopted in literature and well established for the measurement of illicit drugs in Europe, its application for the measurement of alcohol and tobacco in a European perspective has been more limited. At this moment, WBE is an underemployed tool for monitoring substance use in Eastern European countries and still has a lot of potential in this geographical area to provide a complementary measurement of substance use.

METH and MDMA where the most abundant illicit drugs used. This study revealed a dumping event of AMP in the sewer system, which was verified by chiral analysis of AMP and target and non-target screening for drug precursors. Alcohol use in Lithuania proved to be higher compared to other European countries and it has slightly increased from 2018 to 2019. In contrast, the use of tobacco remained quite stable throughout both years and was similar to the amounts reported in other European WBE studies. Interestingly, this study found significant discrepancies between WBE data and sales statistics of tobacco. A potential hypothesis could be the illegal trade of cigarettes and tobacco heating products from neighbouring countries (e.g. Belarus, Ukraine). However, more research is needed with regards to conversion factors to decrease the uncertainty associated with WBE back-calculations.

CRediT authorship contribution statement

Tim Boogaerts: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing. **Lina Jurgelaitiene**: Conceptualization, Methodology, Resources, Writing - Review & Editing. **Catalina Dumitrascu**: Investigation, Writing - Review & Editing. **Barbara Kasprzyk-Hordern**: Methodology, Resources, Writing - Original Draft. **Andrew Kannan**: Methodology, Investigation, Writing - Review & Editing. **Frederic Been**: Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing. **Erik Emke**: Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing. **Pim de Voogt**: Resources, Writing - Original Draft, Writing - Review & Editing. **Adrian Covaci**: Conceptualization, Resources, Writing - Original Draft, Writing - Review & Editing. **Alexander L.N. van Nuijs**: Conceptualization, Methodology, Resources, Writing - Original Draft, Writing - Review & Editing.

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.2021.145914.

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