CHAPTER FIFTEEN

The estimation of cannabis consumption through wastewater analysis

Lubertus Bijlsma[a,*], Daniel A. Burgard[b], Frederic Been[c], Christoph Ort[d], João Matias[e], Viviane Yargeau[f]

[a]Research Institute for Pesticides and Water, University Jaume I, Castellón, Spain
[b]Department of Chemistry, University of Puget Sound, Tacoma, WA, United States
[c]KWR Water Research Institute, Nieuwegein, The Netherlands
[d]Eawag, Urban Water Management, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
[e]European Monitoring Centre for Drugs and Drug Addiction, Lisbon, Portugal
[f]Department of Chemical Engineering, McGill University, Montreal, QC, Canada
[*]Corresponding author: e-mail address: bijlsma@uji.es

Contents

1. Wastewater-based epidemiology

Wastewater-based epidemiology (WBE) profits from raw influent wastewater samples that are collected routinely at the inlet of most sewage treatment plants (STPs). In these "pooled urine samples", the concentrations of illicit drugs and their metabolites can be quantified to estimate the total amount of drugs consumed by a community. This provides a non-invasive, near-real-time analysis of drug use within the area of a sewer network connected to a STP.

In the 1990s, liquid-chromatography coupled to mass spectrometry (LC-MS) was initially used as a technique to monitor micropollutants, such as

Comprehensive Analytical Chemistry, Volume 90
ISSN 0166-526X
https://doi.org/10.1016/bs.coac.2020.04.005

pharmaceuticals, personal care products and pesticides, and to study the impact of treated and untreated liquid household waste in the environment. It was, at that time, suggested to use the quantified data of pharmaceuticals to estimate its consumption by the general public or in hospitals from a catchment of a STP, and WBE had its first "official" mention in 2001 [1]. The approach has rapidly developed over the last decade to monitor a myriad of illicit drug residues in near-real-time with numerous methodological and applied publications (>250, Keywords "sewage epidemiology" OR "wastewater-based epidemiology" SCOPUS 14.2.2020). WBE has established itself as a useful routine application in estimating temporal and geographical trends in illicit drug use and strongly complements the various sources of information on the drug situation, where each source has different uses and strengths. In comparison with survey methods, wastewater analysis is not subject to response and non-response bias. By formally testing wastewater, this method is expected to be closer to the true spectrum of drugs being consumed rather than relying on individual recollection or belief. It also has the potential to provide timely information, within short time frames, on geographical and temporal trends. However, it cannot provide information on prevalence and frequency of use, numbers of users, types of user and purity of the drugs. Triangulation of data from wastewater analysis with data obtained through other indicators is an important area of continuing work that will help establish the merits and validity of both.

WBE consists of five steps (see Fig. 1). (1) Representative daily composite samples of raw wastewater are collected. (2) Concentrations of selected substances are quantified. (3) The concentrations are multiplied with the wastewater volumes measured over the period of sampling to obtain loads of drug residues in sewers. (4) Daily loads are divided by the number of people present in the catchment area of the STP to facilitate comparison among cities based on these population-normalized estimates. Finally, (5) the total daily consumption of a drug is estimated by applying a specific correction factor to the daily sewer loads. The correction factor considers the average excretion rate of a given drug residue and the molecular mass ratio of the parent drug to its metabolite, but can also take the stability or purity of a drug into account. This final step is, however, optional and not needed if trends are the desired outcome, or if for example excretion rates are not available or reliable. Typically, results are reported up to step 4 or 5 and sometimes a sixth step is also performed, i.e., number of doses.

To minimize uncertainties, it is recommended to follow best practice in all steps:

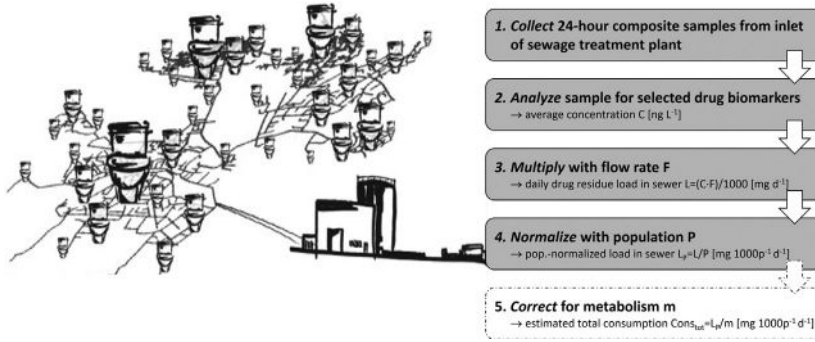

Fig. 1 Key steps for estimating drug consumption at the community level based on raw wastewater collected at the inlet of a sewage treatment plant ($Cons_{tot} = \frac{C \bullet F}{P} \bullet \frac{1}{m}\left[mg\ 1000p^{-1}\ d^{-1}\right]$, where C is the concentration [ng/L], F the flow rate [$m^3\ d^{-1}$], P the population [−] and m the metabolism [excretion rate in %]. *Modified from K. V. Thomas, L. Bijlsma, S. Castiglioni, A. Covaci, E. Emke, R. Grabic, F. Hernández, S. Karolak, B. Kasprzyk-Hordern, R.H. Lindberg, M. Lopez de Alda, A. Meierjohann, C. Ort, Y. Pico, J.B. Quintana, M. Reid, J. Rieckermann, S. Terzic, A.L.N. van Nuijs, P. de Voogt, Comparing illicit drug use in 19 European cities through sewage analysis, Sci. Total Environ. 432 (2012) 432–439. https://doi.org/10.1016/j.scitotenv.2012.06.069.*

Sampling of wastewater. In most modern STPs, a sampling scheme to collect samples on a routine basis is in place. It is typically used to quantify removal rates for traditional compounds such as nutrients or commonly used micropollutants. Ideally, samples are collected at least every 10 min in a flow-proportional mode over a 24-h period to obtain a composite sample adequately representing the daily average concentration [2,3]. Alternatively, a volume-proportional mode can be applied with a similar number of individual samples collected over a day. Time-proportional modes may imply more systematic or random uncertainties depending on the—typically unknown—intra-day variations of concentration profiles [4]. Relevant information on the catchment area and STP under investigation should be collected and documented [5]. For small catchments or even outlets of individual premises, e.g., schools or prisons, requirements are more stringent because an individual toilet flush containing the substance of interest may pass the monitoring station in less than 1 min and are likely being missed with traditional sampling equipment. Also measuring flow to calculate loads and technical implementation of sampling is much more demanding [4,6]. Besides, especially when sampling small communities, ethical principles should be considered to evade stigmatization of a particular group [7].

Selection of biomarkers. The selection of specific drug biomarkers is not an easy task, since an ideal target drug residue needs to fulfil specific requirements to ensure the reliable application of WBE. An appropriate biomarker is (i) excreted as a high fraction of the consumed dose, (ii) a human metabolite specific for consumption to differentiate from unconsumed drugs (e.g. disposal), (iii) stable during in-sewer transport and (iv) detectable in raw wastewater.

Estimation of population size. An important uncertainty when estimating per capita consumption of illicit drugs by means of wastewater analysis relates to the size and variability of the de facto population in a catchment [5,8]. Several methods based on census data, mobile device data and measuring hydrochemical parameters or specific substances in wastewater are currently employed to estimate the population [5,8,9]. The most reliable procedure would be to use all possible information and weight it according to certainty of individual estimates. With the refinement of the other contributors, population size has become one of the largest uncertainties in wastewater analysis [5,10].

Analytical methods. The chemical analysis of urinary biomarkers of illicit drugs in untreated wastewater is an analytical challenge. Drug residues are often present at very low concentration levels (ng/L) and wastewater is a complex sample matrix that contains particulate matter and compounds that may interfere with the analysis of the target analytes. Hence, a sample treatment step, which consists in a filtration step and solid phase extraction (SPE) is typically applied prior to analysis, in order to remove matrix interferences and to pre-concentrate target biomarkers.

LC coupled to tandem MS (LC-MS/MS) with triple quadrupole (QqQ) mass analyzers is currently the most popular analytical technique for the quantitative determination of illicit drug residues in wastewater samples. These instruments have a wide dynamic range, reach high sensitivity and selectivity, are relatively easy to operate, and allow multi-residue analysis in a single run, which permits reducing analysis time and costs. The QqQ instruments operate in MS/MS mode, where at least two specific precursor-to-product ion transitions for each target analyte should be monitored [5]. A quantification transition, most often the most sensitive one to favour the quantification at lower concentrations, and a confirmation transition. It is generally accepted that for a reliable positive finding in the sample, both transitions need to show a chromatographic peak at the same retention time as the reference standards as well as the compliance of the ion ratio between the two transitions [11]. Moreover, the use of isotope-

labelled internal standards (ILIS) for each target analyte is pivotal for wastewater analysis and added to the sample prior to sample treatment (i.e. as surrogate), to correct for matrix effects and compensate for potential errors associated with sample preparation. The performances of the analytical methodologies need to be fully validated for all target analytes in terms of linearity, accuracy, precision and limits of quantification (LOQ), and it is imperative to analyse internal quality controls (QCs) for daily method variations and perform regular checks of external QCs to guarantee the reporting of reliable WBE data. The latter can be done by participating in inter-laboratory exercises, such as those that are yearly organized by SCORE (www.score-network.eu) [12,13].

Nowadays, applying WBE for estimating cocaine, amphetamine, methamphetamine and MDMA use is well established and the related uncertainties are well known [5]. However, when applying it for estimating the use of cannabis, several specific challenges need to be carefully considered. In the following sections, an overview of the applications will be given, the utility and potential of WBE for cannabis, but also the limitations and bottlenecks with particular emphasis on the analytical methodology applied.

