

1 **Assessing the impact of a major electronic music festival on the consumption patterns of**
2 **illicit and licit psychoactive substances in a Mediterranean city using wastewater analysis**

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Highlights

- Psychoactive substance consumption patterns studied using a multiparametric approach
- The electronic music festival contributed to an increase of stimulants and alcohol use
- A 30-fold increase was observed for MDMA
- The impact of the festival on other drugs, tobacco and opioid use was negligible
- WBE-based estimates agree with national surveys, except for heroin and amphetamines

21 **Abstract**

22 The consumption patterns of five categories of psychoactive substances (PS), including
23 “conventional” illicit drugs, new psychoactive substances (NPS), therapeutic opioids, alcohol and
24 nicotine, were studied in the city of Split, Croatia, using wastewater-based epidemiology (WBE), with
25 an emphasis on the impact of a large electronic music festival. The study involved the analysis of 57
26 urinary biomarkers of PS in raw municipal wastewater samples collected in three characteristic
27 periods, including the festival week in the peak-tourist season (July) and reference weeks in the
28 peak-tourist season (August) and the off-tourist season (November). Such a large number of
29 biomarkers allowed the recognition of distinct patterns of PS use associated with the festival, but
30 also revealed some subtle differences between summer and autumn seasons. The festival week was
31 characterized by markedly increased use of illicit stimulants (MDMA: 30-fold increase; cocaine and
32 amphetamine: 1.7-fold increase) and alcohol (1.7-fold increase), while consumption of other
33 common illicit drugs (cannabis and heroin), major therapeutic opioids (morphine, codeine and
34 tramadol) and nicotine remained rather constant. Interestingly, NPS and methamphetamine clearly
35 contributed to the festival PS signature in wastewater, but their prevalence was rather low
36 compared to that of common illicit drugs. Estimates of cocaine and cannabis use were largely
37 consistent with prevalence data from national surveys, whereas differences were found for typical
38 amphetamine-type recreational drugs, particularly MDMA, and for heroin. The WBE data suggest
39 that the largest proportion of morphine came from heroin consumption and that the percentage of
40 heroin users seeking treatment in Split is probably rather low. The prevalence of smoking calculated
41 in this study (30.6%) was consistent with national survey data for 2015 (27.5 – 31.5%), while the
42 average alcohol consumption per capita >15 years (5.2 L) was lower than sales statistics suggest (8.9
43 L).

44

45 **Keywords:** Cotinine, Ethyl sulfate, Illegal drugs, Sewage epidemiology, Substances of abuse,
46 Wastewater-based epidemiology, Tobacco

47 **1. Introduction**

48 The use of psychoactive substances (PS), such as illicit drugs, alcohol and tobacco, poses a significant
49 health risk and can cause numerous public health, criminal justice and social service problems.
50 According to the United Nations Office on Drugs and Crime, 284 million people worldwide used
51 drugs in 2020 (UNODC World Drug Report 2022). The European Monitoring Centre for Drugs and
52 Drug Addiction (EMCDDA) estimates that more than 83 million (29%) of adults aged 15 – 64 in the
53 EU have used illicit drugs at least once in their lives (European Drug Report 2022). An additional risk
54 comes from the large number of poorly regulated new psychoactive substances (NPS) that have
55 appeared on the drug market in recent years. At the end of 2021, the EMCDDA was monitoring
56 around 880 NPS (European Drug Report 2022). On the other hand, alcohol and tobacco are the most
57 commonly used legal PS in the world and cause many deaths every year. The World Health
58 Organization (WHO) estimates that excessive alcohol consumption is responsible for 5.1% of the
59 global burden of disease and 5.3% of all deaths in the world (WHO, 2022a). *Per capita* alcohol
60 consumption in Croatia is slightly lower than the EU average (WHO, 2016), but the country has one
61 of the highest rates of everyday alcohol consumers (RARHA, 2016) and an increasing trend in
62 consumption among young people (ESPAD Group, 2020). Tobacco use causes up to 8 million deaths
63 and numerous health problems worldwide each year (WHO, 2022b), and its prevalence in Croatia is
64 one of the highest in the EU (Croatian Employers' Association, 2021).

65 Illicit drug use is usually assessed by surveys, seizure data and treatment program statistics, whereas
66 alcohol and tobacco use can be estimated from surveys or sales data. However, these databases
67 tend to be incomplete, and their accuracy and reliability can be compromised by biased reporting. In
68 addition, spatial and temporal patterns of consumption are often missing. Therefore, a
69 complementary approach, based on wastewater-based epidemiology (WBE), is becoming
70 increasingly important for estimating illicit drug and NPS use (González-Mariño et al., 2020; Bijlsma
71 et al., 2020; Zuccato et al., 2008) and, more recently, the use of legal PS, such as alcohol (López-
72 García et al., 2020; Ryu et al., 2016) and nicotine (Castiglioni et al., 2015; Montes et al., 2020).

73 WBE has been used to study drug abuse in the general population (e.g., González-Mariño et al.,
74 2020; Terzic et al., 2010) and, to a lesser extent, in specific communities, such as schools (Zuccato et
75 al., 2017) and prisons (Postigo et al., 2011). In addition, several WBE studies have focused on the
76 impact of special events on PS consumption, including music and school graduation festivals (Bade et
77 al., 2020; Benaglia et al., 2020; Bijlsma et al., 2020; Brett et al., 2022; Devault et al., 2019; Jiang et al.,
78 2015; Lai et al., 2013b; Mackuľak et al., 2019), holidays (Foppe et al., 2018; Krizman-Matasic et al.,
79 2019; Lai et al., 2013a), city festivals (Andrés-Costa et al., 2016; Causanilles et al., 2017), carnivals
80 (Sodré et al., 2022) and sporting events (Gerrity et al., 2011; Sodré et al., 2017).

81 Although the impact of music festivals on drug use has been investigated by several authors, the
82 studies conducted so far have mostly focused on a rather limited number of substances, such as
83 “conventional” illicit drugs (Benaglia et al., 2020; Bijlsma et al., 2014; Mackuľak et al., 2014), while
84 relatively few studies included some therapeutic opioids and/or NPS (Bade et al., 2020; Bijlsma et al.,
85 2020; Brandeburová et al., 2020; Brett et al., 2022; Jiang et al., 2015; Lai et al., 2013b; Mackuľak et
86 al., 2019), alcohol and/or tobacco (Bade et al., 2020; Brandeburová et al., 2020; Mackuľak et al.,
87 2015). However, none of them simultaneously studied a broad range of PS, including the main
88 representatives of common illicit drugs along with a wide range of NPS, therapeutic opioids, alcohol
89 and nicotine biomarkers during the same music festival.

90 Therefore, the main objective of this work was to investigate the impact of a major electronic music
91 festival, as well as summer tourist season, on the consumption patterns of a broad range of target
92 PS, including 57 urinary biomarkers, in the city of Split, a popular tourist destination on the Adriatic
93 coast with about 210,000 inhabitants, which has seen a rapid increase in the number of visitors in
94 recent years. The city hosts one of the largest electronic music festivals in Europe (Ultra Europe),
95 which is attended by many visitors from all over the world. Moreover, Split is one of the Croatian
96 cities with the highest rate of registered drug addicts (Katalinić and Huskić, 2017), so one of the
97 objectives of the study was to assess PS use among the city's permanent residents and compare
98 these data with traditional epidemiological surveys.

99 2. Materials and methods

100 2.1. Chemicals and materials

101 The study included 57 urinary biomarkers excreted after the consumption of a broad range of illicit
102 and licit PS classified into five categories, including “conventional” illicit drugs, new psychoactive
103 substances (NPS), therapeutic opioids, alcohol, and nicotine (Table 1). Standard stock solutions of
104 illicit drugs, NPS and therapeutic opioid biomarkers, as well as the isotopically labeled analogues,
105 used as internal standards (IS), were obtained from Lipomed AG (Switzerland), whereas the
106 standards of alcohol and nicotine biomarkers (including their IS) were from Santa Cruz Biotechnology
107 (CA, USA). LC grade methanol, acetonitrile and ammonia solution (25% in water) were purchased
108 from Merck (Germany). Ammonium acetate, sodium acetate, phosphoric acid (85%) and formic and
109 acetic acids (both LC-MS grade) were obtained from Fluka (Switzerland). Tetrabutylammonium
110 bromide (TBA) and the enzyme β -glucuronidase (from *Helix pomatia*, type H2) were provided by
111 Sigma-Aldrich (Germany). Milli-Q water generators (Merck Millipore, Germany) were used to
112 produce ultrapure water. Oasis MCX cartridges (150 mg/6 mL) were purchased from Waters (USA),
113 whereas Strata NH₂ cartridges (200 mg/3 mL) and HPLC columns (Synergi Polar 4 μ m, 150 mm \times 3
114 mm; Kinetex PFP 2.6 μ m, 100 mm \times 2.1 mm; Synergi Fusion RP 4 μ m, 100 mm \times 2.0 mm) were
115 manufactured by Phenomenex (CA, USA). Glass fiber filters (1.2 μ m – GF/C and 0.7 μ m – GF/A) were
116 purchased from Whatman (UK), while 0.22 μ m nitrocellulose filters were supplied by Merck
117 Millipore.

118

119 2.2. Collection of wastewater samples

120 The 24-hour composite samples of raw wastewater (influent) were collected from the two main
121 wastewater collectors (Stupe and Katalinica Brig), which serve more than 90% of the population of
122 the city of Split, Croatia, with approximately 210,000 inhabitants. Samples were collected using
123 automatic sampling devices working in time-proportional mode (time interval: 15 min) with
124 sampling storage at 5 – 8 °C. The sampling scheme included the sample collection on 7 consecutive

125 days, from 8 am to 8 am of the following day, in three distinctive periods of 2016: A) music festival
126 week during the peak-tourist season: July 13 – 19, B) peak-tourist reference week without special
127 events: August 24 – 30, and C) off-tourist reference week: November 9 – 15. After sampling, samples
128 were immediately frozen and stored at -20 °C until analysis.

