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Long-term monitoring of drug consumption patterns in a large-sized European city using wastewater-based epidemiology: Comparison of two sampling schemes for the assessment of multiannual trends

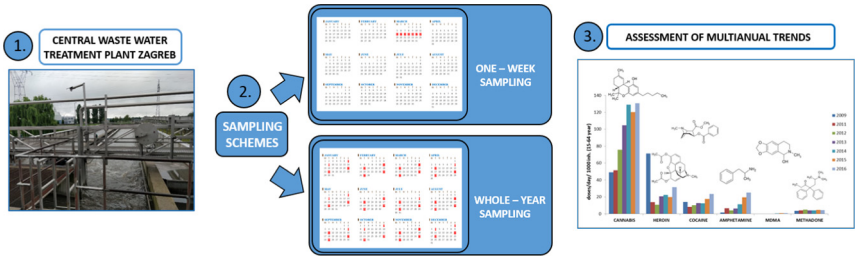
Ivona Krizman-Matasic, Ivan Senta, Petra Kostanjevecki, Marijan Ahel, Senka Terzic *

Division for Marine and Environmental Research, Rudjer Boskovic Institute, Bijenicka c. 54, 10000 Zagreb, Croatia

HIGHLIGHTS

- Drug consumption patterns were studied using wastewater-based epidemiology.
- The 8-year study was performed in a large European city.
- Comparison of one-week and whole-year sampling strategies was made.
- Significant multiannual drug consumption changes were determined.
- A comparison with epidemiological data was performed.

GRAPHICAL ABSTRACT



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ABSTRACT

A comprehensive study aimed at monitoring of temporal variability of illicit drugs (heroin, cocaine, amphetamine, MDMA, methamphetamine and cannabis) and therapeutic opiate methadone in a large-sized European city using wastewater-based epidemiology (WBE) was conducted in the city of Zagreb, Croatia, during an 8-year period (2009–2016). The study addressed the impact of different sampling schemes on the assessment of temporal drug consumption patterns, in particular multiannual consumption trends and documented the possible errors associated with the one-week sampling scheme. The highest drug consumption prevalence was determined for cannabis (from 59 ± 18 to 156 ± 37 doses/day/1000 inhabitants 15–64 years), followed by heroin (from 11 ± 10 to 71 ± 19 doses/day/1000 inhabitants 15–64 years), cocaine (from 8.3 ± 0.9 to 23 ± 4.0 doses/day/1000 inhabitants 15–64 years) and amphetamine (from 1.3 ± 0.9 to 21 ± 6.1 doses/day/1000 inhabitants 15–64 years) whereas the consumption of MDMA was comparatively lower (from 0.18 ± 0.08 to 2.7 doses ±0.7 doses/day/1000 inhabitants 15–64 years). The drug consumption patterns were characterized by clearly enhanced weekend and Christmas season consumption of stimulating drugs (cocaine, MDMA and amphetamine) and somewhat lower summer consumption of almost all drugs. Pronounced multiannual consumption trends were determined for most of the illicit drugs. The investigated 8-year period was characterized by a marked increase of the consumption of pure cocaine (1.6-fold), THC (2.7-fold), amphetamine (16-fold) and MDMA (15-fold) and a concomitant decrease (2.3-fold) of the consumption of pure heroin. The heroin consumption decrease was associated with an increase of methadone consumption (1.4-fold), which can be linked to its use in the heroin substitution therapy. The estimated number of average methadone doses consumed in the city of Zagreb was in a good agreement with the prescription data on treated opioid addicts in Croatia.

* Corresponding author.
E-mail address: terzic@irb.hr (S. Terzic).

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1. Introduction

Abuse of illicit drugs has become a major global problem with numerous negative consequences including increase in crime rate, negative impacts on public health, economic damage as well as costs of treatment of drug addicts (EMCDDA, 2009). Consequently, knowing the extent and patterns of drug abuse is very important for planning timely and effective actions to mitigate these problems. The official data about illicit drug consumption usually include the information about the amount and purity of seized drugs, number of treated drug addicts and general population survey data, whose frequency in different countries may be rather different. In recent years, wastewater-based epidemiology (WBE) has been used as a complementary approach for the estimation of drug consumption across the world (e.g. Bijlsma et al., 2016; Bones et al., 2007; Huerta-Fontela et al., 2008; Khan et al., 2014; Kankaanpää et al., 2014; Kasprzyk-Hordern et al., 2009; Irvine et al., 2011; Lai et al., 2013a, 2016; Metcalfe et al., 2010; Postigo et al., 2010; Terzic et al., 2010; van Nuijs et al., 2009; Zuccato et al., 2008).

The main advantages of the WBE approach are objectivity and suitability for near-real-time monitoring. In order to improve and expand the WBE approach, several publications addressed the problem of uncertainties associated with sample collection (Ort et al., 2010), sample stability (McCall et al., 2016; van Nuijs et al., 2012; Senta et al., 2014) as well as back-calculation of drug consumption (Castiglioni et al., 2013; Gracia-Lor et al., 2016; Lai et al., 2011). A number of studies have already demonstrated the potential of WBE to provide information about the spatial (Been et al., 2016; Bijlsma et al., 2016; Kankaanpää et al., 2016; Nefau et al., 2013) and temporal (Bade et al., 2018; Been et al., 2016; Lai et al., 2016; Mastroianni et al., 2017; Tscharke et al., 2016) drug consumption patterns, including large international comparative studies (Ort et al., 2014a; Thomas et al., 2012), which showed a pronounced regional and temporal variability of drug abuse across the Europe. In several studies, the potential of this approach as a complementary tool to support epidemiological and seizure data (Baz-Lomba et al., 2017; Been et al., 2016; Zuccato et al., 2016) was demonstrated. The WBE approach was also successfully applied to study the differences in drug consumption patterns between the large and small cities (Krizman et al., 2016; van Nuijs et al., 2009), with a clear indication that large cities represent communities with significantly enhanced drug consumption and, consequently, are very suitable for the investigation of the drug consumption patterns.

Regarding temporal variability, a significant emphasis of existing studies was on short-term consumption variability, especially regarding so-called recreational stimulating drugs. A number of WBE studies performed in different countries confirmed an enhanced consumption of stimulating illicit drugs during the weekend (e.g. Krizman et al., 2016; Terzic et al., 2010; Thomas et al., 2012), large sport events (Gerrity et al., 2011), music festivals (Bijlsma et al., 2014; Jiang et al., 2015; Lai et al., 2013b; Mackuľak et al., 2014) and the peak of tourist season in the vacation areas (Krizman et al., 2016; Lai et al., 2013c). In contrast, only few reports addressed the issue of multiannual changes in drug consumption patterns within the selected population (e.g. Kankaanpää et al., 2016; Mastroianni et al., 2017; Ort et al., 2014a; Tscharke et al., 2016; Zuccato et al., 2016). Most of the published multiannual studies were based on the comparison of one-week wastewater sampling campaigns in a given time-period (Kankaanpää et al., 2016; Mastroianni et al., 2017; Ort et al., 2014a; Zuccato et al., 2016.). In such cases, possible week-to-week variability during the particular year was not taken into account, which might increase the uncertainties related to the annual consumption estimates. In order to get a more accurate estimate, representative of average annual drug consumption, a recent study by Ort et al. (2014b) recommended the use of stratified annual sampling to minimize the errors associated with day-to-day variability. The importance of sampling scheme for the assessment of consumption was also discussed in Humphries et al. (2016).

In this study we investigated the multiannual trends in the consumption of 6 illicit drugs (cannabis, cocaine, heroin, MDMA, amphetamine, methamphetamine) and one therapeutic opioid (methadone) in the city of Zagreb in the period 2009–2016, by applying two different sampling schemes (one-week sampling scheme and a whole-year sampling scheme). The city of Zagreb is the capital and the largest Croatian city, representing almost 20% of Croatia's population. Furthermore, an initial WBE study conducted in Zagreb (Terzic et al., 2010) indicated specific drug consumption patterns which were different from those reported for most of the other European cities, in particular regarding comparatively higher prevalence of heroin consumption and lower prevalence of cocaine and amphetamine drug consumption.