2. Cannabis biomarkers

Δ^9-Tetrahydrocannabinol (THC), the psychoactive ingredient in cannabis can be absorbed by diverse routes of administration such as smoking, oral, oromucosal, rectal, transcutaneous, and intravenous, while elimination from the body is equally diverse such as faeces, urine, sweat, oral fluid, and hair [14]. THC pharmacokinetic processes are dynamic and may be affected by a person's frequency and magnitude of use. THC is metabolized by microsomal hydroxylation to 11-hydroxy-THC (THC-OH) which is both a potent psychoactive metabolite and an intermediate for further metabolism to 11-Nor-9-carboxy-THC (THC-COOH) by liver alcohol-dehydrogenase enzymes (Fig. 2) [15]. THC is a highly lipophilic compound with storage in the body in adipose tissue, the extent to which appears to be determined by the frequency of use, leading to different detection windows in biological matrices among types of user [16]. Although there are more positive urine tests for cannabinoids than for any other drug class in workplace drug testing, a scarcity of urinary excretion data from controlled clinical studies of cannabis exist [17]. As noted above, there are a variety of routes of administration, and major differences exist in the ratio of the concentration of metabolites depending on these routes [15].

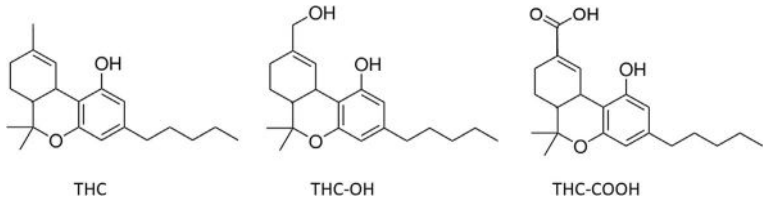

Fig. 2 Chemical structures of THC, THC-OH and THC-COOH.

Khan and Nicell identified 19 suitable studies that reported on various relevant aspects of THC excretion [18]. These studies involved a large range of doses, frequency of use, routes of administration, and numbers of participants, yielding a large range in the excretion profiles of the original THC dose in either/both urine and faeces. THC-COOH is the primary urinary metabolite and final point in the metabolization process and although a significant fraction of urinary THC-COOH is excreted as the glucuronide conjugate, conversion back to free THC-COOH in wastewater is expected due to sewer conditions and microbial activity in the conveyance system [18–20]. Hence, from a WBE perspective, THC-COOH is most frequently selected and determined in wastewater (see also Section 3 and Table 3). THC and THC-OH have been detected at a high rate in sewage sludge [21] owing to their more lipophilic nature, but THC-COOH is not expected to sorb onto wastewater influent suspended solids, and almost the entire faecal load of THC-COOH is expected to partition into the aqueous phase [18]. As will be discussed in later sections, THC-COOH can still be problematic to quantify compared to other drugs of abuse, because even in the carboxylated form, its lipophilicity in terms of Log D is still greater (Table 1) than drugs such as amphetamine LogD = −0.79 at pH 7.4 [22].

Several criteria have to be met for a biomarker to be suitable for WBE studies [23]. Besides that, a biomarker must be unique to human activity and excreted in substantial amounts, it must also be stable in wastewater during in-sewer transport and during storage until analysis.

The hydraulic residence times in the sewers can be from tens of minutes to nearly 24 h. The conveyance system consists of gravity sewers with an air column above the water surface and/or pressurized sewers. These contain biofilms with different microbial communities. Once the wastewater reaches the entrance to the treatment plant, a sample is collected and may spend up to 24 h in a container as a representative composite is obtained. Auto sampler containers are typically maintained at 4 °C. An aliquot is obtained from the composite and then either analysed immediately or more

Table 1 Predicted LogD values for THC, its metabolites and other illicit drugs as reference.

Compound	pH = 2	Log D (pH = 7.4)	Log D (pH = 8.0)
THC	5.94	5.94	5.92
THC-OH	4.66	4.66	4.64
THC-COOH	5.13	1.98	1.72
Methamphetamine	−1.1	−0.39	0.06
Amphetamine	−1.2	−0.79	−0.17
Cocaine	−1.3	1.8	2.1
Benzoylecgonine	−1.3	−0.7	−0.81

https://chemaxon.com/

often frozen at −20 °C and processed days, weeks or even months later. McCall et al. provide a literature review and found only a handful of studies that have investigated the stability of THC-COOH either in-sewer or in-sample [24]. THC-COOH has been shown to have <20% transformation in-sewer with variable in-sample results over five different studies [24]. The variety of stability results are due to differences in storage temperatures (20 °C, 4 °C, −20 °C) and length of periods studied. However, the larger issue with THC-COOH appears to come from the pH of the samples. Many WBE multi-residue analysis procedures call for acidification to pH 2 as a preservative technique, which has little effect on most illicit drugs. However, for THC-COOH, pH 2 results in a protonation of the carboxylic acid and a neutral molecule, which in theory leads to a much higher tendency towards sorption to particles or container walls.

One strength of the WBE approach is the estimation of parent drug consumption through back-calculation using wastewater loads (grams/day), parent/metabolite molar mass ratio, and pharmacokinetic excretion factors. This was first applied for THC-COOH by Zuccato et al. [25], using a correction factor of 152, which assumed a 0.6% of the THC dose excreted as THC-COOH and taking into account the molar mass ratio of parent drug to metabolite. Eq. 1 shows the calculation of a correction factor.

$$cor.factor = \frac{mass_{consumed.parentdrug}}{mass_{excreted.metabolite}} \times \frac{molarmass_{parent.drug}}{molarmass_{metabolite}} \quad (1)$$

This excretion factor seems to come primarily from a study involving six healthy male participants with a history of cannabis use, smoking two

different amounts [17]. The results from this study showed the percent of the administered dose excreted in urine was 0.54 ± 0.12, but no results were published concerning the THC-COOH fraction from the participant's faeces. The 0.6% value has been widely cited in the field as an excretion fraction, which can be used to back-calculate THC consumption from THC-COOH loads [26-28,25,29]. However, Postigo et al. [30] proposed instead 2.5% (correction factor of 36.4), a larger excretion factor for the aqueous THC-COOH available in wastewater. This larger factor was established due to this particular study finding no THC-OH in the wastewater yet 2% had been reported as the fraction of THC-OH excreted in urine [30,31]. Since no THC-OH was detected it was assumed that there was rapid oxidation to THC-COOH and thus the sum of THC-COOH (0.5%) and oxidized THC-OH (2%) was used. Gracia-Lor et al. [32] published a refined correction factor for the back-calculation of THC. This study again references 18 studies reporting THC metabolite excretion in urine from various routes and 14 studies concerning THC metabolite excretion in faeces. After reviewing the available literature, the "refined" mean excretion rate of THC-COOH was reported lower at 0.5% and a correction factor of 182. This new, smaller excretion factor appears to be dominated by urine THC-COOH levels from smoked cannabis. Furthermore, Been et al. [33] used the previously mentioned clinical studies to derive an average excretion rate for THC-COOH. In this study, authors combined all the available study results in a Bayesian hierarchical model, which included prevalence data from surveys and THC-COOH loads measured in wastewater. Combining all the data in a single model, the authors calculated posterior distributions of user prevalence, total cannabis consumption and excretion rates. While they assumed smoking as the most predominant administration route for Switzerland (according to available survey data), the authors estimated an excretion rate for THC-COOH of 7.0% (95%-CI range 4.2–10.6%) and 0.04% (95%-CI range 0.01–0.08%) through faeces and urine, respectively. Finally, Burgard et al. [10] monitored THC-COOH loads in wastewater over a 3 year period corresponding to the new legalized recreational market in Washington State, USA. This study specifically did not perform back-calculations to report THC consumed owing to the discrepancy among reported excretion rates in urine and faeces, the types of users, and the 440% difference that comes with different routes of administration. Smoked cannabis reportedly yields 0.5% urinary excretion while oral doses yield $2.2 \pm 1.2\%$ [18]. In addition to wastewater loads, Burgard et al. [10] tracked recreationally purchased THC mass and

compared these two indicators during the same time period. While only an estimate of the contribution from the residual black market could be made, they concluded that the excretion estimate of 2.5% [30] seems much more reasonable for use with WBE calculations. The obtained excretion rate is also in the same order of magnitude as the combined faeces and urine excretion rate estimated by Been et al. [33]. Furthermore, it is important to realize the possible shifting cannabis markets and the changes in desirable routes of administration. During the Washington State study, initially 90% of the sold cannabis was smokable products, but in under 3 years that had dropped to 83% of the market. Changing user demographics may lead to less smoking of cannabis i.e., A total of 48% of eighth grade survey respondents reported routes other than smoking preferred (eating, drinking, vaping, etc.) [10]. Less smoking and more oral use of cannabis will lead to larger excretion rates of the consumed THC dose. Moreover, an important distinction needs to be made with regard to the back-calculated amounts, namely whether the goal is to estimate total THC use or total cannabis use. In the latter case, an additional factor taking into account the average purity of sold cannabis needs to be included in the calculations.

Finally, cannabidiol (CBD) has gained in popularity due to its ability to alleviate certain medical conditions and is used as a medicine, an ingredient in foods, and as a dietary supplement. Numerous CBD products marketed may also contain small percentages of THC and could thus contribute to the total load of THC in wastewater. However, this contribution is uncertain, but seems small compared to THC used for recreational purposes. Therefore, at this point, it might be assumed that the small fractions of THC in CBD products is not a significant component of the overall THC load. Nevertheless, when monitoring THC, the additional fraction coming from CBD products adds to the overall uncertainty.