129

130 *2.3. Sample preparation and instrumental analysis*

131 Previously developed and validated analytical methods were used to determine biomarkers of illicit
132 drugs, NPS and therapeutic opioids (Krizman-Matasic et al., 2018; Senta et al., 2015, 2013). In all
133 methods, 125 mL of raw wastewater was spiked with IS (120 ng L⁻¹). After equilibration (15 min),
134 samples were filtered (GF/C filter), acidified to pH 2 with phosphoric acid and enriched on Oasis MCX
135 cartridges. Final extracts were analyzed by liquid chromatography – tandem mass spectrometry (LC-
136 MS/MS) on a Surveyor HPLC system coupled to a TSQ AM triple quadrupole mass spectrometer
137 (Thermo Electron, CA, USA) using an electrospray ionization (ESI) source. Multiple Reaction
138 Monitoring (MRM) mode was used for the detection and quantification of the target compounds.
139 For the determination of THC-COOH, a Kinetex PFP column (100 x 2.1 mm; 2.6 µm) and negative
140 ionization polarity were used, while all other illicit drugs, as well as NPS and therapeutic opioids,
141 were separated on a Synergi Polar-RP column (150 x 3 mm; 4 µm) and analyzed in positive ionization
142 polarity. Note that the method for NPS includes EPH and PEPH, which are legal compounds
143 chemically related to the target NPS.

144 Alcohol and nicotine biomarkers were determined by direct injection of the filtered wastewater
145 samples, which were first passed through GF/A and then through nitrocellulose filters. For nicotine
146 biomarker analysis, the method developed by Rodríguez-Álvarez et al. was used (Rodríguez-Álvarez
147 et al., 2014b). Briefly, filtered samples (1.14 mL) were acidified to pH 5 with 0.15 mL of sodium
148 acetate buffer, spiked with 300 units of β-glucuronidase and IS (20 µg L⁻¹) and heated at 37 °C for 5 h.
149 After deconjugation, extracts were analyzed by LC-MS/MS using a Varian HPLC system (CA, USA)
150 coupled to a Varian 320-MS triple quadrupole mass spectrometer equipped with an ESI source.

151 Separation was performed on a Synergi Fusion RP column (100 x 2 mm; 4 μm), and MS/MS analysis
152 in MRM mode in positive ionization polarity. The alcohol biomarker ethyl sulfate (EtS) was
153 determined according to the method developed by Rodríguez-Álvarez et al. (Rodríguez-Álvarez et al.,
154 2014a). Filtered samples (1.5 mL) were spiked with IS (10 $\mu\text{g L}^{-1}$) and TBA (50 mM) and analyzed by
155 LC-MS/MS using the same HPLC-MS system applied for nicotine biomarkers determination. A Synergi
156 Fusion-RP column (100 x 2 mm; 4 μm) was used for separation, while MRM mode in negative
157 ionization polarity was used for detection and quantification.

158 Analyses of all target biomarkers included in this study were performed within three months of the
159 last sampling campaign and each analytical batch included quality control samples.

160

161 *2.4. Estimation of drug consumption*

162 The estimation of PS consumption was based on the methodology proposed by Zuccato et al.
163 (Zuccato et al., 2008). The daily mass load of the selected urinary biomarker was calculated by
164 multiplying its concentration by the daily volume of wastewater entering the collector. When the
165 concentration of the biomarker was below the detection limit (LOD), the value of LOD/2 was used to
166 calculate the mass loads for statistical analysis. Mass loads were determined separately for each
167 collector and then summed to obtain the total load for the entire city of Split. Total loads were
168 normalized to population by dividing the mass load by the total number of residents and tourists in
169 each period. The average number of tourists in the city of Split, based on official data, is shown in
170 Fig. S1.

171 For those PS for which reliable excretion data were available from the literature, consumption was
172 estimated by multiplying biomarker mass loads by the appropriate correction factors compiled in
173 Table S1. Illicit drug and MTHD use was also calculated as the number of doses consumed per 1000
174 inhabitants, using the corresponding average doses in Table S1.

175 Nicotine consumption was estimated separately from COT and HCOT loads. Since nicotine
176 consumption was fairly similar with both biomarkers, the average value was assumed to be

177 representative. The number of cigarettes smoked was estimated assuming an average intake of 0.8
178 mg nicotine per cigarette (St.Charles et al., 2010). Alcohol consumption was also expressed in liters
179 of pure alcohol per 1000 inhabitants, taking into account ethanol density (0.789 kg L⁻¹).
180 The WBE-based prevalence for illicit drugs was calculated assuming that each person consumes one
181 dose per day, with the exception of heroin (3 doses per day) (Reichman et al., 1973), while the
182 smoking prevalence was calculated assuming that the average number of cigarettes smoked daily
183 among regular users in Croatia is 15.9 (Dečković Vukres et al., 2015).

184

185 *2.5. Statistical analysis*

186 Statistica 7.0 (StatSoft Europe, Germany) was used for statistical analysis, which was performed
187 using the population-normalized mass loads. Because of the relatively small sample size per period
188 ($n=7$), non-parametric tests with a 95% confidence level were used. The Kruskal-Wallis test was used
189 to compare the three periods. In case of significant differences, the Mann-Whitney two-tailed U test
190 was additionally used to compare periods in pairs. Hierarchical cluster analysis and principal
191 component analysis (PCA) were performed with normalized data sets to avoid bias due to the
192 extremely different concentration ranges of PS. Hierarchical cluster analysis was performed using 1-
193 Person r as the distance measure and unweighted pair-group average as the clustering algorithm.

194

195 **3. Results and discussion**

196 *3.1. Occurrence of target urinary biomarkers and their mass loads in wastewater*

197 Concentrations of the target compounds quantified in this study varied in a rather wide range, from
198 1.2 ng L⁻¹ to 34 µg L⁻¹. Of the 57 urinary biomarkers of PS included in the study, 30 were found at
199 concentrations higher than the LOQ (typically about 1 ng L⁻¹, corresponding to an excretion of less
200 than 0.1 g day⁻¹ for the entire city of Split). Taking into account the considerable population of the
201 city of Split (about 210,000 inhabitants), this indicates that the methodology used allows for rather
202 sensitive detection of possible PS use. The complete concentration data set for measurable PS

203 biomarkers is given in the Supplementary Material (Tables S2 – S7). It is interesting to note that both
204 the concentrations and the composition of biomarkers in the two wastewater collectors (Stupe and
205 Katalinica Brig) were rather similar. The most prominent representatives of illicit drug biomarkers
206 were amphetamines (MDMA, AMP and MAMP), followed by COC (BE and COC), HER (MOR, M3G,
207 M6G and norMOR) and cannabis (THC-COOH) (Tables S2 and S3). The detection frequency and
208 concentrations of NPS were generally much lower. In fact, of the 22 target NPS and chemically
209 related compounds, only five (KET, norKET, 4-FA, MDA and PEPH) were found in measurable
210 concentrations (Table S4), indicating their rather low consumption prevalence. This is consistent
211 with the qualitative analyses of PS in the urine samples collected from the stadium chemical toilets
212 during the Ultra Festival (Sutlović et al., 2021). The concentrations of biomarkers of common opioid
213 analgesics, such as TRA, O-TRA, N-TRA, COD, MET and EDDP, were much higher than those of BUP,
214 DMOR, SFNT, PP, OC and norHC (Tables S5 and S6). As expected, alcohol and nicotine biomarkers
215 were detected in all samples and reached low $\mu\text{g L}^{-1}$ concentrations (Table S7).

216 The daily mass loads of the measurable PS biomarkers are listed in Table S8, while Figure S2 show
217 the variability of individual biomarkers during the sampling weeks. Based on their weekly dynamics,
218 they can be divided into two main groups. Most PS biomarkers showed relatively weak variability
219 within the week, while biomarkers of stimulants (MDMA, AMP, BE and COC) often showed an
220 increase on weekends compared to weekdays. This feature has already been described in numerous
221 studies (e.g. Bijlsma et al.; 2014; Krizman et al., 2016; Mackuľak et al., 2014; Terzic et al., 2010;) and
222 will not be discussed in detail here. The lack of systematic variability during the week for biomarkers
223 of HER and cannabis, therapeutic opioids and nicotine is also consistent with previous studies (e.g.,
224 Krizman et al., 2016; Terzic et al., 2010; Rice et al., 2020; Montes et al., 2020), although occasional
225 exceptions have been observed at specific sites (Mackuľak et al., 2016; van Wel et al., 2016). In
226 contrast, elevated weekend mass loads were observed for the alcohol biomarker EtS, consistent with
227 other WBE studies (e.g., Brandeburová et al., 2020; López-García et al., 2020; Ryu et al., 2016; van
228 Wel et al., 2016).

229

230 *3.2. Seasonal consumption patterns and the impact of an electronic music festival*

231 To investigate the possible differences between the three sampling periods, the population-
232 normalized daily mass loads were subjected to additional statistical analysis using hierarchical cluster
233 analysis. Results are shown in Fig. 1. As it can be seen, the analysis provided almost perfect
234 separation of samples by sampling period and confirmed remarkable differences in biomarker mass
235 loads between the festival week and the two reference weeks. Moreover, a more subtle difference
236 between the summer and autumn reference weeks was also clearly reflected in the dendrogram.
237 This suggests a high-resolution power of the multiparametric approach as well as the
238 appropriateness of the sampling strategy used in this study.