The specific goals of the present study included: a) long-term study of the weekday-related drug consumption patterns; b) impact of the holiday season on drug consumption patterns; c) seasonal changes in drug consumption patterns; d) testing different sampling schemes for the assessment of multiannual trends; e) tracking the multiannual changes of the drug consumption over a period of 8 years and comparison with the available epidemiological data.

2. Materials and methods

2.1. Selection of target compounds

The selection of target compounds was based on the available data on drug consumption patterns in Croatia (Glavak Tkalic et al., 2013) and in the city of Zagreb (Krizman et al., 2016; Terzic et al., 2010). Selected analytes included morphine (MOR), morphine 3 glucuronide (M3G) and 6-acetylmorphine (6-AM) as principal heroin-derived substances as well as benzoylecgonine (BE), amphetamine (AMP), methamphetamine (MAMP), 3,4 methylendioximethamphetamine (MDMA), 11 nor 9 carboxy tetrahydrocannabinol (THC-COOH) and 2 ethylidene 1,5 dimethyl 3,3 diphenylpyrrolidine (EDDP) as principal biomarkers of cocaine, amphetamine, methamphetamine, MDMA, cannabis and methadone consumption, respectively.

2.2. Chemicals and materials

Standard solutions of all target analytes (1 g/L) and their deuterated analogues (0.1 g/L) were purchased from Lipomed AG (Switzerland). Mixed standard solutions of the analytes and their deuterated analogues, used as surrogate standards, were prepared in methanol (MeOH) at concentrations of 10 mg/L and 2 mg/L, respectively, and kept in the dark at −20 °C. Aqueous ammonia solution (NH_3, 25%) and LC-MS grade MeOH were purchased from Merck AG (Darmstadt, Germany). Acetic acid (CH_3COOH), also LC-MS grade, formic acid (HCOOH) and phosphoric acid (H_3PO_4) were purchased from Fluka (Switzerland). Milli-Q water was obtained by purifying with an Elix-Mili-Q-system (Millipore, Bedford, USA). Oasis MCX cartridges (150 mg/6 mL) were purchased from Waters (Milford, MA, SAD) whereas Strata NH_2 (200 mg/3 mL) cartridges as well as HPLC columns used for the chromatographic separation (Synergi Polar; 4 μm, 150 mm × 3 mm, Kinetex PFP; 2.6 μm, 100 mm × 2.1 mm) were purchased from Phenomenex (Torrance, California, USA). Glass-fiber filters (GF/C) were purchased from Whatman (USA).

2.3. Wastewater sampling and analysis

The 24-h composite samples (from 8 a.m. of the previous day to 8 a.m. of the sample collection day) of untreated wastewater were collected at the inlet of the central WWTP of the city of Zagreb in the period 2009–2016, except in 2010. All collected samples were time-proportional, with the sampling time interval of 15 min. A total number of 282 samples, having an average pH of 7.6 ± 0.2, was collected. Depending on the specific research goals, different sampling schemes were applied to cover both short-time and long-term variability: one-week sample scheme, a whole-year sampling-scheme and Christmas season sampling scheme.

All investigated years included at least one one-week sampling period (25 March–2 April 2009; 26 August–3 September 2009, 9–15 March 2011, 17–24 March 2012, 6–12 March 2013, 24 July–31 August 2013, 11–18 March 2014, 17–23 March 2015, 9–15 March 2016).

In addition, in 2009 and further throughout the period 2012–2016, samples were also collected over the whole year, two to four times per month, and uniformly covered all seasons (whole-year sampling scheme). In principle, a whole-year sampling scheme included a collection of equal number of weekend (Sunday) and weekday (Tuesday) samples. The total number of samples collected within one whole-year sampling scheme varied from 21 to 46. Special time-periods such as Christmas holiday season and major festivals were avoided within the one-week and whole-year sampling schemes. Christmas season sampling scheme included two Christmas holiday seasons in the period: 21 December 2012–4 January 2013 ($n = 15$) and 20 December 2013–3 January 2014 ($n = 14$). The samples collected within the one-week sampling scheme and the Christmas holiday sampling scheme were frozen immediately after collection and kept frozen until analyses, whereas all other samples were processed within a few hours after collection. Since the study covers a rather long time-period, some of the data, resulting from the sampling campaigns described above, were partially used in previously published studies (e.g. Krizman et al., 2016; Ort et al., 2014b; Terzic et al., 2010; Thomas et al., 2012).

The sample preparation and LC-MS/MS analysis were performed by applying already published and validated analytical method (Senta et al., 2013). The performance of the method was repetitively confirmed in 6 international intercalibration studies performed during the period 2011–2016 (van Nuijs et al., 2018). Briefly, samples of wastewater (125 mL) were spiked with surrogate standards (120 ng/L) and after equilibration filtered using GF/C filters. After filtration, samples were enriched on Oasis MCX cartridges. The basic drugs were eluted with 6 mL of 0.5% NH_3 in MeOH whereas THC-COOH was eluted with methanol and additionally cleaned-up using Strata NH_2 cartridges. These two fractions were analyzed separately by triple-quadrupole liquid chromatography tandem mass spectrometry (Quantum AM, Thermo Electron, USA). Chromatographic separation of basic drug biomarkers was performed using a gradient elution on Synergy 4 μ POLAR-RP 80 Å column (Phenomenex, 150 × 3 mm), whereas for the analyses of THC-COOH, Kinetex 2.6 μm PFP 100 Å (Phenomenex, 100 × 2.1 mm) column was used. Eluents used for the separation of basic analytes included 0.1% acetic acid in H_2O (v/v) and 0.1% acetic acid in MeOH (v/v), whereas THC-COOH analyses were performed using H_2O and MeOH as eluents. THC-COOH was analyzed in negative ionization mode (NI) whereas the analyses of all other analytes were performed in positive ionization mode (PI). Identification and quantification was performed using two characteristic transitions for each analyzed compound (MRM mode). Quantitation of all analytes was performed using corresponding deuterated internal standards for all analytes.

2.4. Estimation of drug consumption

Estimation of drug consumption was performed as described earlier by Krizman et al. (2016), applying the methodology originally proposed by Zuccato et al. (2008). The representative average mass loads (X_{rp}) and their corresponding standard deviations (S_{RP}) used for the assessment of drug consumption were calculated using the following equations:

$$XRP = \frac{5}{7}x\,(workday) + \frac{2}{7}\,x\,(weekend)$$

$$SRP = \sqrt{\left(\frac{5}{7}S\,(workday)\right)^2 + \left(\frac{2}{7}S\,(weekend)\right)^2}$$

in which X (workday), S (workday), X (weekend) and S (weekend) represent the average values and standard deviations of workday and weekend daily mass loads. The concentration equal to the half of the detection limit was applied in all cases when the analyzed urinary biomarkers were not detectable.

The number of inhabitants as well as the number of inhabitants of age 15–64 years, served by the WWTP, was based on 2011 Census data. The normalized consumption of individual drugs, expressed as the number of doses per 1000 inhabitants, was calculated using the corresponding average dose size listed in Table 1.

The amounts of street-purity drugs which circulated on the illegal market in Zagreb were calculated from the estimated annual consumption of pure drugs (expressed in kg/year), which were divided by the corresponding drug purity presented in Table S1.

Most of the correction factors used in the calculation of drug consumption were taken from the paper published by Gracia-Lor et al. (2016). The estimation of cocaine consumption was made by using the correction factor of 3.6 (Castiglioni et al., 2013), whereas heroin consumption was calculated from 6-AM mass loads, using a correction factor of 86.9 (van Nuijs et al., 2011).

2.5. Statistical evaluation

Statistical analysis of the data was performed using Sigma Plot 12.0 (Systat software Inc., SAD). Depending on data distribution, parametric (*t*-test, One-way ANOVA) and non-parametric tests (Mann-Whitney, Kruskal-Wallis test) were applied. In order to examine differences among multiple groups, One-way ANOVA and Kruskal-Wallis tests were used (with follow-up Holm-Sidak and Dunn's method post-hoc testing, respectively) while for testing the differences between two groups, *t*-test and Mann-Whitney test were used.