3. Analytical methodology

The analytical methodologies applied in WBE studies commonly include multiple substances. The quantitative determination of several illicit drug biomarkers in a single analysis is more practical, faster and thus cost-effective. Multi-residue methods do not only regard to the analysis, but also concern the sample collection, storage and sample treatment. However, when measuring multiple compounds, a compromise of the experimental conditions is often required.

Cannabis biomarkers have lower polarity, i.e., higher lipophilicity, compared to other illicit drugs and metabolites (see also Table 1). Despite the different physico-chemical properties, biomarkers of cannabis use have been included in multi-residue methodologies for the analysis of influent wastewater. Specifically, its main human urinary metabolite THC-COOH, has been the most reported biomarker of cannabis consumption in WBE publications. Yet, results of the inter-laboratory exercises performed by SCORE revealed difficulties related to the chemical analysis of THC-COOH in wastewater suggesting that concentrations found might be underestimated [12]. In fact, the authors illustrated that whilst laboratories performed well when analysing THC-COOH in methanol (i.e. relative standard deviation (RSD) <25% from the group's mean), underreporting of up to 90% (from the group's mean) were reported for tap water samples. In the latter case, laboratories were asked to process the samples according to their established procedures, which generally involved SPE and, in some cases, also sample acidification (as will be discussed further on, this can dramatically reduce the recovery of THC-COOH from water samples). Moreover, other non-instrumental factors, such as possible sorption to sample containers, partition on biofilms and particulate matter and excretion rates should be better understood to provide more accurate back-calculation estimates of the total amount of cannabis/THC used by a population [11,34]. Nevertheless, monitoring consumption trends using excreted THC-COOH in wastewater can give unique and timely information [10], but the data and methods used to generate these data should be carefully evaluated and interpreted.

In this section, focus has been put on the analytical methodology. In total 29 peer-reviewed articles were published since 2006 describing validated analytical methodologies for the determination of cannabis biomarkers in wastewater. Furthermore, important progress has been made in relation to the initial aspects of the analytical procedure, where it has been highlighted that the order of the initial sample preparation steps after sample collection is crucial [34]. Tables 2 and 3 show the sample preparation steps and instrumental parameters of the reported analytical methods, respectively.

The collection of representative 24 h composite samples is pivotal in the WBE approach (see Section 1). Different sample container materials have been used to collect and store samples such as amber glass, high-density polyethylene (HDPE), polypropylene (PP) and polyethylene terephthalate (PET) (Table 2). After sample collection and prior to SPE, a filtration and/or centrifuged step is often performed to prevent the SPE cartridge sorbent from clogging. A wide range of different filter types with pore sizes from

Table 2 Sample preparation steps.

Year, Ref	Sample container	Filtration/ Centrifugation	Acidification (Y/N)	ILIS addition	SPE	CF
2006, [35]	Glass	F: GF/A 1.6 µm	Y, prior to SPE	After F, prior to SPE	Oasis MCX (3 mL, 60 mg)	250
2007, [36]	n/a	F: GF/A	N	After F, prior to SPE	Oasis HLB (6 mL, 200 mg)	400
2008, [37]	Glass	F: GF 1 µm, Nylon 0.45 µm	N	After F, prior to SPE	Online Oasis HLB Prospekt-2 (10.4 mg)	–
2009, [38]	HDPE	C: 5 min, 4500 rpm	Y, prior to SPE	After C, prior to SPE	Oasis MCX (6 mL, 150 mg)	10
2010, [39]	Glass	F: GF BF 85/70	Y, prior to F	After F	Direct injection	–
2010, [40]	Glass	F: GF, NC 0.45 µm	Y, prior to SPE	After F, prior to SPE	Oasis HLB (6 mL, 200 mg)	500
2011, [41]	Glass	F: n/a	Y, prior to F	After F, prior to SPE	Oasis MCX (6 mL, 150 mg)	n/a
2011, [30]	PET	F: GF 1 µm, Nylon 0.45 µm	N	After F, prior to SPE	Online Oasis HLB Prospekt-2 (10.4 mg)	–
2012, [42]	n/a	F: GF, NC 0.45 µm	Y, prior to SPE	After F, prior to SPE	Oasis MCX (6 mL, 150 mg)	200
2012, [43]	Glass	F: NC 0.45 µm	Y, prior to SPME	After F, prior to SPME	SPME: DVB-CAR-PDMS fibre	–
2013, [44]	Glass	F: GF/A 1 µm, PES 0.45 µm	N	After F, prior to SPE	Oasis HLB (6 mL, 150 mg)	200
2013, [45]	n/a	F: RC 0.45 µm	N	After F, prior to SPE	Inline C_{18} Hypersil gold 20 × 2.1 mm 12 µm	–

Continued

Table 2 Sample preparation steps.—cont'd

Year, Ref	Sample container	Filtration/ Centrifugation	Acidification (Y/N)	ILIS addition	SPE	CF
2013, [26]	PP	F: GF/B 1 mm	N	After F, prior to SPE	Oasis HLB (6 mL, 500 mg)	500
2013, [46]	n/a	F: GF/D 2.7 µm	Y, prior to SPE	After F, prior to SPE	Oasis MCX (6 mL, 150 mg) + Strata NH_2 (3 mL, 200 mg)	250
2014, [47]	PET	F: RC 0.45 µm	N	Y, 15 min before F	Strata X (6 mL, 500 mg)	250
2014, [48]	HDPE	F: CEM 0.45 µm	N	Before F, prior to SPE	Oasis HLB (3 mL, 60 mg)	25
2014, [27]	PP	F: GF/D 2.7 µm	N	After F, prior to SPE	Oasis HLB (6 mL, 500 mg)	200
2014, [49]	HDPE	F: GF 0.7 µm	N	Before F, prior to SPE	Oasis HLB (3 mL, 60 mg)	250
2015, [28]	PET	F: GFC 1 µm, GF 0.5 µm	N	After F	Online Hypersep RetainPEP 20 × 2.1 mm 12 µm	–
2016, [50]	n/a	F: n/a	Y, prior to LLE	After F, prior to LLE	LLE	175
2017, [51]	n/a	F: GF/F	N	No ILIS	Chromabond HR-X (6 mL, 500 mg)	n/a
2018, [52]	Glass	C: 5 min, 4500 rpm, F: GF	N	After F, prior to SPE	Oasis HLB (6 mL, 200 mg)	100
2018, [53]	n/a	F: GF/A 0.7 µm	N	Before F, prior to SPE	Oasis MCX (6 mL, 150 mg)	1000
2018, [54]	PP	–	Y, with LLE	n/a	LLE	100

Table 2 Sample preparation steps.—cont'd

Year, Ref	Sample container	Filtration/ Centrifugation	Acidification (Y/N)	ILIS addition	SPE	CF
2018, [55]	Glass	C: 5 min, 4500 rpm, F: GF	N	After F, prior to SPE	Oasis HLB (n/a)	100
2019, [10]	HDPE	F: RC syringe 0.2 μm	N	Before F, prior to SPE	Strata-CX (3 mL, 60 mg)	n/a
2019, [56]	HDPE	F: GF 1.6 mm	Y, prior to SPE	Before F, prior to SPE	Strata-XC 33 μm (6 mL, 200 mg)	250
2019, [57]	Glass	F: GF 0.7 μm	N	After F, prior to SPE	Oasis HLB (6 mL, 500 mg)	100
2019, [58]	HDPE	F: GF 0.7 μm, Nylon 0.45 μm	N	Before F, prior to SPE	Oasis HLB (3 mL, 60 mg)	50

CF, Concentration Factor; n/a, information not available.

0.2 μm to 1.6 mm have been reported: glass fibre (GF), cellulose ester membrane (CEM), regenerated cellulose (RC), nitro cellulose (NC) and nylon membranes (Table 2). For multi-residue drug analysis, it was recommended to adjust the pH to acidic conditions after sample collection to decrease possible degradation and increase the in-sample stability for the majority of the illicit drug biomarkers [5]. However, at acidic pH, THC-COOH is present in its non-charged hydrophobic form, which means that it has potential to sorb to sample container or filter materials. Causanilles et al. [34] demonstrated that sorption of THC-COOH to container walls occurred more rapidly and to a higher extend at pH 2.5 for glass and PP containers. Furthermore, THC-COOH recovery after filtration was highly dependent on the pH [46,34]. When filtering large volumes of wastewater at neutral pH, recovery losses around 30% were reported independent of the filter material, but at acidic pH losses during filtration were above 75% [34]. Therefore, it is not advisable to acidify the samples, if it is not required by the selected protocol. Hence, a best-practice protocol for the initial sample preparation steps has been proposed, i.e., first addition of ILIS, second filtration and third acidification (if required) [34]. Table 2 shows that five out of the eight (62.5%) articles published in the years 2018 and 2019, thus

Table 3 Analytical parameters.