239 A summary of population-normalized daily mass loads for individual PS biomarkers is shown in Table
240 2. Mass loads revealed significant differences between PS use patterns in different periods. The
241 electronic music festival had a dramatic impact on population-normalized MDMA loads, which
242 increased 30-fold compared with the off-tourist reference week and 15-fold compared with the
243 peak-tourist reference week, reaching 610 g day^{-1} ($2.7 \text{ g day}^{-1} 1000 \text{ inhabitants}^{-1}$). Mass loads of
244 AMP, MAMP and BE were also higher in the festival week, but the differences were much less
245 pronounced (up to 1.7-fold increase for AMP and BE, and up to 2.2-fold increase for MAMP) and, in
246 most cases, not statistically significant. Elevated loads of MDMA, AMP and BE during music festivals
247 have already been reported (Benaglia et al., 2020; Bijlsma et al., 2020, 2014; Lai et al., 2013b;
248 Mackuřak et al., 2019, 2014), but not to the extent found in this study. Mass loads of stimulant drugs
249 in the peak-tourist reference week showed a slight increase compared to the off-tourist reference
250 week, which is consistent with our study in the city of Zadar, another popular tourist destination on
251 the Adriatic coast (Krizman et al., 2016), and some other studies in vacation areas (Lai et al., 2013a).
252 In contrast, population-normalized mass loads of cannabis (THC-COOH) and HER biomarkers (MOR
253 and its metabolites) showed no increasing trend during the festival. In fact, the loads of THC-COOH
254 in the festival week were significantly lower ($p < 0.05$) than in the two reference weeks. This came as

255 a surprise since some previous studies reported a 10-fold increase of THC-COOH during a pop/rock
256 festival in Portugal (Bijlsma et al., 2020). However, such trend was not found in other studies (Lai et
257 al., 2013b; Mackuľak et al., 2014), which could be related to the type of music festival. In fact,
258 cannabis use has been reported to increase primarily at pop/rock festivals (Mackuľak et al., 2019),
259 which might explain the markedly different use pattern observed in our study (electronic music
260 festival). Moreover, in this study, THC-COOH loads during the peak-tourist season were slightly but
261 significantly lower than in the off-tourist reference week, which is also consistent with our previous
262 study in Zadar (Krizman et al., 2016) and the study by Lai et al. (Lai et al., 2013a).

263 Mass loads of NPS were generally much lower than those of “conventional” illicit drugs, suggesting
264 that their use is not yet as widespread in the region. The highest individual load, reaching 16 g day^{-1}
265 ($71 \text{ mg day}^{-1} 1000 \text{ inhabitants}^{-1}$), was estimated for MDA. However, this compound was detected in
266 only three samples collected on festival days and most likely reflects MDMA use, since 1% of MDMA
267 consumed is excreted from the human body as MDA (Baselt, 2008). Another amphetamine-like drug,
268 4-FA, was also quantified in some samples collected during the festival week, but its loads were
269 comparatively lower. The mass loads of KET biomarkers (KET and norKET) were also rather low, in
270 line with those reported in larger Italian cities (Castiglioni et al., 2015). However, a significant
271 increase was observed during the festival week ($p < 0.05$). The relatively low detection frequency and
272 mass loads of NPS, especially during “regular” periods without special events, suggesting low
273 consumption in Split, are consistent with national survey data on lifetime prevalence of NPS use in
274 the general population (Glavak Tkalić, 2018). Nevertheless, it should be stressed that only a small
275 percentage of possible NPS was included in our study and it cannot be ruled out that some common
276 NPS, such as N-ethylpentylone, 4-chloromethcathinone, 3-fluorophenmetrazine and 3-
277 methylmethcathinone, remained undetected.

278 Mass loads of PEPH were much higher than those of the other chemically closely related NPS from
279 the list (Table 1). This is not surprising, as this compound is a common ingredient in some popular
280 over-the-counter medications used to reduce nasal congestion and other symptoms of flu, colds,

281 and similar illnesses. This may also explain the fact that the highest PEPH loads were found in
282 November, when use of decongestants is higher than in summer.

283 With the exception of MOR, for which 85 – 90% has been shown to be derived from HER
284 consumption (Krizman et al. 2018), the highest mass loads of therapeutic opioid biomarkers were
285 determined for TRA and its metabolites O-TRA and N-TRA, COD, MTHD and its metabolite EDDP. In
286 addition, several minor opioid biomarkers, including BUP, PP, SFNT, norHC, OC and DMOR, were
287 found at very low levels (mostly below 1 g day⁻¹). TRA and COD are used to treat moderate to severe
288 pain in adults, and therefore it is not surprising that the mass loads of their biomarkers were
289 relatively high, consistent with some previous studies (Mackul'ak et al., 2016; Rice et al., 2020).
290 MTHD and BUP are mainly used to treat HER addicts. In fact, about 85% of registered HER addicts in
291 Croatia receive substitution therapy based on MTHD or BUP (Katalinić and Huskić, 2017). Mass loads
292 of MTHD and EDDP were much higher than mass loads of BUP and norBUP, which could be mainly
293 due to the different dosing and/or pharmacokinetics.

294 The population-normalized mass loads of the alcohol biomarker EtS were significantly higher in both
295 summer weeks than in the off-tourist reference week. The increase was especially pronounced
296 during the festival week (up to 1.7-fold). Increased alcohol consumption was also observed during a
297 dance music festival and a multicultural festival in Slovakia (Brandeburová et al., 2020). In contrast,
298 mass loads of both nicotine biomarkers were similar in the three periods, indicating rather constant
299 tobacco use without clear seasonal trends. Significantly higher cotinine loads were observed during
300 rock/metal and dance festivals in Slovakia, but not during a folk festival in the Czech Republic
301 (Mackul'ak et al., 2015).

302 To better understand the factors that influence PS usage patterns in different periods, the collected
303 data set was further analyzed using PCA. Results are shown in Fig. 2. and Fig. S3. The first three
304 factors (principal components) describe 72% of the total variance with principal components 1, 2
305 and 3 describing 36%, 25% and 11% of the total variance, respectively. Projection of the variables
306 (i.e., mass loads of PS biomarkers) to the factor-plane (1 x 2) shows that the variables with strong

307 positive correlation factors that contribute most to the variance associated with PC1 include
308 biomarkers of major stimulants (MDMA, COC, AMP), but also of minor stimulant PS (MAMP, KET), as
309 well as some opioid analgesics (norHC, OC and DMOR) and alcohol (Fig. 2). In fact, these compounds
310 represented the specific signature of increased PS use during the festival week, so PC1 can be
311 attributed to the predominant impact of the festival. Interestingly, the highest negative correlation
312 with factor 1 was found for THC-COOH, PEPH, MOR glucuronides (M3G and M6G) and opioid
313 addiction treatment drugs (MTHD and BUP), consistent with slightly lower mass loads of these
314 biomarkers during the festival. The third group, clearly separated by PCA in the factor-plane (1 x 2),
315 included mainly biomarkers of therapeutic opioids (TRA, COD, EDDP) and nicotine, which contribute
316 little to the variance of PC1, but are positively related with PC2. The projection of the sampling days
317 to the factor-plane (1 x 2) confirms the clear separation of the corresponding data sets, with the
318 main festival days (17-07 and 18-07) being the most distant points on the graph with respect to the
319 off-tourist reference week. The projection of the variables to the factor plane (2 x 3) shows much
320 weaker correlations of the analyzed PS biomarkers with the PC3 (Fig. S3). Only a few compounds,
321 including M3G, M6G and PEPH, showed moderate positive correlation. These signatures most closely
322 describe the PS usage pattern in the autumn.

323

324 *3.3. Assessment of psychoactive substance use and comparison with conventional epidemiological* 325 *data*

326 Data obtained by WBE and conventional epidemiological methods are rarely directly comparable.
327 The WBE approach provides objective data on collective drug use, but it is difficult to assess
328 prevalence. In contrast, conventional methods, such as surveys, can provide information on
329 prevalence, but reliable quantitative data on use are not easily obtained. Nevertheless, general
330 patterns and trends in drug use can be better understood when two complementary approaches are
331 combined. WBE-based consumption was estimated for all “conventional” illicit drugs, MTHD, alcohol
332 and nicotine. As mentioned in the previous sections, the consumption of PS determined during the

333 festival week cannot be considered representative for permanent residents. Therefore, to avoid the
334 impact of the festival, consumption estimates were based on the data from the two reference
335 weeks. Consumption of selected PS, expressed in mg/g/kg per day per 1000 inhabitants, is shown in
336 the Supplementary Material (Table S9), and results for illicit drugs are additionally expressed as the
337 number of doses consumed per day per 1000 inhabitants aged 15 – 64 (Table 3), to allow
338 comparison with traditional epidemiological data on drug consumption prevalence. It should be
339 pointed out that the correction factors for cannabis and alcohol biomarkers are high (182 and 3047,
340 respectively), which may affect the reliability of the calculated doses for cannabis and the amount of
341 alcohol consumed. Nevertheless, such estimates could be very useful when interpreted in
342 combination with official epidemiological data. Furthermore, it should be noted that HER
343 consumption was estimated from mass loads of total morphine (MOR_{tot}), obtained by summing the
344 daily mass loads of MOR, M3G and M6G, as previously described (Krizman-Matasic et al., 2018). A
345 more specific urinary biomarker of HER consumption, 6-AM, was not used for estimation due to its
346 lower stability at elevated temperatures typical of summer ($>20\text{ }^{\circ}\text{C}$) (McCall et al., 2016; Baker and
347 Kasprzyk-Hordern, 2011; Krizman-Matasic et al., 2018.). Moreover, in the present study, it was
348 necessary to freeze the samples to minimize degradation during transport to the laboratory. The
349 labile compounds, such as 6-AM, are susceptible to additional losses during deep freeze storage at -
350 $20\text{ }^{\circ}\text{C}$ (Krizman-Matasic et al., 2018). Since MOR is also used for therapeutic purposes, this
351 contribution was subtracted from the load of MOR_{tot} before calculating HER consumption. According
352 to official data from local health authorities, therapeutic consumption of MOR in Split in 2016 was
353 893 g, which corresponds to an average consumption of 2.45 g day^{-1} . About 75% of this amount
354 (1.84 g day^{-1}) is excreted as MOR, M3G and M6G (Baselt, 2008). In addition, a possible contribution
355 to MOR_{tot} from the metabolic transformation of COD (Dinis-Oliveira, 2019) was also taken into
356 account. The COD consumption was calculated from the COD daily mass loads using the correction
357 factor from Table S1. According to Hedenmalm et al. (1997), after the intake of COD, 6.5% of the
358 dose is excreted in urine as MOR and this percentage was used to calculate the contribution of COD-