3. Results and discussion

3.1. Occurrence of drug biomarkers in municipal wastewater of the city of Zagreb

The analyses included selected drug biomarkers which are excreted after the consumption of 6 illegal drugs (cannabis, heroin, cocaine, amphetamine, MDMA and methamphetamine) and methadone which is primarily used in the treatment of heroin users. The analyses performed between 2009 and 2016 showed that most of the investigated drug biomarkers were rather common constituents in the wastewater of the city of Zagreb (Table 2). The most frequently detected biomarkers were MOR, BE, THC-COOH and EDDP, which were determined in all analyzed wastewater samples ($n = 270$–282). Very high frequency of detection was obtained also for 6-AM (98%), M3G (97%), AMP (96%) and MDMA (99%; $n = 282$), whereas MAMP was the least frequently detected drug biomarker (83%). Regarding abundances, the highest average annual concentrations were determined for MOR (from 74 ± 29 ng/L to

Table 1
Selected drug biomarkers and data used for estimation of drug consumption.

Drug	Biomarker for estimation of consumption	Percentage of drug doses excreted as drug biomarker	Molar ratio	Correction factor	Dose (mg)
Heroin	6-AM	1.3	1.13	86.9[a]	10[d]
Cocaine	BE	29	1.05	3.6[b]	30[d]
Amphetamine	AMP	36	1.00	2.8[c]	10[c]
MDMA	MDMA	22.5	1.00	4.4[c]	97[d]
THC (Cannabis)	THC-COOH	0.5	0.91	182[c]	125[e]
Methadone	EDDP	25	1.12	3.6	80[f]

[a] van Nuijs et al., 2011.
[b] Castiglioni et al., 2013.
[c] Gracia-Lor et al., 2016.
[d] Office for Combating Narcotic Drug Abuse of the Government of the Republic of Croatia, data for 2013.
[e] Zuccato et al., 2008.
[f] Croatian Institute of Public Health, data for Zagreb for 2010.

Table 2
Mass loads of urinary biomarkers (MOR_{tot}, MOR, M3G, 6-AM, MAMP, AMP, MDMA, BE, MDMA, THC-COOH, EDDP) in raw wastewater of the city of Zagreb (Croatia) in the period 2009–2016.

Urinary drug biomarker	Year	n[a]	FD[b]	Concentration range (ng/L)	Average ± SD (ng/L)	Mass load range (g/day)	Average ± SD (g/day)
MOR_{tot}	2009	39	100	161–476	294 ± 83	45–106	75 ± 15
	2011	7	100	130–160	142 ± 10.8	30–36	32 ± 2.4
	2012	54	100	26–183	95 ± 37	11–61	27 ± 12
	2013	72	100	33–167	90 ± 32	17–50	28 ± 6.8
	2014	54	100	25–129	80 ± 28	17–62	28 ± 7.5
	2015	30	100	45–144	94 ± 23	16–39	30 ± 5.2
	2016	26	100	49–147	97 ± 22	18–44	35 ± 6.2
MOR	2009	39	100	160–476	294 ± 83	45–106	75 ± 15
	2011	7	100	109–135	120 ± 9.3	25–31	27 ± 2.1
	2012	54	100	19–183	94 ± 37	11–61	27 ± 12
	2013	72	100	26–166	86 ± 32	15–50	27 ± 7.0
	2014	54	100	22–127	74 ± 29	13–61	26 ± 7.3
	2015	30	100	41–141	91 ± 22	16–38	29 ± 5.0
	2016	26	100	42–143	91 ± 23	17–41	32 ± 5.7
M3G	2009	0	NA	NA	NA	NA	NA
	2011	7	100	3.3–5.5	4.7 ± 0.7	0.7–1.3	1.1 ± 0.2
	2012	54	85	<0.2–10.5	1.6 ± 2.2	<0.03–6.8	0.5 ± 1.1
	2013	72	100	<0.3–19	6.6 ± 4.9	<0.1–8.9	2.3 ± 2.0
	2014	54	100	<0.3–29	8.5 ± 6.7	<0.1–15	3.4 ± 3.3
	2015	30	100	<0.3–16	3.9 ± 4.2	<0.1–6.5	1.2 ± 1.5
	2016	26	100	<0.3–24	9.9 ± 6.7	<0.1–11	3.9 ± 3.0
6-AM	2009	39	100	3.3–28	12 ± 4.7	0.7–6.0	3.1 ± 1.2
	2011	7	100	2.3–4.2	3.3 ± 0.6	0.5–0.96	0.8 ± 0.1
	2012	54	91	<0.1–16	2.0 ± 2.4	<0.01–3.7	0.5 ± 0.6
	2013	72	100	0.1–14	3.1 ± 1.9	0.1–3	1.1 ± 0.5
	2014	54	100	0.1–7.0	3.1 ± 1.04	0.1–2.3	1.2 ± 0.4
	2015	30	93	<0.1–7.6	3.4 ± 1.8	<0.04–1.9	1.1 ± 0.5
	2016	26	100	2.2–16	5.0 ± 2.9	0.7–4.1	1.7 ± 0.7
MAMP	2009	0	NA	NA	NA	NA	NA
	2011	0	NA	NA	NA	NA	NA
	2012	54	83	<0.2–4.0	0.7 ± 0.9	<0.1–0.96	0.2 ± 0.2
	2013	72	78	<0.2–3.8	1.1 ± 1.0	<0.1–2	0.4 ± 0.5
	2014	54	78	<0.2–2.8	0.63 ± 0.66	<0.1–1.9	0.25 ± 0.3
	2015	30	100	<0.4–5.9	1.4 ± 1.8	<0.1–1.7	0.4 ± 0.5
	2016	26	89	<0.2–12	1.3 ± 2.3	<0.1–3.7	0.5 ± 0.7
AMP	2009	39	72	<1.3–35	7.5 ± 7.5	<0.3–7.6	1.9 ± 1.8
	2011	7	100	32–62	42 ± 10.3	7.2–13	9.5 ± 2.1
	2012	54	100	7.2–58	27 ± 15	2.3–17	7.5 ± 4.1
	2013	72	100	6.3–235	45 ± 38	2.7–63	13 ± 8.4
	2014	54	100	14–149	51 ± 26	6.1–74	18 ± 12
	2015	30	100	34–320	100 ± 70	12–111	32 ± 23
	2016	26	100	25–295	109 ± 58	15–89	38 ± 19
MDMA	2009	39	79	<1.1–33	6.8 ± 7.7	<0.2–7.4	1.7 ± 1.7
	2011	7	100	5.3–16	9.4 ± 4.6	1.2–3.6	2.1 ± 1.0
	2012	54	98	<0.1–96	26 ± 22	<0.03–21	7.1 ± 4.9
	2013	72	100	3.4–260	30 ± 40	1.8–62	8.5 ± 8.7
	2014	54	100	8.0–133	38 ± 30	3.4–67	15 ± 12
	2015	30	100	23–316	91 ± 68	7.6–92	28 ± 19
	2016	26	100	18–215	92 ± 58	8.9–80	32 ± 20
BE	2009	39	100	89–325	186 ± 59	27–77	47 ± 12
	2011	7	100	100–189	143 ± 34	22–43	32 ± 7.6
	2012	54	100	52–497	196 ± 94	24–166	56 ± 29
	2013	72	100	57–769	203 ± 125	31–224	60 ± 27
	2014	54	100	35–399	150 ± 66	24–197	57 ± 29
	2015	30	100	114–474	236 ± 96	45–125	75 ± 24
	2016	26	100	92–520	273 ± 101	52–173	97 ± 32
THC-COOH	2009	30	100	21–128	60 ± 23	7.3–31	16 ± 5.5
	2011	7	100	71–100	87 ± 10.4	16–22	20 ± 2.2
	2012	54	100	34–183	107 ± 36	18–52	30 ± 7.4
	2013	72	100	44–214	133 ± 43	16–74	42 ± 11
	2014	54	100	38–312	137 ± 54	19–117	49 ± 16
	2015	30	100	52–309	141 ± 58	15–88	45 ± 17
	2016	24	100	60–363	156 ± 66	32–105	54 ± 14
EDDP	2009	27	100	71–156	128 ± 20	24–38	30 ± 3.6
	2011	7	100	177–196	184 ± 6.5	40–45	42 ± 1.8
	2012	54	100	61–330	190 ± 67	25–69	52 ± 10.4
	2013	72	100	60–220	140 ± 43	31–67	43 ± 7.9
	2014	54	100	44–220	121 ± 41	29–92	43 ± 11
	2015	30	100	85–205	145 ± 24	25–67	47 ± 8.9
	2016	26	100	67–194	128 ± 34	26–60	45 ± 7.2

NA – not applicable.
[a] Number of analyzed samples.
[b] Frequency of detection.