Year, Ref	Biomarkers	Instrument	Ionization	Quantification	Confirmation
2006, [35]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI -	343 > 299	343 > 245
2007, [36]	THC-COOH, THC	RP-LC-MS/MS (QqQ)	ESI +	345 > 327; 315 > 193	345 > 193; 315 > 123
2008, [37]	THC-COOH, THC, THC-OH	RP-LC-MS/MS (QqLIT)	ESI -	343 > 299; 313 > 245; 329 > 311	343 > 191; 313 > 191; 329 > 268
2009, [38]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI +	345 > 193	345 > 299, 345 > 327
2010, [39]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI +	345 > 327	345 > 299
2010, [40]	THC-COOH, THC	GC–MS (Ion Trap)	EI	473 > 355; 386 > 371	-; 386 > 330, 386 > 315
2011, [41]	THC-COOH, THC	RP-LC-MS/MS (QqQ)	ESI +/−	343 > 299 (−); 315 > 193 (+)	343 > 245 (−); 315 > 123 (+)
2011, [30]	THC-COOH, THC, THC-OH	RP-LC-MS/MS (QqLIT)	ESI -	343 > 299; 313 > 245; 329 > 311	343 > 191; 313 > 191; 329 > 268
2012, [42]	THC-COOH, THC	RP-LC-HRMS (QTOF)	ESI -	343.1915; 313.2173	299.2017; 245.1547
2012, [43]	THC-COOH, THC	GC–MS (Q)	EI	371; 386	473,488; 303, 371
2013, [44]	THC-COOH, THC, THC-OH	RP-LC-HRMS (LTQ-Orbitrap)	ESI +	345.2060; 315.2319; 331.2268	327, 299; 259, 193; 313

2013, [45]	THC-COOH	RP-LC-HRMS (Q-Orbitrap)	ESI +	345.2060 > 299.2006	345.2060 > 327.1953
2013, [26]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI -	343 > 299	343 > 245
2013, [46]	THC-COOH, THC-OH	RP-LC-MS/MS (QqQ)	ESI -	343 > 245; 329 > 311	343 > 191; 329 > 173
2014, [47]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI +	345 > 41	345 > 327
2014, [48]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI +	345 > 193	345 > 299, 345 > 327
2014, [27]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI -	343 > 299	343 > 245
2014, [49]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI -	343 > 299	343 > 245
2015, [28]	THC-COOH, THC	RP-LC-HRMS (Q-Orbitrap)	ESI +	345.2060; 315.2319	RT ILIS ±0.03 min, isotopic fit
2016, [50]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI -	343 > 299	343 > 245
2017, [51]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI +	345 > 299	–
2018, [52]	THC-COOH, THC, THC-OH	RP-LC-MS/MS (QqQ)	ESI +	345 > 327; 315 > 193; 331 > 313	345 > 299; 315 > 123; 331 > 193

Continued

Table 3 Analytical parameters.—cont'd

Year, Ref	Biomarkers	Instrument	Ionization	Quantification	Confirmation
2018, [53]	THC-COOH, THC-OH	RP-LC-MS/MS (QqQ)	ESI +	345 > 327; 331 > 313	345 > 299; 331 > 193
2018, [54]	THC-COOH, THC, THC-OH, $THC(OH)_2$	SFC-MS/MS (QqQ)	ESI +	345 > 299; 315 > 193; 331 > 313; 315 > 193	345 > 193; 315 > 123; 331 > 201; 315 > 123
2018, [55]	THC-COOH, THC, THC-OH	RP-LC-MS/MS (QqQ)	ESI +	345 > 327; 315 > 193; 331 > 313	345 > 299; 315 > 123; 331 > 193
2019, [10]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI -	343 > 299	343 > 245
2019, [56]	THC-COOH, THC	RP-LC-MS/MS (QqQ)	ESI +	345 > 299; 315 > 193	345 > 193; 315 > 123
2019, [57]	THC-COOH, THC, THC-OH	RP-LC-MS/MS (QqQ)	ESI +	345 > 299; 315 > 123; 331 > 313	345 > 193; 315 > 193; 331 > 193
2019, [58]	THC-COOH	RP-LC-MS/MS (QqQ)	ESI +	345 > 299	345 > 193

after the recommendations were made, applied this best-practice protocol, whereas only three of 19 (15.8%) applied the correct order of steps in earlier publications. Two articles applied either centrifugation [38], or the order of initial steps was unknown [51].

Concentration levels of most illicit drug biomarkers in wastewater are in the ng/L–mg/L range, and despite that the sensitivity of modern instruments is excellent, a pre-concentration step is generally needed in order to reach the required quantification limits. In addition, matrix components that might co-elute with the analyte and interfere with the analytical measurement leading to a suppression or enhancement of the analyte response (i.e. matrix effects) may also be removed. Off-line SPE is most often applied for sample pre-concentration and clean-up, but also fully automated large volume injections [39] and on-line SPE applications [37,30,45,28] have been reported. Polymeric-based SPE sorbents with reversed phase (RP) properties built of generic hydrophilic and lipophilic balanced (Oasis HLB) monomers or strong cation-exchange mixed mode sorbents built upon RP copolymers (Oasis MCX or Strata-XC) were most popular in multi-residue methods (Table 2). Although cation-exchange mode cartridges allow improved selectivity towards basic analytes, THC-COOH in its neutral form at low pH may also be retained by the mixed RP characteristics resulting in satisfactory recoveries. Furthermore, some alternative sample preparation protocols can be found in the literature: liquid-liquid extraction (LLE) and solid-phase micro extraction (SPME) were both proposed for extracting anionic THC-COOH from wastewater [43,50,54]. The main drawback, however, is the limited applicability to measure multiple compounds. Yet, for the determination of cannabis only in wastewater, these specific sample preparation protocols could be good alternatives.

The use of internal standards, preferably the labelled form of the analyte of interest, is essential and compulsory in WBE studies. ILIS should be added to the sample as surrogate, i.e., just after sample collection, to correct for matrix effects and for potential errors associated with sample manipulation and storage. All reported methodologies used deuterated analogues of the cannabis biomarkers as ILIS for more accurate quantification, except one study that did not use any ILIS [51].

THC-COOH was always included as biomarker of cannabis consumption when analysing wastewater (Table 3) and the majority use LC-MS/MS with QqQ for its determination (22 of 29, 76%). Reversed phase (RP) analytical columns based on C18 were mostly used for chromatographic

separation of drug biomarkers including THC-COOH. Acid was often added to mobile phases to favour the formation of protonated molecules, but acid can deteriorate the sensitivity and chromatographic performance of THC-COOH [48]. QqQ analyzers are known for their robustness and excellent sensitivity and selectivity, but hybrid systems with high-resolution mass spectrometry (HRMS) such as time-of-flight or Orbitrap analyzers have also shown good performances for both qualitative and quantitative analysis [42,44,45,28]. However, HRMS instrument are more expensive and require experienced operators. Furthermore, gas chromatography coupled to mass spectrometry (GC–MS) has been applied for the analysis of THC-COOH and THC in wastewater providing high levels of selectivity and sensitivity [40,43], yet a derivatization step is required to make them compatible with GC. Consequently, sample treatment is more laborious and time-consuming. An alternative could be supercritical-fluid chromatography (SFC) coupled to tandem mass spectrometry [54], but these instruments are often not standard available in laboratories.

For a reliable positive finding of THC-COOH in wastewater using tandem MS instruments, it is recommended that a minimum of at least two specific MS/MS transitions is monitored, whereas for HRMS instruments, at least two ions need to be monitored [5,11]. THC-COOH has been measured in both negative-ion and positive-ion mode. In principle, more abundant ionization would be expected in negative mode, due to higher trend towards the ionization of the acidic group. However, sensitivity seems manufacturer dependent, for example, Waters instruments seem to perform better in positive electrospray ionization (ESI) mode. Thus, a better ionization towards the basic group [38]. In positive ESI mode, m/z 345 > 327 has been selected as quantification transition in five occasions (Table 3). Although this transition probably was the most sensitive one, and would thus favour the quantification of THC-COOH at lower concentrations, it also corresponds to the non-specific loss of water. Therefore, this transition might be more problematic and prone to be interfered when analysing wastewater [11]. Similar is the selection of m/z 345 > 299 (or m/z 343 > 299 in negative ESI mode) corresponding to a non-specific loss of the carboxylic acid group. Hence, the selection of a less abundant, but more specific transition m/z 345 > 193 can be beneficial, presenting better signal-to-noise (s/n) ratios and thus sensitivity (Fig. 3). Chromatographic separation might not be an important issue when using MS/MS for detection, although it can be essential to avoid or minimize matrix effects, especially in complex influent wastewater samples. LC separation becomes, however, a crucial issue when