359 derived MOR in the samples. The resulting amount was also subtracted from the MOR_{tot} to obtain
360 the final estimate of HER-derived MOR. The average contribution of COD-derived MOR to MOR_{tot}
361 calculated from the data in this work was 10.1±1.5%, which means that about 90% of MOR derived
362 from heroin consumption.

363 WBE estimates in Table 3 indicate a relatively high prevalence of some illicit drugs, such as cannabis,
364 COC, AMP, and HER, with an average consumption of 202, 27, 12 and 7.8 daily doses per 1000
365 inhabitants aged 15 – 64 years, respectively. In contrast, MDMA and MAMP use (3.5 and 0.55 daily
366 doses per 1000 inhabitants aged 15 – 64 years, respectively) was much lower.

367 Unfortunately, there are no specific data on drug prevalence in the city of Split, so only a comparison
368 with Croatian national statistics was possible. According to the EMCDDA report, the most prevalent
369 drug in Croatia in 2015 was cannabis (lifetime prevalence among adults aged 15 – 64: 19.4%),
370 followed by amphetamines (3.5%), MDMA, (3%) and COC (2.7%) (European Drug Report 2020). In
371 2019, prevalence increased for all drugs (cannabis: 22.9%, amphetamines: 4.6%, MDMA: 4.2%,
372 cocaine: 4.8%) (European Drug Report 2022). The prevalence estimates from the two methods agree
373 very well for cocaine and cannabis (Table 3), whereas a very large discrepancy was found for MDMA
374 and, to a lesser extent, amphetamines, as surveys suggest a higher prevalence than WBE-based data.
375 This discrepancy could be due to regional differences, uncertainty related to dose size, and, even
376 more likely, the fact that survey data refer to lifetime prevalence, whereas MDMA and
377 amphetamines, as typical recreational drugs, are used only occasionally.

378 A rather large discrepancy between WBE and national survey data was also found for HER. According
379 to WBE estimates, the prevalence of HER use in the city of Split is 0.78%, which is about twice the
380 national prevalence (0.4%) assessed through regular surveys (Glavak Tkalić et al., 2013). However,
381 national data on the prevalence of HER use are somewhat outdated (2011) and represent the
382 average prevalence for the whole country, while recent WBE studies suggest substantial spatial and
383 temporal variation in HER consumption over the past decade (Krizman et al., 2016; Krizman-Matasic
384 et al., 2019). Nonetheless, our WBE results are also inconsistent with official statistics on HER users

385 included in medical treatment and rehabilitation programs in 2016 (Katalinić and Huskić, 2017).
386 Although the population-normalized number of registered opioid addicts was higher in the city of
387 Zagreb and Zadar County, WBE data from this study suggest that HER use is much higher in the city
388 of Split. It should be noted that treatment and rehabilitation data refer to counties, not cities, and it
389 is possible that this rate is higher in the city of Split than in the county as a whole. However, it could
390 also mean that the percentage of registered HER addicts in Split is lower than in other cities, which
391 could be important information for health authorities.

392 The average consumption of MTHD, which is widely used in substitution therapy of HER addicts, was
393 0.33 doses per 1000 inhabitants 15 – 64 years, which means that the prevalence is three times
394 higher than the official data on the number of addicts treated with this therapeutic opioid in Split
395 County (Katalinić and Huskić, 2017). This may be related to the higher percentage of treated addicts
396 in the city (compared to the county as a whole), as well as illicit MTHD use.

397 The number of cigarettes smoked daily per 1000 inhabitants >15 years was estimated to be about
398 4,750, which is very similar to the average value determined in Italy (Castiglioni et al., 2015) and in
399 other European cities (Baz-Lomba et al., 2016; Montes et al., 2020). However, it should be
400 emphasized that the uncertainty of this calculation can be high due to the different amounts of
401 nicotine absorbed by the human body, which is related to differences in the nicotine content in
402 cigarettes (Zheng et al., 2017) and some other factors. Some authors have used a conversion factor
403 of up to 1.25 mg per cigarette for back-calculations (Castiglioni et al., 2015; van Wel et al., 2016),
404 which means that the uncertainty can be up to 40% (Montes et al., 2020). Another potential source
405 of error and limitation of this study relates to the assessment of nicotine from other sources, such as
406 nicotine gums and patches. This contribution is likely small but could be assessed using the specific
407 tobacco biomarker anabasine (Zheng et al., 2023). Taking into account the average number of
408 cigarettes smoked daily by regular smokers (15.9), the calculated smoking prevalence is 30.6%,
409 which agrees very well with the prevalence determined in the 2014 – 2015 national survey (27.5% of
410 daily smokers and 31.5% overall) (Dečković Vukres et al., 2015).

411 The estimated daily alcohol consumption, expressed as the volume of pure ethanol consumed *per*
412 *capita* (>15 years), in the peak-tourist and off-tourist reference weeks was 17 mL and 12 mL,
413 respectively. The resulting average annual *per capita* consumption of 5.2 L was fairly similar to the
414 average consumption in 13 Spanish cities (4.7 L) (López-García et al., 2020), and many other cities in
415 Europe (Baz-Lomba et al., 2016). This is lower than WHO's estimate of 8.9 L per year for the whole
416 country (adjusted for tourist consumption) in 2016 (WHO, 2018). One of the possible explanations
417 for this discrepancy is regional and/or seasonal differences. However, very similar discrepancies
418 were found in national studies in Belgium (Boogaerts et al., 2016) and Spain (López-García et al.,
419 2020), where consumption estimates from WHO (largely derived from sales data) were also higher
420 than WBE-derived consumption. This could be partly related to the stability of EtS. Although EtS is
421 stable in wastewater (Rodríguez-Álvarez et al., 2014a), it can degrade to some extent in sewage
422 systems (Gao et al., 2018; Banks et al., 2018), which could lead to an underestimation of alcohol
423 consumption.

424

425 **4. Conclusion**

426 This is the first comprehensive WBE study to simultaneously address the impact of an electronic
427 music festival on patterns of illicit drug, NPS, therapeutic opioid, alcohol, and nicotine use,
428 superimposed on seasonal dynamics in a coastal Mediterranean city. The study revealed that the
429 festival had a major impact on the increased use of stimulant drugs and alcohol, with an
430 approximately 30-fold increase for MDMA. In contrast, the consumption of other illicit drugs
431 (cannabis and heroin), therapeutic opioids and tobacco remained rather constant. The prevalence of
432 NPS was low, suggesting that they are not yet widely used in this region. The prevalence estimates
433 obtained using the WBE-approach are mostly consistent with conventional epidemiological studies,
434 except for typical amphetamine-type recreational drugs and HER. Our study also revealed that HER
435 consumption in Split may be higher than official data suggest and that the percentage of registered
436 HER addicts may be rather low, which could be important information for health authorities. Overall,

437 this study confirms the suitability of the WBE approach for assessing differences in collective drug
438 use at fine temporal resolution.

439

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451

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673

Table 1. List of the target biomarkers included in the study.

Category	Drug	Target biomarker	Abbreviation
"Conventional" illicit drugs	Heroin (HER)	Morphine	MOR
		Morphine-3- β -D-glucuronide ^a	M3G
		Morphine-6- β -D-glucuronide ^a	M6G
		Normorphine ^a	norMOR
	Cocaine	Benzoylcegonine	BE
		Cocaine	COC
	Amphetamine	Amphetamine	AMP
	Methamphetamine	Methamphetamine	MAMP
MDMA ^b	MDMA	MDMA	
Cannabis	THC-COOH ^c	THC-COOH	
New psychoactive substances (NPS)	Ephedrine	Ephedrine	EPH
	Pseudoephedrine	Pseudoephedrine	PEPH
	Ketamine	Ketamine	KET
		Norketamine	norKET
	Cathinone	Cathinone	CAT
	Methcathinone	Methcathinone	MCAT
	Flephedrone	Flephedrone	4-FMC
	4-Fluoroamphetamine	4-Fluoroamphetamine	4-FA
	MDA ^d	MDA	MDA
	Methylone	Methylone	MDMC
	Methedrone	Methedrone	PMMC
	4-Methylamphetamine	4-Methylamphetamine	4-MA
	PMMA ^e	PMMA	PMMA
	Mephedrone	Mephedrone	4-MMC
	Butylone	Butylone	bk-MBDB
	4-Methylethcathinone	4-Methylethcathinone	4-MEC
	4-Ethylmethcathinone	4-Ethylmethcathinone	4-EMC
	2C-T-2 ^f	2C-T-2	2C-T-2
	2C-I ^g	2C-I	2C-I
	MDPV ^h	MDPV	MDPV
2C-T-7 ⁱ	2C-T-7	2C-T-7	
Naphyrone	Naphyrone	O-2484	
Therapeutic opioids	Dihydromorphine	Dihydromorphine	DMOR
	Oxymorphone	Oxymorphone	OM
	Hydromorphone	Hydromorphone	HM
	Ethylmorphine	Ethylmorphine	EMOR
	Codeine	Codeine	COD
		Norcodeine	norCOD
	Dihydrocodeine	Dihydrocodeine	DCOD
	Oxycodone	Oxycodone	OC
		Noroxycodone	norOC
	Hydrocodone	Hydrocodone	HC
		Norhydrocodone	norHC
	Tramadol	Tramadol	TRA
		<i>O</i> -demethyl- <i>cis</i> -tramadol	O-TRA
		<i>N</i> -demethyl- <i>cis</i> -tramadol	N-TRA
	Fentanyl	Fentanyl	FNT
		Norfentanyl	norFNT
	Sufentanil	Sufentanil	SFNT
Buprenorphine	Buprenorphine	BUP	