294 ± 83 ng/L), BE (from 143 ± 34 ng/L to 273 ± 101 ng/L) and EDDP (from 121 ± 41 ng/L to 190 ± 67 ng/L), followed by AMP (from 7.5 ± 7.5 ng/L to 109 ± 58 ng/L) and MDMA (from 6.8 ± 7.7 ng/L to 92 ± 58 ng/L). The lowest concentrations were determined for MAMP (from 0.6 ± 0.6 ng/L to 1.4 ± 1.8 ng/L), M3G (from 1.6 ± 2.2 ng/L to 9.9 ± 6.7 ng/L) and 6-AM (from 2.0 ± 2.4 ng/L to 12 ± 4.7 ng/L).

3.2. Drug consumption patterns

3.2.1. Workday/weekend drug consumption patterns

Possible differences in workday to weekend consumption patterns of individual drugs have been evaluated based on the ratios of weekend and workday daily mass loads of selected drug biomarkers for individual years

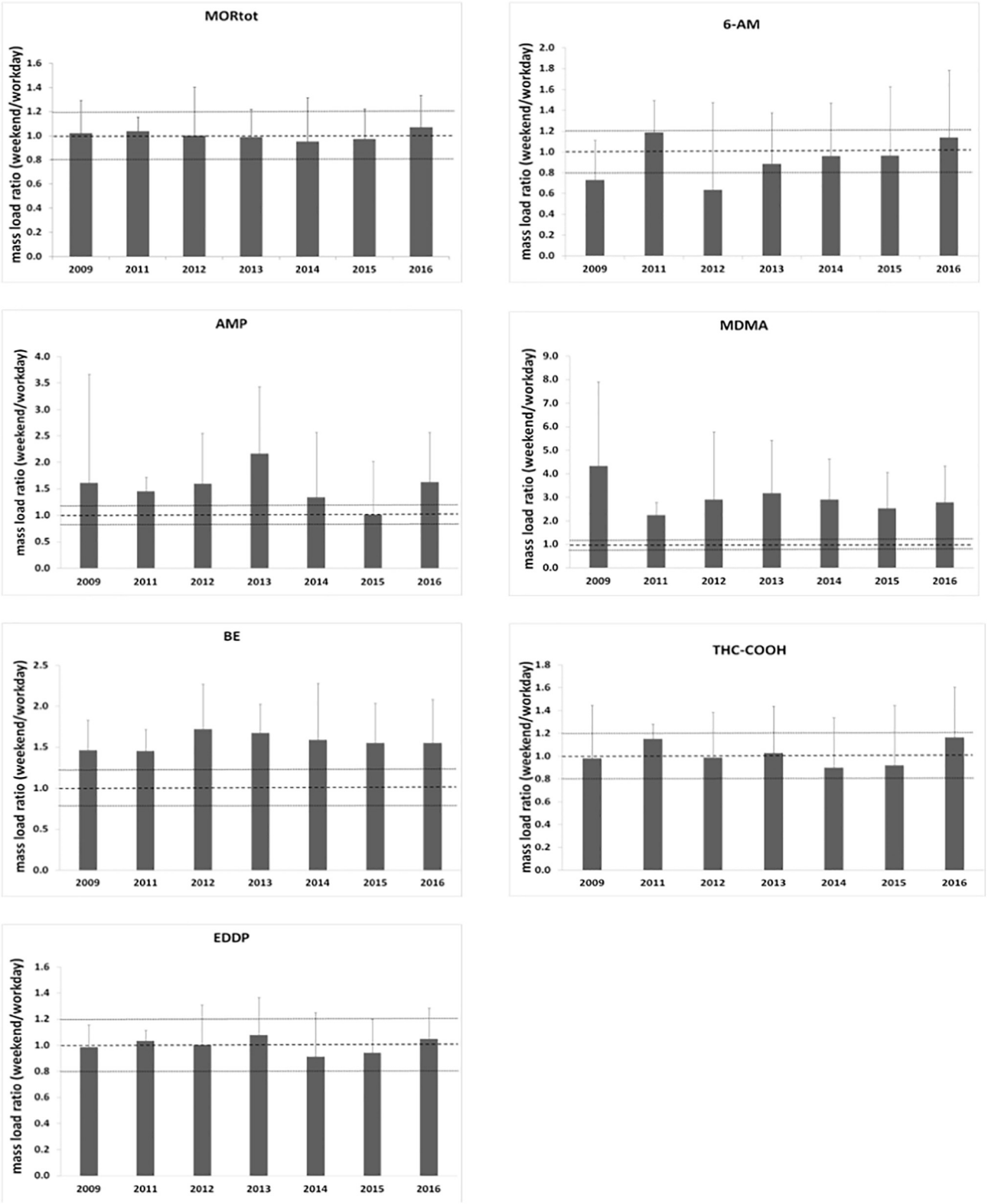

Fig. 1. Ratios of weekend and workday average mass loads of selected urinary drug biomarkers (MOR_{tot}, 6-AM, AMP, MDMA, BE, THC-COOH, EDDP) determined in the period from 2009 to 2016. Error bars represent standard deviations. Horizontal lines represent arbitrarily assumed weekend to workday mass load ratio of 1.0 ± 0.2.

(Fig. 1). It was arbitrarily assumed that a ratio significantly different from 1.0 ± 0.2 was a confirmation of some specific weekday-related consumption pattern. It should be stressed that the ratio for MOR consumption was calculated from the corresponding mass loads of the total morphine (MOR_{tot}). The MOR_{tot} mass loads were obtained by summing up the daily mass loads of MOR and M3G (taking into account the molar ratio to MOR of 1.62).

Almost all ratios of the weekend and workday average daily mass load of stimulating drug biomarkers, BE (1.5 ± 0.3 to 1.7 ± 0.5), MDMA (2.3 ± 0.5 to 4.3 ± 3.6) and AMP (1.0 ± 1.0 to 2.2 ± 1.3), were significantly (*t*-test) different from 1.0 ± 0.2. By contrast, most of the ratios for MOR_{tot} (0.95 ± 0.4 to 1.1 ± 0.3), 6-AM (0.6 ± 0.8 to 1.2 ± 0.3), THC-COOH (0.9 ± 0.4 to 1.2 ± 0.4) and EDDP (0.9 ± 0.3 to 1.1 ± 0.3) indicated a rather uniform consumption of heroin, cannabis and methadone throughout the week. The observed weekend-related drug consumption patterns of stimulating drugs (MDMA, cocaine and AMP) documented in this study not only fully support the results obtained in a number of previous studies based on 7 consecutive days sampling scheme (e.g. Krizman, 2015; Ort et al., 2014a; Terzic et al., 2010; Thomas et al., 2012), but also confirm the robustness of the applied whole-year sampling scheme to demonstrate the importance of weekday-weekend dynamics at long-term time scales.