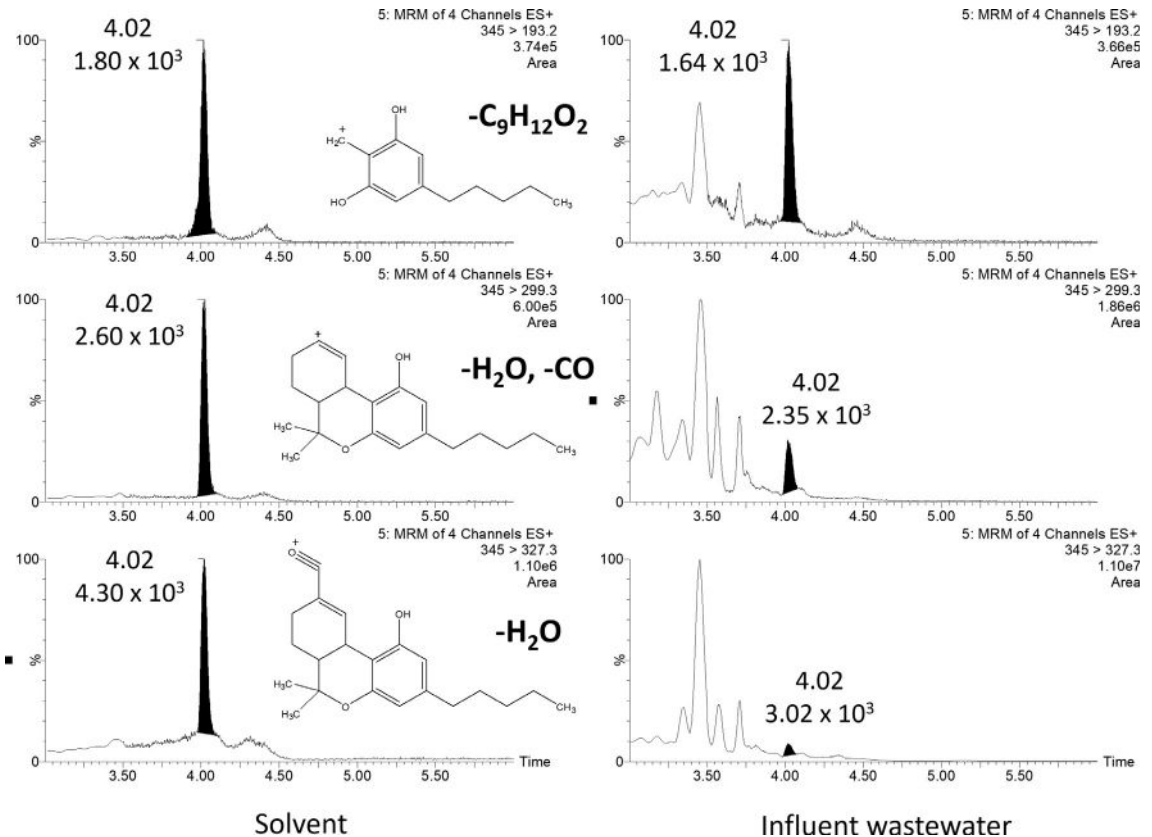

Fig. 3 Selectivity of THC-COOH transitions. *From F. Hernandez, S. Castiglioni, A. Covaci, P. de Voogt, E. Emke, B. Kasprzyk-Hordern, C. Ort, M. Reid, J. V Sancho, K. V Thomas, A.L.N. van Nuijs, E. Zuccato, L. Bijlsma, Mass spectrometric strategies for the investigation of biomarkers of illicit drug use in wastewater, Mass Spectrom. Rev. 37 (2018) 258–280.*

compounds have the same MS/MS transitions [38]. THC and CBD present common transitions, but more importantly for monitoring cannabis consumption through WBE, their metabolites THC-COOH and CBD-7-COOH also share their MS/MS transitions. Therefore, co-elution needs to be avoided, especially since CBD has become more popular and can thus be present in wastewater.

Limits of detection (LODs) and limits of quantification (LOQs) of THC-COOH in wastewater are generally in the ng/L—μg/L range and usually estimated based on *s/n* ratios of 1:3 and 1:10, respectively [11]. The estimation of realistic and comparable LODs and LOQs is, however, complicated in wastewater, as notable variations in chemical composition between samples occur. LOQs would need to be estimated from samples containing biomarkers at low level, where the *s/n* in the chromatograms and matrix effects can be taken into account. Furthermore, LOQs should be estimated at *s/n* 10 for the quantification transition, but also at *s/n* 3 for the confirmation transition to ensure not only the quantification of the compound but also its reliable identification [48]. The ultimate and most homogeneous approach is to estimate LOD and LOQ from inter-laboratory exercises where all participants analyse the same samples with their own analytical methodology [11].

4. Wide-scale use of WBE for cannabis estimates in Canada and US.

In the context of an increasing proportion of the population considering that consuming cannabis should not be a criminal offence, going from 51% in 2001 [59] to 70% in 2016 [60] in Canada, of a search for strategies to defeat the black market and to serve public health goals, several countries have adopted a policy of decriminalization to make simple possession a non-criminal offence and other countries or jurisdictions, such as Canada, Uruguay, the USA states Colorado, California and Illinois, and the Australian Capital Territory in Australia have legalized possession and use of recreational cannabis. In 2013, for the first time, the majority of United States population polled favoured legalizing cannabis, which followed the first legalization of a recreational cannabis market in the states of Washington and Colorado in 2012 [61]. Now 11 states and Washington D.C. have legal recreational markets. The changes in legislation increased the needs for the monitoring of cannabis consumption in the populations

affected by these changes. This led to innovative strategies to collect data to track the prevalence of cannabis prior to and following the legalization.

One objective of implementing new methods to collect statistics was to identify sources of data with a lower risk of relying on underestimated consumption levels prior to legalization, which might be significant prior to legalization due to stigma associated with use of illegal drugs and the reluctance to disclose purchases from non-regulated suppliers. One strategy that was implemented in Canada and in Washington State, USA, was the use of WBE.

The implementation of the WBE approach at the scale of a country, which in the case of Canada involved 15 STPs up to about 6000 km apart, collecting wastewater monthly, over a period of a year, in five large urban centres across the country, and representing nearly 8.4 million people, was associated with few challenges, such as shortage and type of container material, sample storage and logistics. Tests using sampling bottles made of PET, the material recommended by SCORE [5,13] and HDPE (the most widely available) demonstrated that no significant difference was observed between the two bottles materials over a period of 7 days of storage. The amount of THC-COOH in the wastewater collected in this study in Canada was quantified applying an extraction [62] and LC-MS method [63], which are based on a procedure previously reported by Rodayan et al. [64].

The deployment of WBE at large scale provided data over extended periods of time for both Canada and Washington State, USA. For Canada, the results of the monitoring for cannabis and other drugs are available online [65]. Fig. 4A shows the average weekly loads of THC-COOH per capita over a year for each geographical area included in the pilot project and Fig. 4B shows the annual trend of the average weekly loads of THC-COOH per capita over the whole country (combined sites) of the pilot project. Unfortunately, significant variability of the monthly results was observed (Fig. 4A) and at the scale of the country, it was not possible to clearly identify a trend that might be associated with the legalization of cannabis in 2018 (Fig. 4B).

Focusing on a smaller geographical area can provide a different perspective. To evaluate the significance of the trend in consumption observed over time for a given population, the results obtained using WBE were compared to the self-reported data collected over the same period of time by Statistics Canada [66] for the corresponding population. Based on self-reported consumption (Table 4), a slight increase in prevalence was observed for the quarter during which cannabis was legalized (Q4 2018), but it decreased

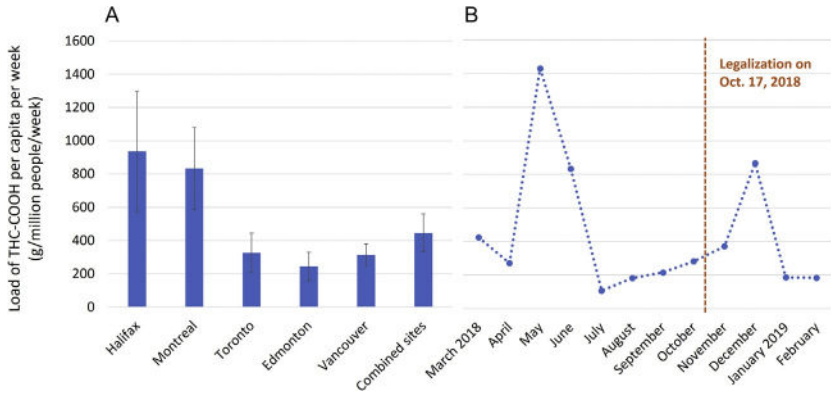

Fig. 4 (A) Average weekly loads of THC-COOH per capita (g/million people/week) by city over a one-year period of the pilot study (March 2018 to February 2019) ($n = 12$, error bars = lower and upper limits over the 12 months) and (B) Average weekly loads of THC-COOH per capita (g/million people/week) by month over the whole country (combined sites), adapted from Statistics Canada [65], 26/08/2019. *This does not constitute an endorsement by Statistics Canada of this product*.

Table 4 Prevalence of cannabis use in the selected Canadian province using self-reported information and the corresponding estimates of consumption for the corresponding metropolitan area included in the WBE pilot project.

Time period	Percentage of people reporting consumption (95% confidence interval)	WBE estimates of consumption based on THC-COOH loads
Q3 2018	10.1% (7.7–13.3)	July 2018: 411 g/week August 2018: 445 g/week September 2018: 1561 g/week
Q4 2018[a]	13.6% (10.9–16.8)	October 2018: 920 g/week November 2018: 768 g/week December 2018: 4396 g/week
Q1 2019	11.0% (8.8–13.8)	January 2019: 665 g/week February 2019: 588 g/week March 2019: *Not available*

[a]Legalization was on October 17, 2018, at the beginning of Q4 2018.
Data extracted from Statistics-Canada, Prevalence of Cannabis Use in the Past Three Months, Self-Reported, (2019). Table 13-10-0383-01. https://doi.org/https://doi.org/10.25318/1310038301-eng.

to very similar percentages of prevalence afterwards. The trend of consumption based on the load of THC-COOH estimated using WBE (Table 4), also suggests that there was an increase in consumption, just before legalization and during the fourth quarter of 2018. The peak of consumption might be explained by an increase interest due to legalization as well as the holiday season. However, the data for the beginning of 2019 do not suggest a sustained increase in consumption.