		Norbuprenorphine	norBUP
	Methadone	Methadone	MTHD
		EDDP ^j	EDDP
	Propoxyphene	Propoxyphene	PP
Alcohol	Ethanol	Ethyl Sulfate	EtS
Tobacco	Nicotine	Cotinine	COT
		<i>Trans</i> -3'-Hydroxycotinine	HCOT

^a Also metabolites of morphine; ^b 3,4-Methylenedioxyamphetamine; ^c 11-Nor-9-carboxy- Δ 9-tetrahydrocannabinol; ^d 3,4-Methylenedioxyamphetamine; ^e 1-(4-Methoxyphenyl)-2-methylaminopropane; ^f 4-Ethylthio-2,5-dimethoxyphenethylamine; ^g 4-Iodo-2,5-dimethoxyphenethylamine; ^h 3,4-Methylenedioxypropylvalerone; ⁱ 4-Propylthio-2,5-dimethoxyphenethylamine; ^j 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

Note: Ephedrine and pseudoephedrine are not NPS but were analyzed using the same analytical method as NPS.

Table 2. Population-normalized daily mass loads of target biomarkers (mg day⁻¹ 1000 inhabitants⁻¹) in two wastewater collectors in the city of Split in 2016.

Biomarker	OFF-TOURIST REFERENCE WEEK			PEAK-TOURIST REFERENCE WEEK			FESTIVAL WEEK		
	Range	Average ± SD	Median	Range	Average ± SD	Median	Range	Average ± SD	Median
MOR	95 – 159	134 ± 20	133	142 – 186	155 ± 15	151	123 – 164	140 ± 17	134
M3G	26 – 119	63 ± 37	62	20 – 52	32 ± 11	32	3.5 – 6.2	4.7 ± 0.9	4.8
M6G	7.1 – 22	12 ± 6	10	8.1 – 11	9.4 ± 1.1	9.2	3.7 – 9.2	6.9 ± 2.3	7.9
norMOR	4.7 – 10	7.5 ± 1.7	7.5	7.3 – 10	8.7 ± 1.3	9.4	6.5 – 7.9	7.3 ± 0.5	7.2
BE	156 – 344	236 ± 75	193	249 – 350	286 ± 37	277	182 – 658	409 ± 179	392
COC	71 – 150	104 ± 30	86	97 – 159	118 ± 22	111	72 – 190	127 ± 43	136
AMP	64 – 112	79 ± 19	67	74 – 161	103 ± 28	102	82 – 192	136 ± 39	123
MAMP	0.93 – 3.0	1.9 ± 0.8	1.8	1.3 – 6.3	3.2 ± 1.9	2.3	1.5 – 13	4.2 ± 4.2	3.2
MDMA	14 – 102	36 ± 31	23	56 – 95	73 ± 16	76	132 – 2723	1078 ± 1097	575
THC-COOH	91 – 118	103 ± 9	105	66 – 99	85 ± 10	88	48 – 79	66 ± 10	71
PEPH	85 – 171	113 ± 28	114	75 – 113	95 ± 13	98	48 – 84	68 ± 12	71
KET	BLD	–	–	0.93 – 2.2	1.5 ± 0.5	1.4	6.3 – 27	16 ± 7	16
norKET	BLD – 1.4	1.0 ± 0.3	0.94	1.4 – 2.7	1.9 ± 0.5	1.7	2.2 – 9.6	5.6 ± 2.5	5.1
4-FA	BLD	–	–	BLD	–	–	BLD – 11	4.1 ± 4.5	1.2
MDA	BLD	–	–	BLD	–	–	BLD – 71	50 ± 26	57
DMOR	BLD	–	–	0.61 – 1.5	1.0 ± 0.39	0.86	0.83 – 1.6	1.3 ± 0.24	1.3
COD	53 – 85	69 ± 9	70	80 – 95	88 ± 6	88	66 – 82	76 ± 6	76
OC	BLD	–	–	BLD	–	–	BLD – 5.0	3.6 ± 1.0	3.6
norHC	BLD	–	–	BLD	–	–	0.58 – 1.2	0.83 ± 0.20	0.86
TRA	155 – 329	216 ± 60	194	186 – 253	223 ± 21	223	182 – 223	193 ± 14	190
O-TRA	114 – 156	143 ± 16	150	186 – 247	221 ± 20	220	182 – 221	195 ± 16	188
N-TRA	44 – 71	58 ± 10	58	55 – 69	64 ± 4	65	56 – 79	64 ± 8	64
SFNT	BLD – 1.5	0.88 ± 0.47	0.59	0.21 – 0.74	0.48 ± 0.15	0.49	0.27 – 0.60	0.47 ± 0.11	0.47
BUP	2.1 – 4.0	3.1 ± 0.7	2.8	2.5 – 3.8	3.1 ± 0.5	3.1	1.2 – 4.3	2.4 ± 1.1	2.0
MTHD	19 – 33	25 ± 5	24	19 – 34	27 ± 5	26	11 – 12	11 ± 1	11
EDDP	36 – 55	44 ± 7	44	42 – 78	53 ± 13	46	35 – 54	45 ± 6	45
PP	BLD – 1.2	0.63 ± 0.38	0.51	BLD – 1.6	0.60 ± 0.50	0.39	0.29 – 0.89	0.56 ± 0.19	0.51
EtS	1908 – 3477	2578 ± 659	2270	2125 – 4244	3664 ± 746	3800	2913 – 7384	4486 ± 1453	4277
COT	865 – 1196	1034 ± 131	1039	854 – 1221	1084 ± 118	1127	901 – 1017	977 ± 41	986
HCOT	1263 – 1742	1535 ± 189	1523	1386 – 1778	1632 ± 168	1712	1377 – 1571	1490 ± 79	1518

BLD – Mass loads below the detection limit.

Table 3. Estimation of consumption and prevalence of drug use in the city of Split in 2016 and comparison with conventional epidemiological methods.

Drug	Unit	CONSUMPTION (WBE)						PREVALENCE / %		
		OFF-TOURIST REFERENCE WEEK			PEAK-TOURIST REFERENCE WEEK			WBE ^a	CONVENTIONAL METHODS	
		Range	Average \pm SD	Median	Range	Average \pm SD	Median	2016	2015	2019
Heroin	doses day ⁻¹ 1000 inhabitants 15–64 years ⁻¹	4.8 – 10	7.9 \pm 1.7	7.7	7.0 – 8.8	7.7 \pm 0.6	7.7	0.78	0.4 ^c	–
Cocaine		16 – 35	24 \pm 8	20	25 – 36	29 \pm 4	28	2.7	2.7 ^d	4.8 ^h
Amphetamine		8.7 – 15	11 \pm 3	9	10 – 22	14 \pm 4	14	1.3 ^b	3.5 ^{b,d}	4.6 ^{b,h}
Methamphetamine		0.20 – 0.65	0.40 \pm 0.17	0.39	0.28 – 1.4	0.69 \pm 0.40	0.50			
MDMA		0.91 – 6.6	2.3 \pm 2.0	1.5	3.7 – 6.1	4.7 \pm 1.0	4.9	0.35	3.0 ^d	4.2 ^h
Cannabis		195 – 252	221 \pm 20	224	142 – 211	183 \pm 22	188	20.2	19.4 ^d	22.9 ^h
Methadone		2.5 – 3.8	3.0 \pm 0.5	3.0	2.9 – 5.3	3.6 \pm 0.9	3.2	0.33	0.11 ^e	–
Nicotine	cigarettes day ⁻¹ 1000 inhabitants >15 years ⁻¹	4013 – 5429	4736 \pm 586	4731	4126 – 5341	4997 \pm 485	5300	30.6	31.1 ^f (27.5 ^g)	–
Alcohol	L day ⁻¹ 1000 inhabitants >15 years ⁻¹	8.7 – 16	12 \pm 3	10	10 – 19	17 \pm 3	17	–	–	–

^a Average value calculated from the two reference weeks; ^b Overall prevalence of amphetamines; ^c Lifetime prevalence for Croatia (Glavak Tkalić et al., 2013; survey data from 2011); ^d Lifetime prevalence for Croatia (European Drug Report 2021; survey data from 2015); ^e Calculated using data from the Croatian Institute of Public Health (2016); ^f Regular and occasional smokers; ^g Regular smokers only; ^h Lifetime prevalence for Croatia (European Drug Report 2021; survey data from 2019)

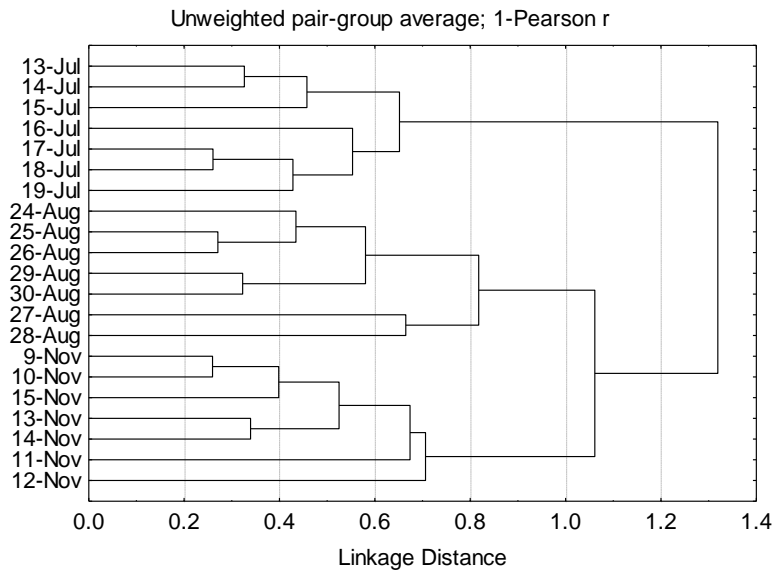


Fig. 1. Hierarchical cluster analysis of sampling periods by pair-group average linkage clustering.