3.2.2. The impact of holiday season on drug consumption patterns

The results of research dealing with the impact of holiday season on drug consumption patterns are presented in Fig. 2, Fig. S1 and Fig. 3. In both periods, the 1st of January (New Year) was characterized by a significantly enhanced daily mass load of BE (224 g/day and 197 g/day), MDMA (62 g/day and 67 g/day) and AMP (42 g/day and 60 g/day), which confirmed an increased consumption of all major stimulating drugs in holiday seasons (Fig. 2). By contrast, the 25th of December (Christmas) was associated with an enhanced excretion of BE (166 g/day and 130 g/day) whereas the Christmas consumption of most amphetamine-type drugs (AMP and MDMA) was not clearly elevated. These results probably reflect the life-style differences of cocaine and amphetamine-type drug consumers within the investigated population. In both holiday season periods, a steady increase of MOR excretion towards Christmas was also observed. However this increase was not associated with the concomitant increase of 6-AM and therefore cannot be unequivocaly related to the enhanced consumption of heroin. Furthermore, unlike for stimulating drugs, holiday-related consumption patterns could not be established for the remaining investigated drugs, such as cannabis and EDDP (Fig. S1). The comparison of the average mass loads during the two holiday season periods with the average weekend and workday mass loads in the corresponding years (Fig. 3) confirmed a significantly higher consumption ($p < 0.05$) of stimulating drugs (BE, MDMA, AMP) during the weekend (n = 19–24) and holiday season period (n = 14–15) as compared to workday periods (n = 19–22). The average mass loads of stimulating drugs during Christmas holiday season were 2–3.9-fold higher than during the average weekday and 1.2–1.9-fold higher than during the average weekend of the corresponding year. This is in a good agreement with previous studies which indicated the enhanced consumption of stimulating drugs during the holidays, festivals, tourist seasons etc. (e.g. Krizman et al., 2016; Lai et al., 2013a; 2013b; van Nuijs et al., 2009) and underlines the ability of the applied WBE approach to address the problem of relative contributions of special events to the overall drug consumption in a particular yearly period.

3.2.3. The seasonal differences in drug consumption patterns

In this study, we compared the average daily mass loads determined in the city of Zagreb in 2 different one-week periods, early spring and

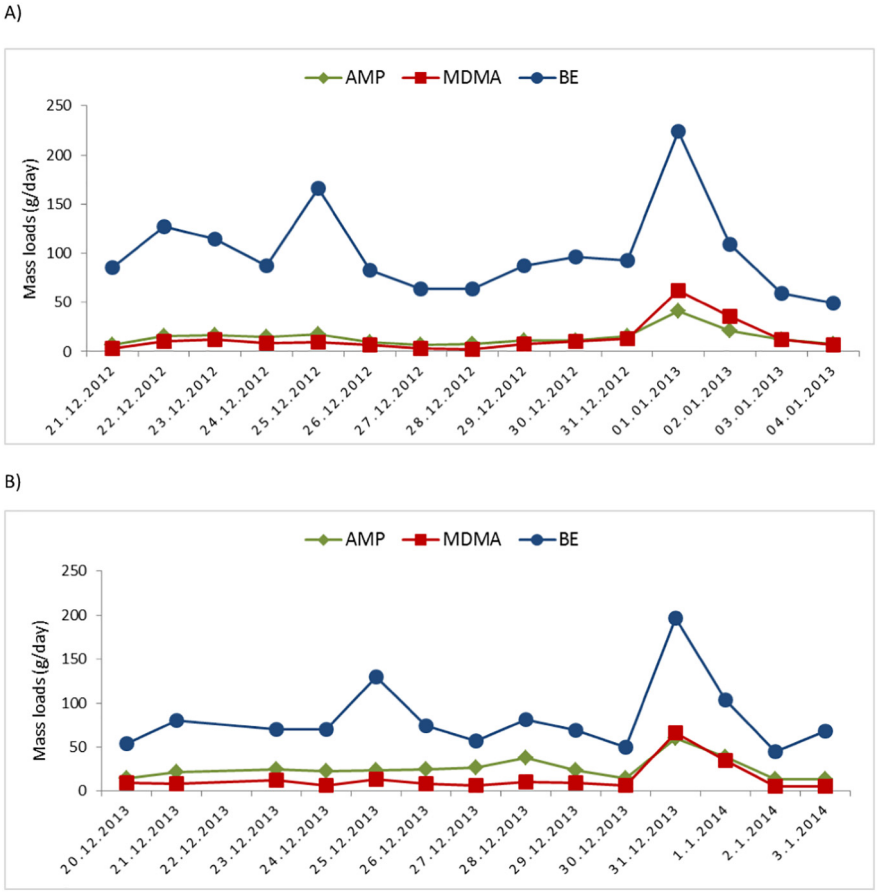

Fig. 2. Mass loads of BE, MDMA and AMP in two different Christmas-New Year holiday periods: A) 2012/2013 and B) 2013/2014.

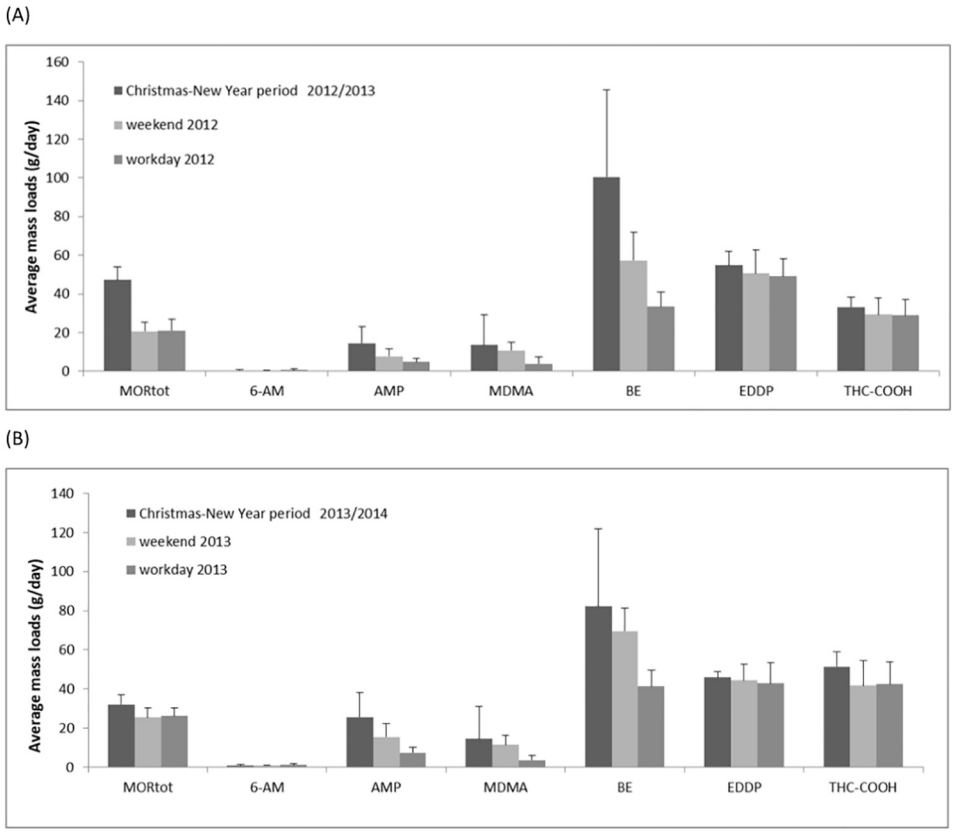

Fig. 3. Average mass loads of selected drug biomarkers determined on workdays, weekend and during two Christmas-New Year periods: A) 2012/2013 and B) 2013/2014. Error bars represent standard deviations.

summer, in 2009 and 2013. The results of this comparison are presented in Fig. 4. In both investigated years, the average summer mass loads of most of the investigated drug biomarkers were lower than those determined in early spring (Fig. 4). However the observed differences were statistically significant ($p < 0.05$; Mann-Whitney test) only for drug biomarkers which exhibit lower intra-week variability (e.g. MOR_{tot}, THC-COOH and EDDP) whereas they were not significant for the biomarkers of stimulating drugs (BE, MDMA), probably due to the comparatively higher intra-week variability. The lower average daily mass loads determined in summer are most likely associated with a disbalanced outward and inward population migrations during the summer vacation season. Namely, the contribution of tourists to the total city population is rather negligible throughout the year (<1%, data from Zagreb Tourist Board), whereas a significant percentage of residential population might be out of town during the peak of summer season. Unfortunately, this assumption cannot be confirmed since the official data related to the outward migrations of the city population were not available. Another possible factor which might have caused the observed differences in spring and summer mass loads is faster in-sewer drug biomarker degradation at higher temperatures (e.g. Devault et al., 2017). However, the model experiments which were performed with the wastewater from the city of Zagreb at 10 °C and 20 °C, indicated rather higher stability of all urinary biomarkers included in this research at the both temperature conditions (Senta et al., 2014). Since the in-sewer wastewater residence time in Zagreb is relatively short (<5 h) and a typical wastewater temperature in March and July/August is 12 °C and 20.5 °C, respectively, it is not very likely that the observed seasonal mass load differences were primarily caused by faster in-sewer degradation in summer. Although the reasons for the observed seasonal differences of the average mass loads are not yet fully understood, they indicated that the total drug consumption might be underestimated if extrapolated from the average daily mass loads determined in summer.