In Washington State, two STPs in a city of 200,000 people were monitored using WBE for THC-COOH for 7 months prior to the first legal recreational cannabis stores opening then followed by 29 months after the new marketplace began. Fig. 5 shows that while the legal THC dispensed in the catchment area of the two STPs increased at nearly 70% per quarter, the THC-COOH metabolite only increased at 9% per quarter. This indicated a much lower increase in cannabis use than new cannabis being introduced into the market and thus a decrease in cannabis from the black market is

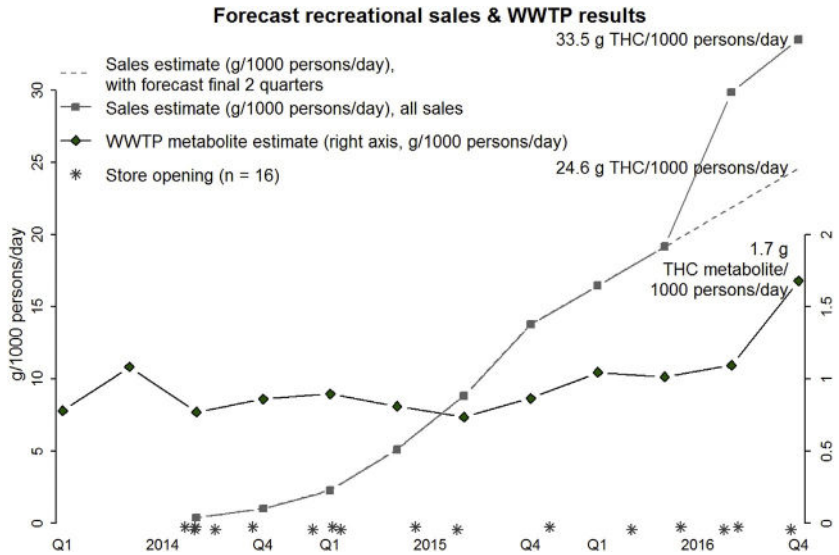

Fig. 5 Quarterly per capita estimates for THC consumption by sales (grey line) and by wastewater THC-COOH metabolite analysis (black line). The dashed line is the forecast continued recreational sales whereas the Q3 and Q4 in 2016 included all sales, including the State tracked medical market that came online starting Q3 2016. *From D.A. Burgard, J. Williams, D. Westerman, R. Rushing, R. Carpenter, A. LaRock, J. Sadetsky, J. Clarke, H. Fryhle, M. Pellman, C.J. Banta-Green, Using wastewater-based analysis to monitor the effects of legalized retail sales on cannabis consumption in Washington State, USA, Addiction 114 (2019) 1582–1590. https://doi.org/10.1111/add.14641.*

concluded. Total THC consumption did indeed increase but the stated goal of legalization to reduce the black market seems to have been achieved. This finding is an example of where a wastewater approach has shown its true value as a complementary approach to traditional used metrics. These other metrics have not yet been able to make any estimates as to an effect on the black market with a direct data source.

5. Future research and current asset

Monitoring cannabis use is highly relevant and interesting, due to its widespread use and ongoing policy changes. The estimation of community drug use through the chemical analysis of specific human biomarkers in wastewater has demonstrated its potential as a useful complementary approach to established drug monitoring tools such as general population surveys on drug use, treatment registry and law enforcement data. The application of WBE to monitor cannabis, however, has been challenging. Therefore, research has recently been centered on refining cannabis estimates, which resulted in a protocol for the storage, handling, and analysis of wastewater samples [34]. Yet, the main focus in this study was on the dissolved phase and did not include suspended solids, which seem to be of particular relevance for lipophilic cannabis biomarkers. Although THC may partition to particulates ultimately accumulating in the biosolids, THC-COOH is expected to partition into the aqueous phase and only predicted to adsorb to suspended solids between 1.1% and 8.5% [33]. However, it is not clear if the fraction excreted in faeces, which is supposedly higher than in urine, remains in the faeces even if they disaggregate in wastewater and are in contact with a high volume of wastewater. Furthermore, differences in wastewater characteristics—i.e., content and type of suspended solids—operation and design of sewer systems and the material of the sampling container may result in variable losses due to sorption of biomarkers and thus lead to variable amounts of chemical loadings measured in the liquid phase [67–69]. Moreover, transformation of cannabis biomarkers (i.e., THC-OH to THC-COOH) in sewer biofilms and during in-sewer transport may also significantly affect the data reported [18,30]. The information currently available is still limited and not conclusive. A better understanding of the possible role of suspended solids in raw wastewater needs attention and ongoing research moves in this direction.

While uncertainties exist around the measurement and quantification of the cannabis active ingredient THC and its metabolites in wastewater, the method does provide useful insights. Wastewater provides one of the only direct measures to estimate community level drug consumption. As shown in this chapter, wide-scale and community level use is a valuable input when assessing major changes to a drug's legal status. Sewer catchments and wastewater properties can vary widely among locations, depending on type of sewer system, special industrial discharges and weather conditions. However, if longitudinal monitoring occurs (i) within the same catchment, (ii) during similar conditions (i.e. dry weather), then relative trends in use can be evaluated even without knowing (iii) the exact sorption to particles, and degradation factors and (iv) average excretion rates since these are expected to remain relatively constant over time. In such situations, trends in use before and after legislative changes are helpful in assessing the effect of the legislation. The Canadian government sees wastewater as a complementary tool to traditional metrics and has invested in its use beyond the inaugural year presented here. Research is currently conducted to identify the potential sources of variability and facilitate the deployment of the method at large scale over extended periods of time. The Washington State case study provides lawmakers with evidence that one of the goals of cannabis legalization, a decrease in the cannabis black-market, appears to be working. This ability to understand the entire cannabis market through community consumption in relation to sales data is a result that would have been difficult to demonstrate applying traditional drug use indicators.

Acknowledgements

This publication was funded by the European Union's Justice Programme—Drugs Policy Initiatives, EuSeME (project number 861602). The Washington State pilot program was supported by the National Institute on Drug Abuse of the National Institutes of Health (R03DA038806). The pilot project in Canada was funded by Statistics Canada and conducted with the support of managers and staff at wastewater treatment plants in the five participating metropolitan areas.

References

[1] C.G. Daughton, Illicit drugs in municipal sewage: proposed new non-intrusive tool to heighten public awareness of societal use of illicit/abused drugs and their potential for ecological consequences, in: C.G. Daughton, T.L. Jones-Lepp (Eds.), Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues, American Chemical Society, 2001, pp. 348–364.

[2] C. Ort, M.G. Lawrence, J. Reungoat, J.F. Mueller, Sampling for PPCPs in wastewater systems: comparison of different sampling modes and optimization strategies, Environ. Sci. Technol. 44 (2010) 6289–6296. https://doi.org/10.1021/es100778d.
[3] C. Ort, M.G. Lawrence, J. Rieckermann, A. Joss, Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review, Environ. Sci. Technol. 44 (2010) 6024–6035. https://doi.org/10.1021/es100779n.
[4] C. Ort, Quality assurance/quality control in wastewater sampling, in: C. Zhang, J.F. Mueller, M. Mortimer (Eds.), Quality Assurance & Quality Control of Environmental Field Sampling, Future Science, London, UK, 2014, pp. 146–168.
[5] S. Castiglioni, L. Bijlsma, A. Covaci, E. Emke, F. Hernandez, M. Reid, C. Ort, K.V. Thomas, A.L.N. van Nuijs, P. de Voogt, E. Zuccato, Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers, Environ. Sci. Technol. 47 (2013) 1452–1460.
[6] C. Ort, J.M. Eppler, A. Scheidegger, J. Rieckermann, M. Kinzig, F. Sörgel, Challenges of surveying wastewater drug loads of small populations and generalizable aspects on optimizing monitoring design, Addiction 109 (2014) 472–481. https://doi.org/10.1111/add.12405.
[7] J. Prichard, W. Hall, P. de Voogt, E. Zuccato, Sewage epidemiology and illicit drug research: the development of ethical research guidelines, Sci. Total Environ. 472 (2014) 550–555. https://doi.org/10.1016/j.scitotenv.2013.11.039.
[8] J.W. O'Brien, P.K. Thai, G. Eaglesham, C. Ort, A. Scheidegger, S. Carter, F.Y. Lai, J.F. Mueller, A model to estimate the population contributing to the wastewater using samples collected on census day, Environ. Sci. Technol. 48 (2014) 517–525. https://doi.org/10.1021/es403251g.
[9] K.V. Thomas, A. Amador, J.A. Baz-Lomba, M. Reid, Use of mobile device data to better estimate dynamic population size for wastewater-based epidemiology, Environ. Sci. Technol. 51 (2017) 11363–11370. https://doi.org/10.1021/acs.est.7b02538.
[10] D.A. Burgard, J. Williams, D. Westerman, R. Rushing, R. Carpenter, A. LaRock, J. Sadetsky, J. Clarke, H. Fryhle, M. Pellman, C.J. Banta-Green, Using wastewater-based analysis to monitor the effects of legalized retail sales on cannabis consumption in Washington State, USA, Addiction 114 (2019) 1582–1590. https://doi.org/10.1111/add.14641.
[11] F. Hernandez, S. Castiglioni, A. Covaci, P. de Voogt, E. Emke, B. Kasprzyk-Hordern, C. Ort, M. Reid, J.V. Sancho, K.V. Thomas, A.L.N. van Nuijs, E. Zuccato, L. Bijlsma, Mass spectrometric strategies for the investigation of biomarkers of illicit drug use in wastewater, Mass Spectrom. Rev. 37 (2018) 258–280.
[12] A.L.N. van Nuijs, F.Y. Lai, F. Been, M.J. Andres-Costa, L. Barron, J.A. Baz-Lomba, J.D. Berset, L. Benaglia, L. Bijlsma, D. Burgard, S. Castiglioni, C. Christophoridis, A. Covaci, P. de Voogt, E. Emke, D. Fatta-Kassinos, J. Fick, F. Hernandez, C. Gerber, I. González-Mariño, R. Grabic, T. Gunnar, K. Kannan, S. Karolak, B. Kasprzyk-Hordern, Z. Kokot, I. Krizman-Matasic, A. Li, X. Li, A.S.C. Löve, M.L. de Alda, A.K. McCall, M.R. Meyer, H. Oberacher, J. O'Brien, J.B. Quintana, M. Reid, S. Schneider, S.S. Simoes, N.S. Thomaidis, K. Thomas, V. Yargeau, C. Ort, Multi-year inter-laboratory exercises for the analysis of illicit drugs and metabolites in wastewater: development of a quality control system, TrAC, Trends Anal. Chem. 103 (2018) 34–43. https://doi.org/10.1016/j.trac.2018.03.009.
[13] C. Ort, K. V. Thomas, S. Castiglioni, L. Bijlsma, M.J. Reid, E. Zuccato, A. Covaci, A. van Nuijs, B. Kasprzyk-Hordern, F. Hernandez, P. de Voogt, E. Emke, Sewage Analysis CORe group Europe (SCORE), accessed February 26, 2020. http://www.score-network.eu/.