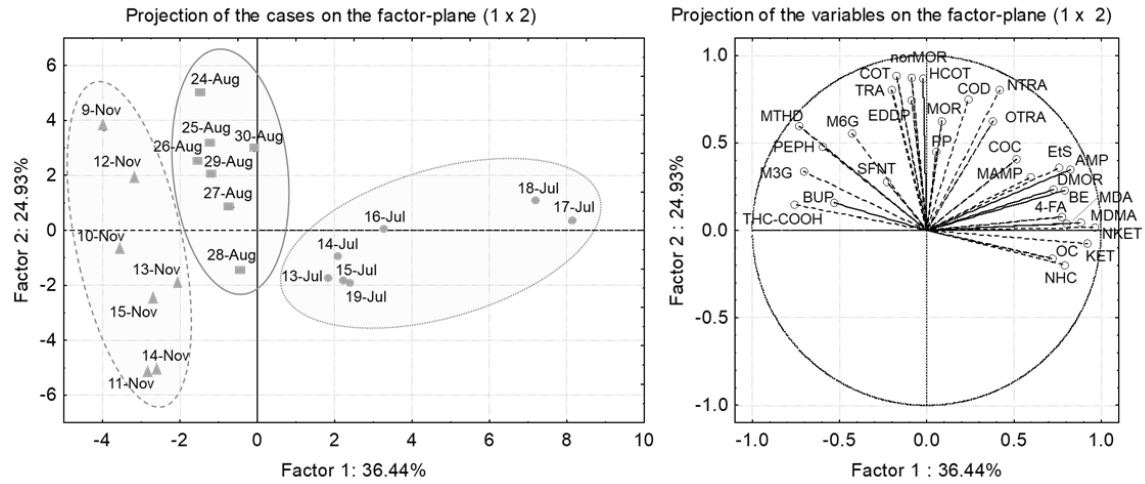


Fig. 2. Principal component analysis (PCA) – projection of cases (sampling dates) and variables (population-normalized mass loads of target biomarkers) on factor 1 vs. 2.

CRedit author statement

Ivan Senta: Investigation, Formal analysis, Validation, Visualization, Writing – original draft. **Ivona Krizman-Matasic:** Investigation, Formal analysis, Validation, Writing – original draft. **Petra Kostanjevecki:** Investigation. **Iria Gonzalez-Mariño:** Investigation, Formal analysis, Writing – review & editing. **Rosario Rodil:** Writing – review & editing, Resources. **José Benito Quintana:** Writing – review & editing, Resources. **Iva Mikac:** Formal analysis. **Senka Terzic:** Conceptualization, Methodology, Formal analysis, Funding acquisition, Resources, Supervision, Writing – review & editing. **Marijan Ahel:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing.

SUPPLEMENTARY MATERIAL

Assessing the impact of a major electronic music festival on the consumption patterns of illicit and licit psychoactive substances in a Mediterranean city using wastewater analysis

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Table of contents

Fig. S1. Average number of tourists per day in the city of Split in 2016. (Page 3)

Fig. S2. Weekly trends of mass loads of target biomarkers in two wastewater collectors in the city of Split. (Pages 4–7)

Fig. S3. Principal component analysis (PCA) – projection of cases (sampling dates) and variables (population-normalized mass loads of target biomarkers) on factor 2 vs. 3. (Page 8)

Table S1. Data used for estimating drug use. (Page 9)

Table S2. Concentrations of “conventional” illicit drug biomarkers in raw wastewater collected at the Stupe wastewater collector in the city of Split in 2016. (Page 10)

Table S3. Concentrations of “conventional” illicit drug biomarkers in raw wastewater collected at the Katalinica Brig wastewater collector in the city of Split in 2016. (Page 10)

Table S4. Concentrations of new psychoactive substance biomarkers in raw wastewater collected at two wastewater collectors in the city of Split in 2016. (Page 11)

Table S5. Concentrations of therapeutic opioid biomarkers in raw wastewater collected at Stupe wastewater collector in the city of Split in 2016. (Page 12)

Table S6. Concentrations of therapeutic opioid biomarkers in raw wastewater collected at Katalinica Brig wastewater collector in the city of Split in 2016. (Page 12)

Table S7. Concentrations of alcohol and nicotine biomarkers in raw wastewater collected at two wastewater collectors in the city of Split in 2016. (Page 13)

Table S8. Daily mass loads of target biomarkers (g day^{-1}) in two wastewater collectors in the city of Split in 2016. (Page 14)

Table S9. Estimation of consumption of illicit drugs, therapeutic opioids, nicotine, and alcohol in the city of Split in 2016. (Page 15)

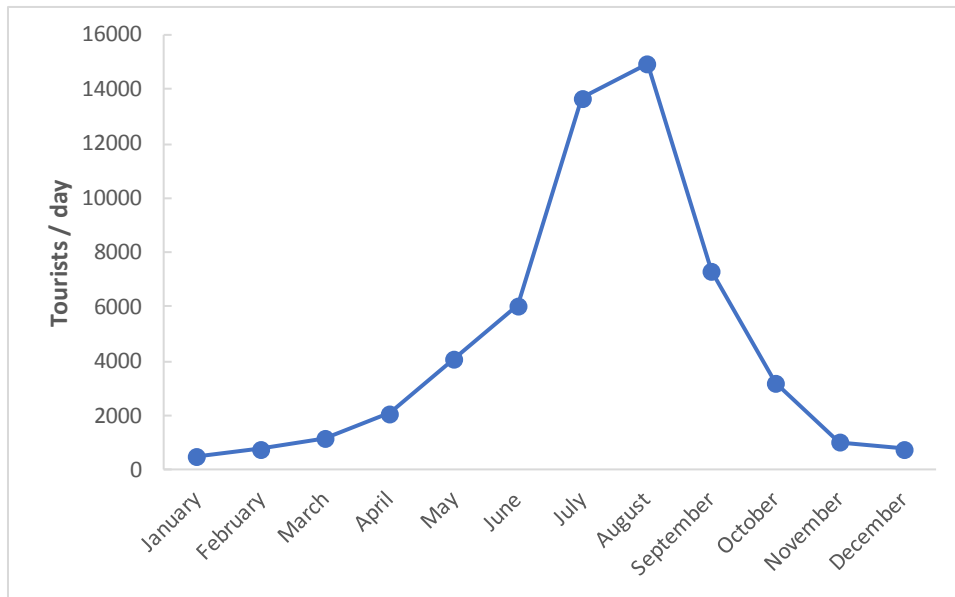


Fig. S1. Average number of tourists per day in the city of Split in 2016.