3.3. Impact of sampling scheme on the estimation of drug consumption in multiannual studies

Most of the previously conducted multiannual WBE studies were based on relatively short one-week sampling periods (e.g. Kankaanpää et al., 2016; Mastroianni et al., 2017; Ort et al., 2014a; Zuccato et al., 2016), which, due to the possible week-to-week variability of daily mass loads, may be associated with a potential error in tracking the drug consumption on an annual basis. In this study, a comparison was made between the representative average daily mass loads of selected drug biomarkers obtained by applying two different sampling schemes: one-week sampling scheme (March/April 2012–2016) and whole-year sampling scheme (Sundays and Tuesdays; sampled either fortnightly in 2012–2014 or monthly in 2015–2016). Based on the extended scheme of the whole-year sampling carried out in 2013 and 2014, which included fortnightly sampling ($n = 48$), it was shown that the reduction of the sample number to half (monthly sampling; $n = 24$) did not significantly affect the estimate of the mass loads (t-test; $p < 0.05$).

The representative daily mass loads of individual drug biomarkers determined by applying the one-week and the whole-year sampling scheme are presented in Fig. 5. Apart from some occasional exceptions, the application of the whole-year sampling scheme was, in principle, associated with somewhat higher day-to-day variability of daily mass loads than the one-week sampling scheme, which is probably a result of higher intra-annual variability of drug consumption. The amphetamine-type drugs (MAMP, AMP and MDMA) exhibited the strongest day-to-day variability within the both sampling schemes, which is most probably associated with a rather irregular consumption pattern of these drugs, characterized by enhanced weekend and holiday consumption rates. Furthermore, the one-week sampling scheme was occasionally associated with relatively high day-to-day variability of

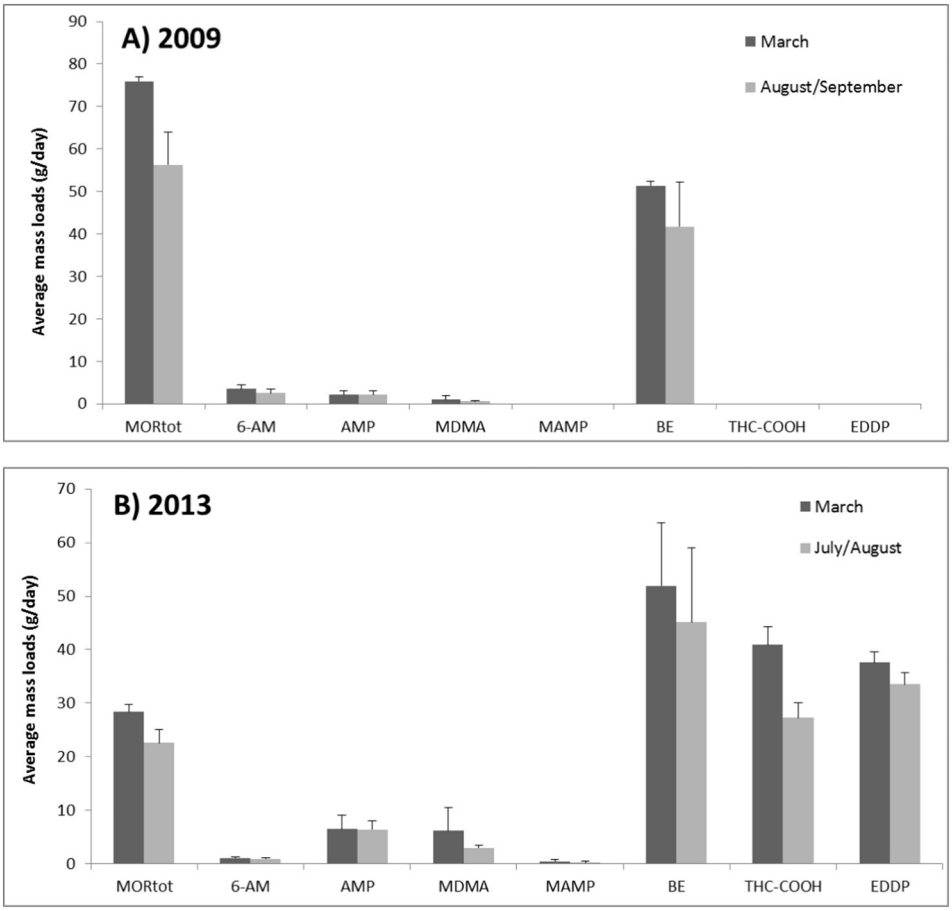

Fig. 4. Variability of average mass loads of selected urinary drug biomarkers in Zagreb during the spring and summer sampling week in A) 2009 and B) 2013. Error bars represent standard deviations.

AMP and MDMA. The statistical analysis of the data exhibited a significant difference ($p < 0.05$) between the representative mass loads of AMP obtained by the two applied sampling schemes in all investigated years. By contrast, the differences for other investigated drug biomarkers were found to be significant ($p < 0.05$) only occasionally. Previous study by Ort et al. (2014b) has shown that the variability of drug consumption in smaller communities (<10,000 inhabitants) is extremely high, requiring very high sampling frequency to achieve the proper estimate of drug consumption. It was estimated that the average annual consumption calculated from 1-week sampling was subject to approximately 60% relative error. In contrast, our study suggests that intra-annual variabilities in larger cities can be significantly smaller allowing detection of relatively small changes (20%) of the drug consumption among different years. Nevertheless, although some previous studies, addressing the issue of multiannual changes, demonstrated the applicability of one-week sampling scheme (Mastroianni et al., 2017; Ort et al., 2014a; Zuccato et al., 2016), our data show that such a scheme is insufficiently reliable for the drugs exhibiting high day-to-day and intra-annual variability, even in case of larger cities like the city of Zagreb.

3.4. Multiannual trends in drug consumption patterns and comparison with available epidemiological data

The back-calculations of drug consumption were based on representative daily mass loads determined for all samples collected within each investigated year, with the exception of those collected during the Christmas-New Year holiday seasons. The consumption was calculated for heroin, cocaine, AMP, MDMA, cannabis (THC) and methadone (MTHD). The results expressed in mg/day/1000 inhabitants of age 15–64 are presented in Fig. 6, whereas the results expressed in other units (e.g. mg/day/1000 inhabitants, doses/day/1000 inhabitants, g/day, kg/year, kg/year of street purity drug) are given in Supplementary Material (Table S2). The highest illicit drug consumption rate was determined for cannabis (from 7368 ± 2197 mg/day/1000 inhabitants 15–64 years to 19,544 ± 4624 mg/day/1000 inhabitants 15–64 years), followed by heroin (from 107 ± 104 mg/day/1000 inhabitants 15–64 years to 712 ± 193 mg/day/1000 inhabitants 15–64 years), cocaine (from 249 ± 27 mg/day/1000 inhabitants 15–64 years to 699 ± 121 mg/day/1000 inhabitants 15–64 years), MDMA (from 17 ± 7.5 mg/day/1000 inhabitants 15–64 years to 259 ± 69 mg/day/1000 inhabitants 15–64 years) and AMP (from 13 ± 8.8 mg/day/1000 inhabitants 15–64 years to 213 ± 61 mg/day/100 inhabitants 15–64 years). The estimated consumption rate of the therapeutic opioid methadone was in the range from 280 ± 26 mg/day/1000 inhabitants 15–64 years to 393 ± 61 mg/day/1000 inhabitants 15–64 years. In principle, the determined drug consumption patterns and rates were rather similar to those determined in some other Mediterranean countries, like Spain and Italy (Mastroianni et al., 2017; Zuccato et al., 2016), although some differences regarding the prevalence of individual drugs as well as regarding the temporal trends were observed. For example, cannabis and cocaine were the most prevalently consumed illicit drugs in Barcelona (Spain) and investigated Italian cities, whereas a heroin consumption was reported to be much lower (Mastroianni et al., 2017; Zuccato et al., 2016).