[14] M.A. Huestis, Human cannabinoid pharmacokinetics, Chem. Biodivers. 4 (2007) 1770–1804. https://doi.org/10.1002/chin.200747256.
[15] M.E. Wall, B.M. Sadler, D. Brine, H. Taylor, M. Perez-Reyes, Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women, Clin. Pharmacol. Ther. 34 (1983) 352–363. https://doi.org/10.1038/clpt.1983.179.
[16] E.L. Karschner, E.W. Schwilke, R.H. Lowe, W.D. Darwin, R.I. Herning, J.L. Cadet, M.A. Huestis, Implications of plasma Δ9-tetrahydrocannabinol, 11-hydroxy-THC, and 11-nor-9-carboxy-THC concentrations in chronic cannabis smokers, J. Anal. Toxicol. 33 (2009) 469–477. https://doi.org/10.1093/jat/33.8.469.
[17] M.A. Huestis, J.M. Mitchell, E.J. Cone, Urinary excretion profiles of 11-nor-9-carboxy-Δ9- tetrahydrocannabinol in humans after single smoked doses of marijuana, J. Anal. Toxicol. 20 (1996) 441–452. https://doi.org/10.1093/jat/20.6.441.
[18] U. Khan, J.A. Nicell, Sewer epidemiology mass balances for assessing the illicit use of methamphetamine, amphetamine and tetrahydrocannabinol, Sci. Total Environ. 421–422 (2012) 144–162. https://doi.org/10.1016/j.scitotenv.2012.01.020.
[19] G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Mancini, R. Mastropasqua, M. Nazzari, R. Samperi, Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities, Sci. Total Environ. 302 (2003) 199–209.
[20] A.L.N. van Nuijs, S. Castiglioni, I. Tarcomnicu, C. Postigo, M.L. de Alda, H. Neels, E. Zuccato, D. Barcelo, A. Covaci, Illicit drug consumption estimations derived from wastewater analysis: a critical review, Sci. Total Environ. 409 (2011) 3564–3577. https://doi.org/10.1016/j.scitotenv.2010.05.030.
[21] N. Mastroianni, C. Postigo, M.L. De Alda, D. Barcelo, Illicit and abused drugs in sewage sludge: method optimization and occurrence, J. Chromatogr. A 1322 (2013) 29–37. https://doi.org/10.1016/j.chroma.2013.10.078.
[22] F. Mack, H. Bönisch, Dissociation constants and lipophilicity of catecholamines and related compounds, Naunyn Schmiedebergs Arch. Pharmacol. 310 (1979) 1–9. https://doi.org/10.1007/BF00499868.
[23] E. Gracia-Lor, S. Castiglioni, R. Bade, F. Been, E. Castrignanò, A. Covaci, I. González-Mariño, E. Hapeshi, B. Kasprzyk-Hordern, J. Kinyua, F.Y. Lai, T. Letzel, L. Lopardo, M.R. Meyer, J. O'Brien, P. Ramin, N.I. Rousis, A. Rydevik, Y. Ryu, M.M. Santos, I. Senta, N.S. Thomaidis, S. Veloutsou, Z. Yang, E. Zuccato, L. Bijlsma, Measuring biomarkers in wastewater as a new source of epidemiological information: current state and future perspectives, Environ. Int. 99 (2017) 131–150. https://doi.org/10.1016/j.envint.2016.12.016.
[24] A.-K. McCall, R. Bade, J. Kinyua, F.Y. Lai, P.K. Thai, A. Covaci, L. Bijlsma, A.L.N. van Nuijs, C. Ort, Critical review on the stability of illicit drugs in sewers and wastewater samples, Water Res. 88 (2016) 933–947.
[25] E. Zuccato, C. Chiabrando, S. Castiglioni, R. Bagnati, R. Fanelli, Estimating community drug abuse by wastewater analysis, Environ. Health Perspect. 116 (2008) 1027–1032. https://doi.org/10.1289/ehp.11022.
[26] T. Nefau, S. Karolak, L. Castillo, V. Boireau, Y. Levi, Presence of illicit drugs and metabolites in influents and effluents of 25 sewage water treatment plants and map of drug consumption in France, Sci. Total Environ. 461–462 (2013) 712–722. https://doi.org/10.1016/j.scitotenv.2013.05.038.
[27] D.A. Devault, T. Néfau, H. Pascaline, S. Karolak, Y. Levi, First evaluation of illicit and licit drug consumption based on wastewater analysis in Fort de France urban area (Martinique, Caribbean), a transit area for drug smuggling, Sci. Total Environ. 490 (2014) 970–978. https://doi.org/10.1016/j.scitotenv.2014.05.090.
[28] N.V. Heuett, C.E. Ramirez, A. Fernandez, P.R. Gardinali, Analysis of drugs of abuse by online SPE-LC high resolution mass spectrometry: communal assessment of consumption, Sci. Total Environ. 511 (2015) 319–330. https://doi.org/10.1016/j.scitotenv.2014.12.043.

[29] S. Terzic, I. Senta, M. Ahel, Illicit drugs in wastewater of the city of Zagreb (Croatia)—estimation of drug abuse in a transition country, Environ. Pollut. 158 (2010) 2686–2693. https://doi.org/10.1016/j.envpol.2010.04.020.
[30] C. Postigo, M.L. de Alda, D. Barceló, Evaluation of drugs of abuse use and trends in a prison through wastewater analysis, Environ. Int. 37 (2011) 49–55. https://doi.org/10.1016/j.envint.2010.06.012.
[31] C. Postigo, M.J. López de Alda, D. Barceló, Drugs of abuse and their metabolites in the Ebro River basin: occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation, Environ. Int. 36 (2010) 75–84. https://doi.org/10.1016/j.envint.2009.10.004.
[32] E. Gracia-Lor, E. Zuccato, S. Castiglioni, Refining correction factors for back-calculation of illicit drug use, Sci. Total Environ. 573 (2016) 1648–1659. https://doi.org/10.1016/j.scitotenv.2016.09.179.
[33] F. Been, C. Schneider, F. Zobel, O. Delémont, P. Esseiva, Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland, Int. J. Drug Policy 36 (2016) 33–42. https://doi.org/10.1016/j.drugpo.2016.06.008.
[34] A. Causanilles, J.A. Baz-Lomba, D.A. Burgard, E. Emke, I. Gonzalez-Marino, I. Krizman-Matasic, A. Li, A.S.C. Love, A.K. McCall, R. Montes, A.L.N. van Nuijs, C. Ort, J.E.B. Quintana, I. Senta, S. Terzic, F. Hernandez, P. de Voogt, L. Bijlsma, Improving wastewater-based epidemiology to estimate cannabis use: focus on the initial aspects of the analytical procedure, Anal. Chim. Acta 988 (2017) 27–33.
[35] S. Castiglioni, E. Zuccato, E. Crisci, C. Chiabrando, R. Fanelli, R. Bagnati, Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry, Anal. Chem. 78 (2006) 8421–8429. https://doi.org/10.1021/ac061095b.
[36] M.R. Boleda, M.T. Galceran, F. Ventura, Trace determination of cannabinoids and opiates in wastewater and surface waters by ultra-performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1175 (2007) 38–48. https://doi.org/10.1016/j.chroma.2007.10.029.
[37] C. Postigo, M.J. Lopez De Alda, D. Barceló, Fully automated determination in the low nanogram per liter level of different classes of drugs of abuse in sewage water by on-line solid-phase extraction-liquid chromatography-electrospray-tandem mass spectrometry, Anal. Chem. 80 (2008) 3123–3134. https://doi.org/10.1021/ac702060j.
[38] L. Bijlsma, J.V. Sancho, E. Pitarch, M. Ibanez, F. Hernandez, Simultaneous ultra-high-pressure liquid chromatography-tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater, J. Chromatogr. A. 1216 (2009) 3078–3089.
[39] J.D. Berset, R. Brenneisen, C. Mathieu, Analysis of llicit and illicit drugs in waste, surface and lake water samples using large volume direct injection high performance liquid chromatography—electrospray tandem mass spectrometry (HPLC-MS/MS), Chemosphere 81 (2010) 859–866. https://doi.org/10.1016/j.chemosphere.2010.08.011.
[40] I. González-Mariño, J.B. Quintana, I. Rodríguez, R. Cela, Determination of drugs of abuse in water by solid-phase extraction, derivatisation and gas chromatography-ion trap-tandem mass spectrometry, J. Chromatogr. A 1217 (2010) 1748–1760. https://doi.org/10.1016/j.chroma.2010.01.046.
[41] F.Y. Lai, C. Ort, C. Gartner, S. Carter, J. Prichard, P. Kirkbride, R. Bruno, W. Hall, G. Eaglesham, J.F. Mueller, Refining the estimation of illicit drug consumptions from wastewater analysis: co-analysis of prescription pharmaceuticals and uncertainty assessment, Water Res. 45 (2011) 4437–4448. https://doi.org/10.1016/j.watres.2011.05.042.