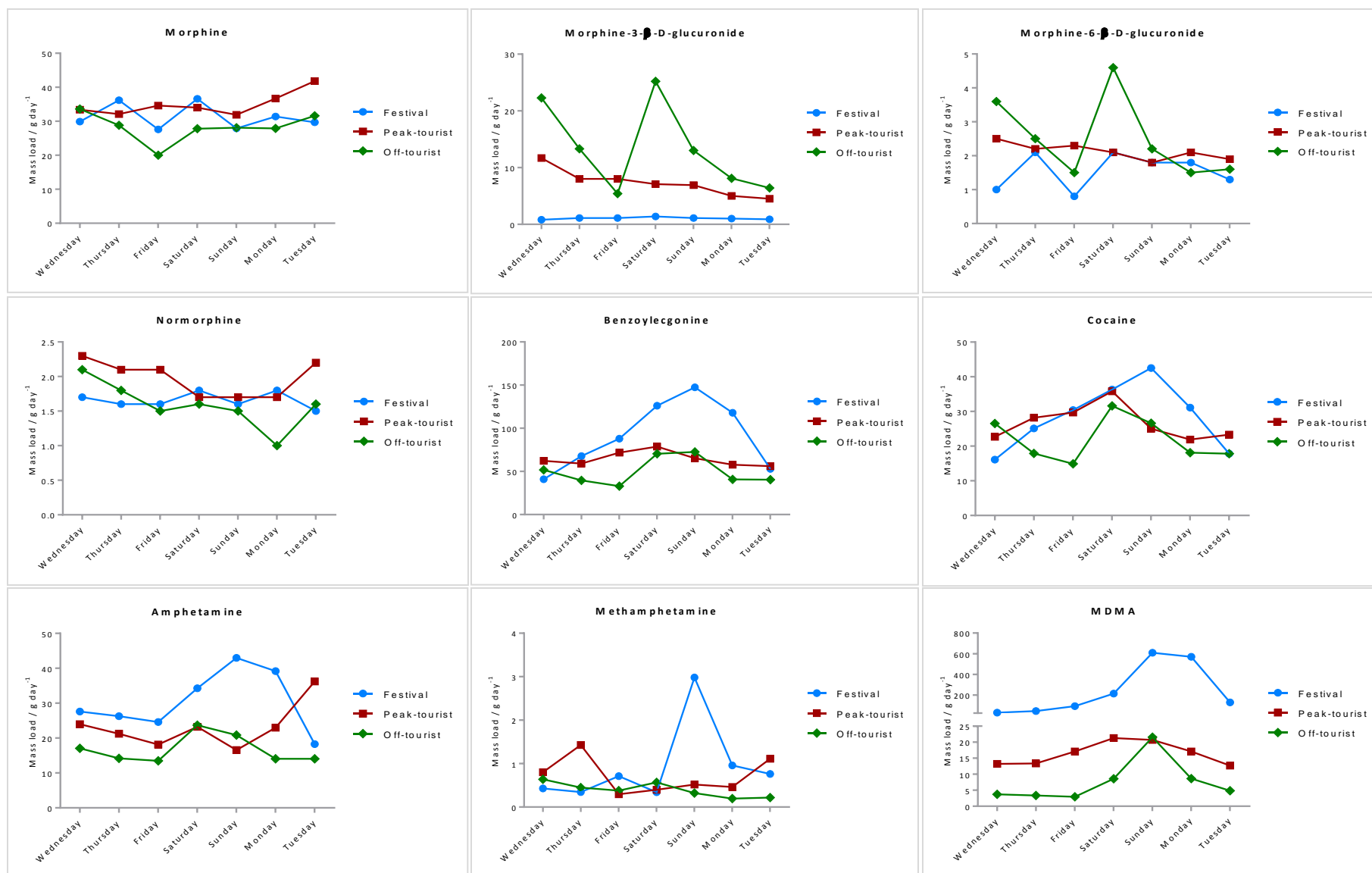


Fig. S2. Weekly trends of mass loads of target biomarkers in two wastewater collectors in the city of Split.