In our study, all investigated illicit drugs, except heroin, exhibited a significant increase ($p < 0.05$) of the consumption rates over the investigated 8-year period (Figs. 6 and 7, Table S2). In 2016, the average

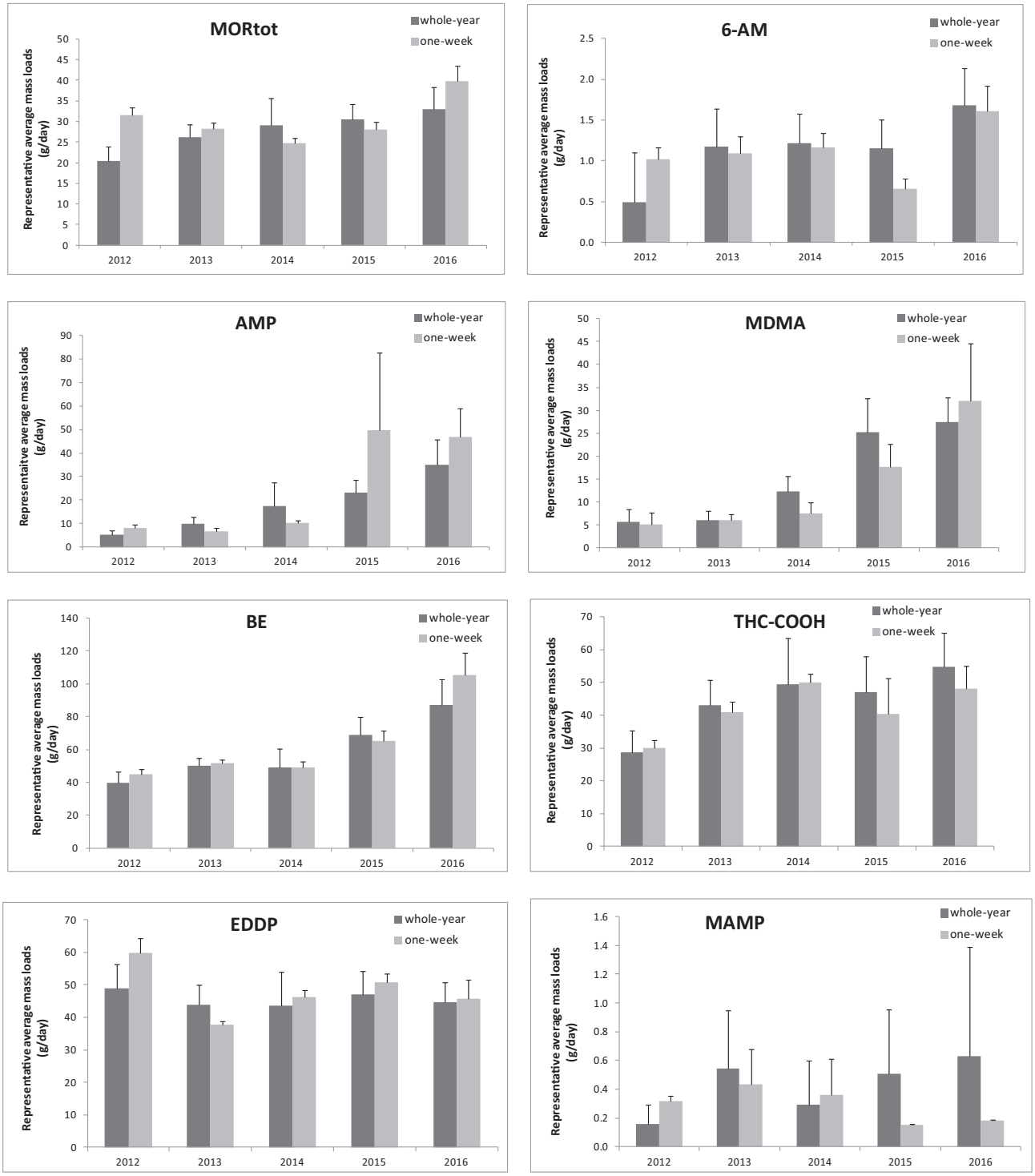

Fig. 5. Impact of the selected sampling schemes (whole-year and one-week monitoring) on the determination of representative mass loads. Error bars represent standard deviations.

consumption rate of pure MDMA, AMP, THC (cannabis) and cocaine, were 15-fold, 16-fold, 3-fold and 2-fold higher than in 2009, respectively. The multiannual consumption patterns of pure AMP and MDMA were characterized by a rather continuing increase of their consumption rates (Fig. 6) over the whole investigated time period, whereas the consumption of THC (cannabis) was characterized by a significant increase in 2009–2014 period ($p < 0.05$, 3-fold increase), and rather stable consumption rate in 2014–2016 period. By contrast, the consumption rate of pure heroin dropped significantly ($p < 0.05$; 5–7-fold) between 2009 and 2011–2012 period, and kept at significantly lower level until 2016 ($p < 0.05$). However, a significant ($p < 0.05$) 2–3-fold increase in pure heroin consumption was recorded between 2011/2012 and 2016, which indicated a gradual recovery of heroin market in that period. Interestingly, a reduction of heroin consumption in the period 2010–2012 was reported for Italy as well (Zuccato et al., 2016).

Based on the estimated amounts of consumed drugs and the official data on purity of seized drugs provided by the Office for Combating

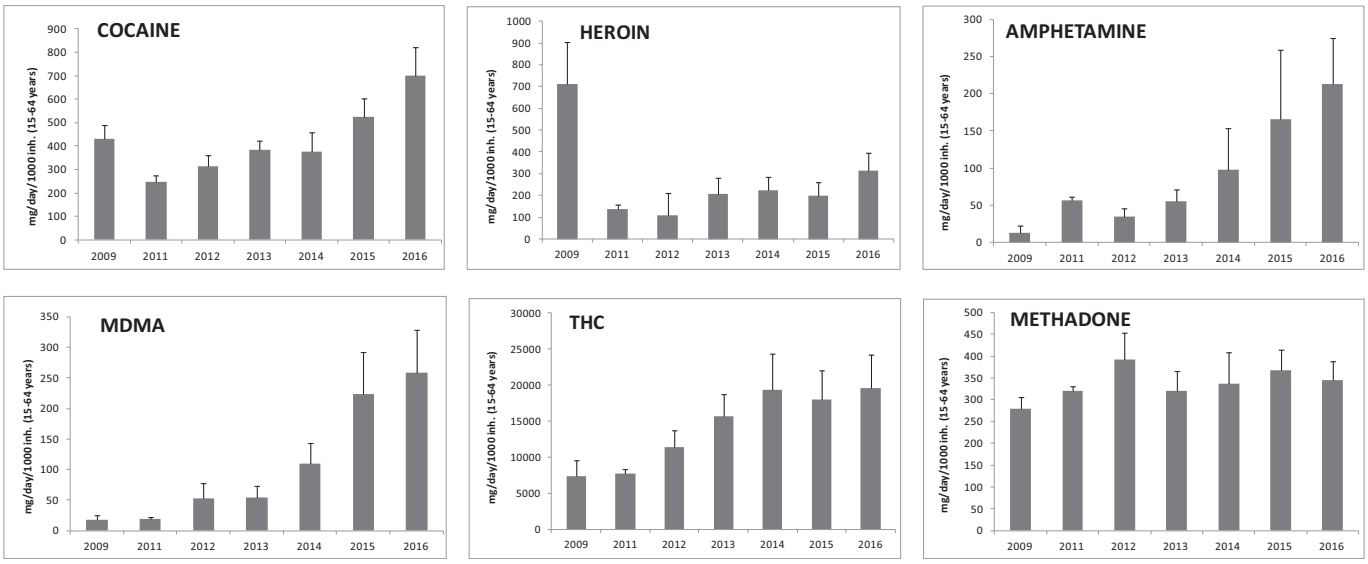

Fig. 6. Consumption of cocaine, heroin, MDMA, amphetamine, THC and methadone in the city of Zagreb in the period 2009–2016. Error bars represent standard deviations.