[42] I. González-Mariño, J.B. Quintana, I. Rodríguez, M. Gonzáez-Díez, R. Cela, Screening and selective quantification of illicit drugs in wastewater by mixed-mode solid-phase extraction and quadrupole-time-of-flight liquid chromatography-mass spectrometry, Anal. Chem. 84 (2012) 1708–1717. https://doi.org/10.1021/ac202989e.

[43] I. Racamonde, E. Villaverde-de-Sáa, R. Rodil, J.B. Quintana, R. Cela, Determination of Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in water samples by solid-phase microextraction with on-fiber derivatization and gas chromatography-mass spectrometry, J. Chromatogr. A 1245 (2012) 167–174. https://doi.org/10.1016/j.chroma.2012.05.017.

[44] L. Bijlsma, E. Emke, F. Hernández, P. De Voogt, Performance of the linear ion trap Orbitrap mass analyzer for qualitative and quantitative analysis of drugs of abuse and relevant metabolites in sewage water, Anal. Chim. Acta 768 (2013) 102–110.

[45] G. Fedorova, T. Randak, R.H. Lindberg, R. Grabic, Comparison of the quantitative performance of a Q-Exactive high-resolution mass spectrometer with that of a triple quadrupole tandem mass spectrometer for the analysis of illicit drugs in wastewater, Rapid Commun. Mass Spectrom. 27 (2013) 1751–1762. https://doi.org/10.1002/rcm.6628.

[46] I. Senta, I. Krizman, M. Ahel, S. Terzic, Integrated procedure for multiresidue analysis of dissolved and particulate drugs in municipal wastewater by liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 405 (2013) 3255–3268. https://doi.org/10.1007/s00216-013-6720-9.

[47] M.J. Andrés-Costa, N. Rubio-López, M. Morales Suárez-Varela, Y. Pico, Occurrence and removal of drugs of abuse in wastewater treatment plants of Valencia (Spain), Environ. Pollut. 194 (2014) 152–162. https://doi.org/10.1016/j.envpol.2014.07.019.

[48] L. Bijlsma, E. Beltran, C. Boix, J.V. Sancho, F. Hernandez, Improvements in analytical methodology for the determination of frequently consumed illicit drugs in urban wastewater, Anal. Bioanal. Chem. 406 (2014) 4261–4272.

[49] A.L.N. van Nuijs, A. Gheorghe, P.G. Jorens, K. Maudens, H. Neels, A. Covaci, Optimization, validation, and the application of liquid chromatography-tandem mass spectrometry for the analysis of new drugs of abuse in wastewater, Drug Test. Anal. 6 (2014) 861–867. https://doi.org/10.1002/dta.1460.

[50] B.J. Tscharke, C. Chen, J.P. Gerber, J.M. White, Temporal trends in drug use in Adelaide, South Australia by wastewater analysis, Sci. Total Environ. 565 (2016) 384–391. https://doi.org/10.1016/j.scitotenv.2016.04.183.

[51] T. Thiebault, L. Fougère, E. Destandau, M. Réty, J. Jacob, Temporal dynamics of human-excreted pollutants in wastewater treatment plant influents: toward a better knowledge of mass load fluctuations, Sci. Total Environ. 596–597 (2017) 246–255. https://doi.org/10.1016/j.scitotenv.2017.04.130.

[52] K.S. Foppe, D.R. Hammond-Weinberger, B. Subedi, Estimation of the consumption of illicit drugs during special events in two communities in Western Kentucky, USA using sewage epidemiology, Sci. Total Environ. 633 (2018) 249–256. https://doi.org/10.1016/j.scitotenv.2018.03.175.

[53] I. González-Mariño, V. Castro, R. Montes, R. Rodil, A. Lores, R. Cela, J.B. Quintana, Multi-residue determination of psychoactive pharmaceuticals, illicit drugs and related metabolites in wastewater by ultra-high performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1569 (2018) 91–100. https://doi.org/10.1016/j.chroma.2018.07.045.

[54] I. González-Mariño, K.V. Thomas, M.J. Reid, Determination of cannabinoid and synthetic cannabinoid metabolites in wastewater by liquid–liquid extraction and ultra-high performance supercritical fluid chromatography-tandem mass spectrometry, Drug Test. Anal. 10 (2018) 222–228. https://doi.org/10.1002/dta.2199.

[55] A.J. Skees, K.S. Foppe, B. Loganathan, B. Subedi, Contamination profiles, mass loadings, and sewage epidemiology of neuropsychiatric and illicit drugs in wastewater and river waters from a community in the Midwestern United States, Sci. Total Environ. 631–632 (2018) 1457–1464. https://doi.org/10.1016/j.scitotenv.2018.03.060.
[56] N. Centazzo, B.M. Frederick, A. Jacox, S.Y. Cheng, M. Concheiro-Guisan, Wastewater analysis for nicotine, cocaine, amphetamines, opioids and cannabis in New York City, Forensic Sci. Res. 4 (2019) 152–167. https://doi.org/10.1080/20961790.2019.1609388.
[57] N. Daglioglu, E.Y. Guzel, S. Kilercioglu, Assessment of illicit drugs in wastewater and estimation of drugs of abuse in Adana Province, Turkey, Forensic Sci. Int. 294 (2019) 132–139. https://doi.org/10.1016/j.forsciint.2018.11.012.
[58] S. Mercan, M. Kuloglu, T. Tekin, Z. Turkmen, A.O. Dogru, A.N. Safran, M. Acikkol, F. Asicioglu, Wastewater-based monitoring of illicit drug consumption in Istanbul: preliminary results from two districts, Sci. Total Environ. 656 (2019) 231–238. https://doi.org/10.1016/j.scitotenv.2018.11.345.
[59] D. Savas, Public Opinion and Illicit Drugs Canadian Attitudes towards Decriminalizing the Use of Marijuana, Fraser Inst. Digit. Publ., 2001, .pp. 1–12. http://oldfraser.lexi.net/publications/books/drug_papers/UDSavas.pdf.
[60] J. Tahirali, 7 in 10 Canadians Support Marijuana Legalization : Nanos Poll, CTV News, 2016. http://www.ctvnews.ca/canada/7-in-10-canadians-support-marijuana-legalization-nanos-poll-1.2968953.
[61] A. Swift, For First Time, Americans Favor Legalizing Marijuana, Gall. Polit., 2013. http://www.gallup.com/poll/165539/first-time-americansfavor-legalizing-marijuana.aspx.
[62] V. Yargeau, B. Taylor, H. Li, A. Rodayan, C.D. Metcalfe, Analysis of drugs of abuse in wastewater from two Canadian cities, Sci. Total Environ. 487 (2014) 722–730. https://doi.org/10.1016/j.scitotenv.2013.11.094.
[63] A. Jacox, J. Wetzel, S.-Y. Cheng, M. Concheiro, Quantitative analysis of opioids and cannabinoids in wastewater samples, Forensic Sci. Res. 2 (2017) 18–25. https://doi.org/10.1080/20961790.2016.1270812.
[64] A. Rodayan, S. Afana, P.A. Segura, T. Sultana, C.D. Metcalfe, V. Yargeau, Linking drugs of abuse in wastewater to contamination of surface and drinking water, Environ. Toxicol. Chem. 35 (2016) 843–849. https://doi.org/10.1002/etc.3085.
[65] T. Werschler, A. Brennan, Wastewater-Based Estimates of Cannabis and Drug Use in Canada: Pilot test Detailed Results, Publication 11-621-M, Stat. Canada Anal. Br., 2019. https://www150.statcan.gc.ca/n1/pub/11-621-m/11-621-m2019004-eng.htm. accessed February 26, 2020.
[66] Statistics-Canada, Prevalence of Cannabis Use in the Past Three Months, Self-Reported. 2019https://doi.org/10.25318/1310038301-eng. Table 13-10-0383-01.
[67] A.K. McCall, A. Scheidegger, M.M. Madry, A.E. Steuer, D.G. Weissbrodt, P.A. Vanrolleghem, T. Kraemer, E. Morgenroth, C. Ort, Influence of different sewer biofilms on transformation rates of drugs, Environ. Sci. Technol. 50 (2016) 13351–13360. https://doi.org/10.1021/acs.est.6b04200.
[68] P. Ramin, A.L. Brock, A. Causanilles, B. Valverde-Pérez, E. Emke, P. De Voogt, F. Polesel, B.G. Plósz, Transformation and sorption of illicit drug biomarkers in sewer biofilms, Environ. Sci. Technol. 51 (2017) 10572–10584. https://doi.org/10.1021/acs.est.6b06277.
[69] P. Ramin, A.L. Brock, F. Polesel, A. Causanilles, E. Emke, P. De Voogt, B.G. Plosz, Transformation and sorption of illicit drug biomarkers in sewer systems: understanding the role of suspended solids in raw wastewater, Environ. Sci. Technol. 50 (2016) 13397–13408. https://doi.org/10.1021/acs.est.6b03049.