Narcotic Drug Abuse of the Government of the Republic of Croatia (see Table S1), we calculated the amounts of the street-purity drugs which circulated on the illegal market in Zagreb in the corresponding years (Table S2). It should be stressed that the street-drug purity of investigated drugs (heroin, amphetamine, MDMA, cocaine) exhibited a pronounced temporal variability (Table S2). The amounts of the most

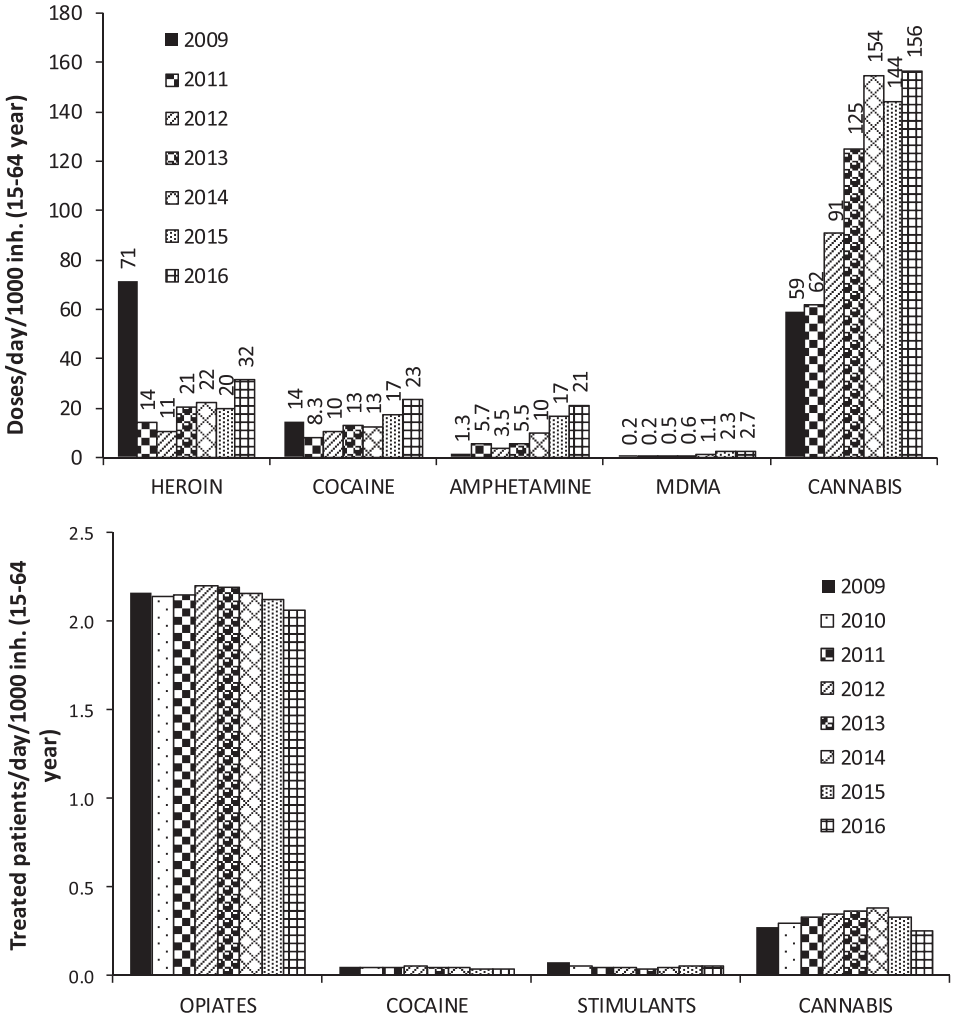

Fig. 7. Comparison of estimated drug consumption in the city of Zagreb with available epidemiological data for Croatia in the period of 2009–2016. Stimulants in the epidemiological figure include amphetamine-type drugs. Opiates include heroin and morphine.

prevalent drugs present on the illegal market in Zagreb were as follows: from 211 to 565 kg/year of heroin, from 157 to 323 kg/year of cocaine, from 44 to 309 kg/year of amphetamine, from 14 to 127 kg/year of MDMA and from 22,853 to 53,988 kg/year of cannabis.

Consequently, the observed multiannual trends in the consumption of pure drugs are probably not impacted exclusively by the changes in drug consumption prevalence but also by the changes in the street drug purity. In this context, it is interesting to note that a significant drop in the heroin consumption rate between 2009 and 2011/2012 was associated with a concomitant decrease of heroin street-drug purity (from 21.5% to 8.4%) and an increase in the consumption of the substitution therapy drug methadone (40%), which then kept a rather stable consumption rate in the subsequent period (2013–2016). The average number of consumed methadone doses estimated in this study (e.g. 3.1 ± 0.4 doses/day/1000 inhabitants in 2015; 80 mg/dose) were in a rather good agreement with the amount of that drug prescribed in the city of Zagreb in 2015 (11.76 DDD/TSD; DDD = 25 mg; 3.7 doses/day/1000 inhabitants for the average dose of 80 mg/L) (Draganic et al., 2017), which confirmed a reliability of WBE approach for tracking the changes of the illicit drug consumption patterns.

The trends in population normalized number of addicts treated due to consumption of different types of drugs did not, however, reflect the multiannual drug consumption trends determined in this study (Fig. 7), probably due to a rather long time-gap between the initial drug consumption and the involvement of the consumers in the treatment.

Furthermore, the drug consumption trends which were determined in the present study were only partially in agreement with the results of general population surveys performed in Croatia in 2011 and 2015, which indicated a significant increase only in the consumption of cannabis (2.9% last-month prevalence in 2011; 5.0% last-month prevalence in 2016) (Glavak Tkalic et al., 2013; Glavak Tkalic et al., 2016), whereas the differences in the consumption prevalence of other illicit drugs were not found to be significant. Our study suggests that the outcome of national population surveys on drug consumption is not necessarily representative for larger cities. Given the fact that the city of Zagreb represents approximately 20% of the whole Croatian population, the drug consumption trends determined in this study imply the need for specific surveys focusing on larger cities. Moreover, the trends observed in the city of Zagreb might be an indication of some trends developing at the national level.

4. Conclusion

The eight-year monitoring period of drug consumption patterns in the city of Zagreb, Croatia, using wastewater-based epidemiology revealed several temporal variability patterns, including weekday-weekend dynamics, holiday season effects and multiannual trends. In agreement with the literature, the enhanced consumption of stimulating drugs was systematically observed during weekends and Christmas holiday season. In addition, a significant multiannual increase of cocaine (1.6-fold), THC (2.7-fold), amphetamine (16-fold) and MDMA (15-fold) consumption with a concomitant decrease (2.3-fold) of the consumption of heroin was observed during the investigated 8-year period (2009–2016). The whole-year sampling scheme showed a clear advantage over the one-week sampling scheme, especially for drugs showing enhanced day-to-day and intra-annual variability. The errors associated with day-to-day and intra-annual variability of BE (<20%) determined in the city of Zagreb (>500,000 inhabitants) study were much smaller from those reported for small communities (Ort et al., 2014b), which indicated enhanced robustness of the estimates obtained for large sized cities. Our data suggest that large sized cities can provide a basis for a reliable detection of relatively small changes in drug consumption over a multi-year period. Consequently, the trends observed in the larger cities could be used as an early warning of the trends developing at the national level.

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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.2018.07.441.

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