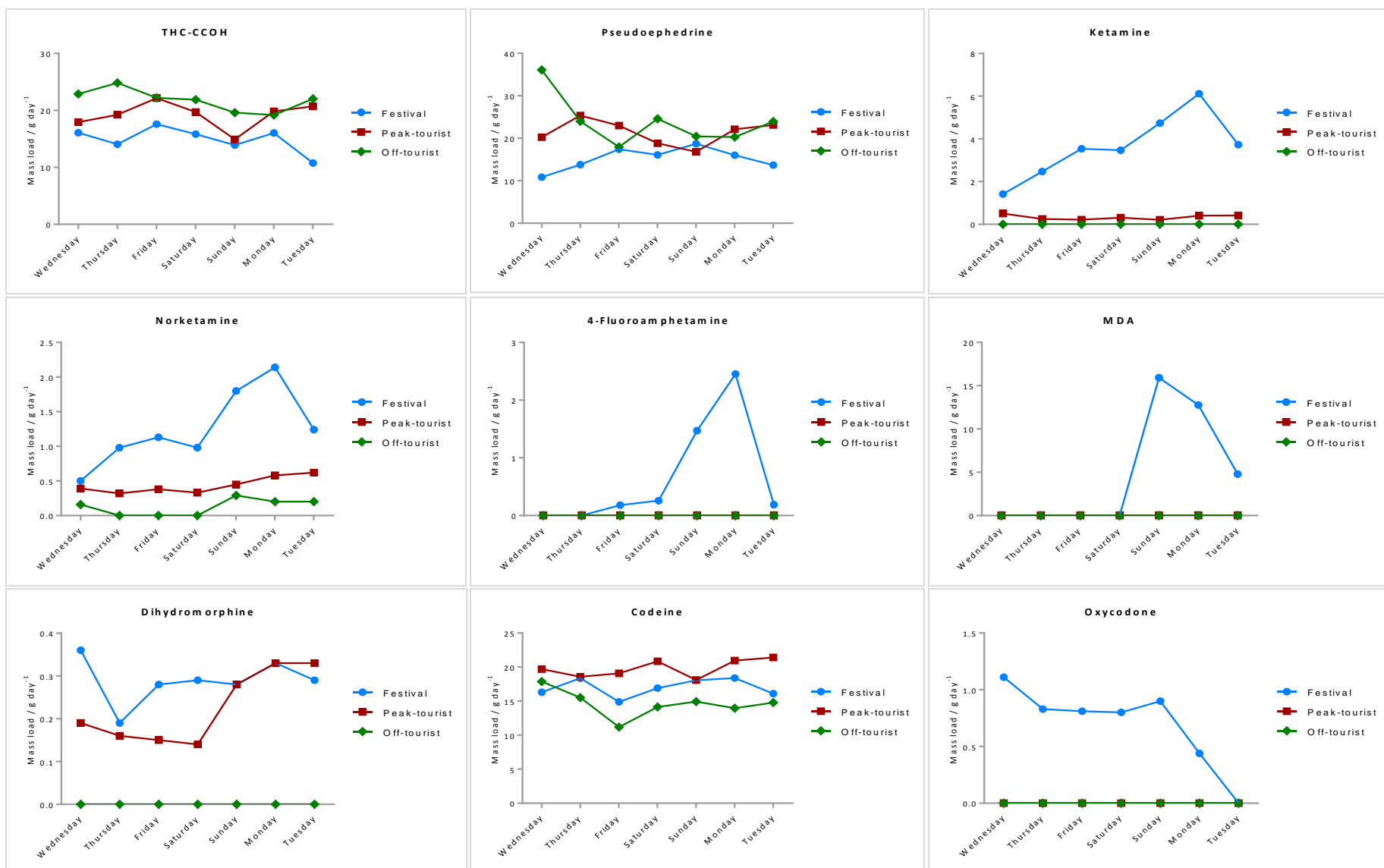


Fig. S2. continued

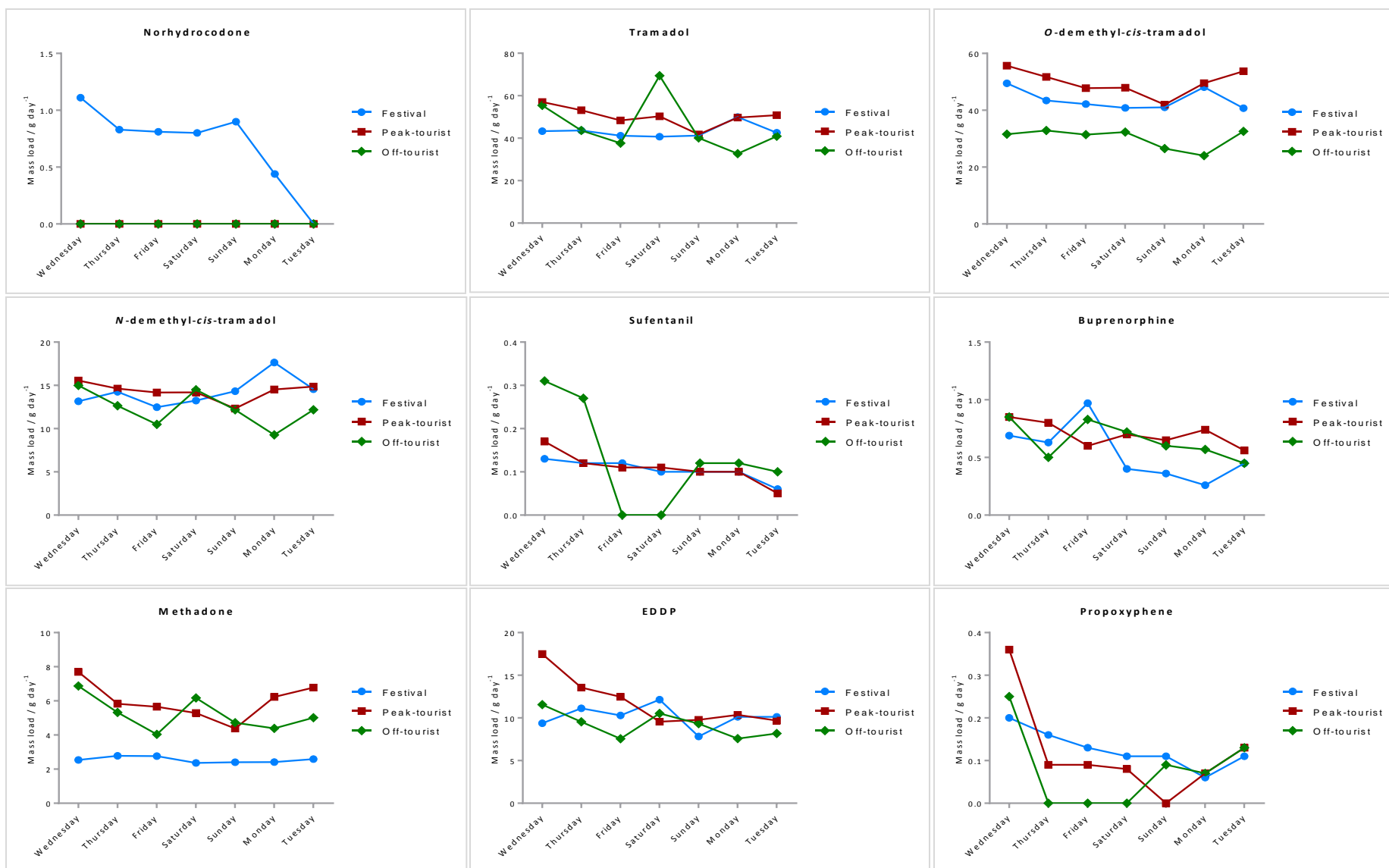


Fig. S2. continued

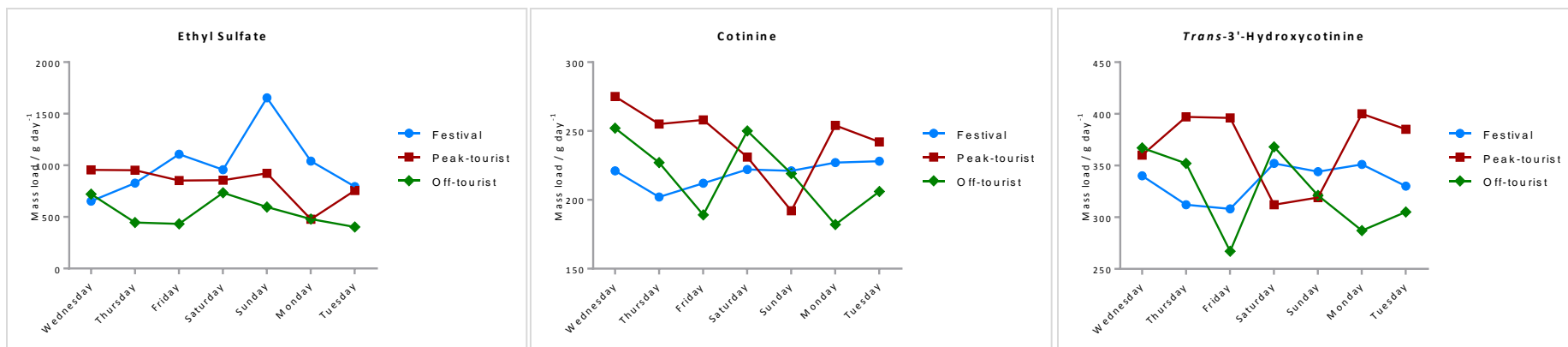


Fig. S2. continued

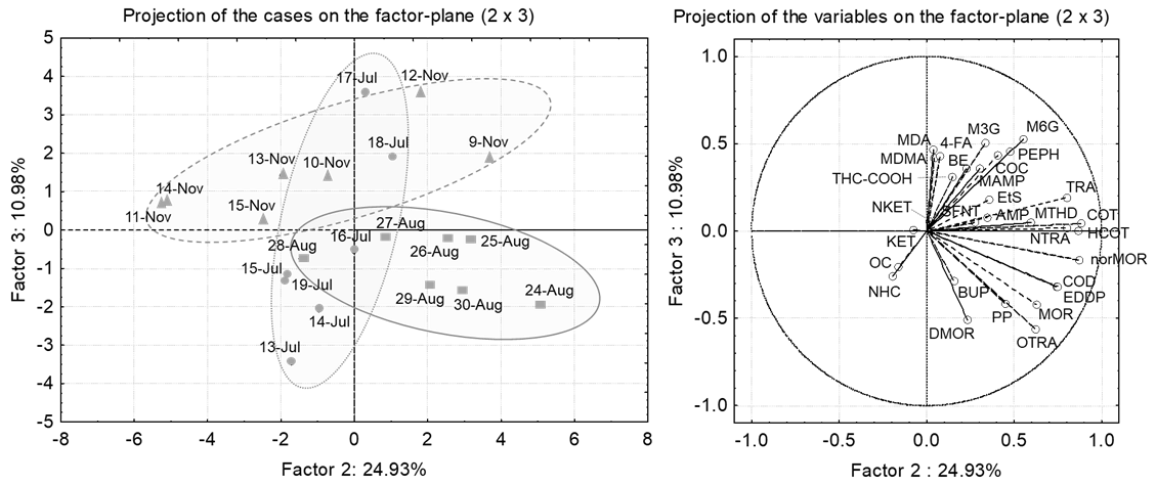


Fig. S3. Principal component analysis (PCA) – projection of cases (sampling dates) and variables (population-normalized mass loads of target biomarkers) on factor 2 vs. 3.

Table S1. Data used for estimating drug use.

Drug	Biomarker used to estimate consumption	Percentage of drug doses excreted as drug biomarker	Molar ratio of drug and target biomarker	Correction factor	Average dose / mg
Heroin	MOR	42 ^a	1.30	3.1	30 ^h
Cocaine	BE	29 ^a	1.05	3.6	52 ^{ij}
Amphetamine	AMP	36.3 ^a	1.00	2.77	30 ^h
Methamphetamine	MAMP	22.7 ^a	1.00	4.4	30 ^h
MDMA	MDMA	22.5 ^a	1.00	4.4	100 ^{h,j}
THC (Cannabis)	THC-COOH	0.5 ^a	0.91	182	125 ^h
Ketamine	KET	20 ^b	1.00	5	–
Pseudoephedrine	PEPH	88 ^c	1.00	1.14	–
Methadone	EDDP	25 ^c	1.12	4.48	98 ^k
Codeine	COD	30 ^d	1.00	3.3	–
Tramadol	TRA	30 ^e	1.00	3.3	–
Alcohol	EtS	0.012 ^f	0.37	3047	–
Nicotine	COT	27 ^g	0.92	3.41	–
Nicotine	HCOT	44.5 ^g	0.84	1.9	–

^a Gracia-Lor, E., Zuccato, E., Castiglioni, S., 2016. Refining correction factors for back-calculation of illicit drug use. *Sci. Total Environ.* 573, 1648–1659, <https://doi.org/10.1016/j.scitotenv.2016.09.179>

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^e Mackul'ak, T., Birošová, L., Gál, M., Bodík, I., Grabic, R., Ryba, J., Škubák, J., 2016. Wastewater analysis: the mean of the monitoring of frequently prescribed pharmaceuticals in Slovakia. *Environ. Monit. Assess.* 188, 18. <https://doi.org/10.1007/s10661-015-5011-7>

^f Rodríguez-Álvarez, T., Rodil, R., Cela, R., Quintana, J.B., 2014a. Ion-pair reversed-phase liquid chromatography–quadrupole-time-of-flight and triple-quadrupole–mass spectrometry determination of ethyl sulfate in wastewater for alcohol consumption tracing. *J. Chromatogr. A* 1328, 35–42. <https://doi.org/10.1016/j.chroma.2013.12.076>

^g Rodríguez-Álvarez, T., Rodil, R., Rico, M., Cela, R., Quintana, J.B., 2014b. Assessment of local tobacco consumption by liquid chromatography–tandem mass spectrometry sewage analysis of nicotine and its metabolites, cotinine and trans-3'-hydroxycotinine, after enzymatic deconjugation. *Anal. Chem.* 86, 10274–10281, <https://doi.org/10.1021/ac503330c>

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ⁱ <https://www.emcdda.europa.eu/publications/drug-profiles/cocaine>

^j Office for Combating Narcotic Drug Abuse of the Government of the Republic of Croatia (data for 2013)

^k Croatian Institute of Public Health (average daily dose; data for Zagreb for 2012)

Table S2. Concentrations of “conventional” illicit drug biomarkers in raw wastewater collected at the Stupe wastewater collector in the city of Split in 2016.

Date	c / ng L ⁻¹									
	MOR	M3G	M6G	norMOR	BE	COC	AMP	MAMP	MDMA	THC-COOH
13.7.	508	12	<0.2	34	626	179	670	11	291	292
14.7.	580	23	25	28	940	295	572	6.8	365	207
15.7.	448	20	<0.2	26	1112	307	434	9.9	720	283
16.7.	737	16	31	39	1848	500	628	4.2	1700	261
17.7.	587	17	28	34	2851	675	909	51	12022	247
18.7.	518	24	25	27	2031	459	670	17	9569	288
19.7.	517	19	17	29	815	282	307	8.5	1583	175
24.8.	519	69	29	40	1055	325	274	7.4	191	234
25.8.	532	53	26	33	942	432	357	19	208	284
26.8.	564	62	23	35	1105	393	267	4.0	188	379
27.8.	524	44	23	30	1334	552	366	4.7	278	345
28.8.	606	48	21	28	1246	478	296	3.5	318	253
29.8.	572	39	23	26	933	339	257	8.7	222	287
30.8.	707	40	19	35	1005	402	562	5.9	190	308
9.11.	493	142	33	34	731	340	235	12	54	294
10.11.	399	264	36	22	480	208	191	3.0	48	320
11.11.	312	85	22	22	614	262	169	7.9	51	318
12.11.	335	184	44	22	866	342	259	11	97	293
13.11.	446	167	36	28	1250	474	290	5.8	345	271
14.11.	586	110	24	20	876	380	259	5.1	135	419
15.11.	590	101	28	30	824	302	227	5.4	81	434

Table S3. Concentrations of “conventional” illicit drug biomarkers in raw wastewater collected at the Katalinica Brig wastewater collector in the city of Split in 2016.

Date	c / ng L ⁻¹									
	MOR	M3G	M6G	norMOR	BE	COC	AMP	MAMP	MDMA	THC-COOH
13.7.	475	14	30	21	710	346	245	3.1	665	236
14.7.	539	13	38	22	1123	459	269	4.1	970	224
15.7.	425	16	27	25	1684	664	341	13	2271	273
16.7.	523	31	39	22	2452	737	548	7.3	5541	280
17.7.	444	22	36	26	2548	851	681	56	10357	258
18.7.	495	7.2	32	30	1771	544	593	14	8809	229
19.7.	439	10	24	18	889	291	282	16	2569	172
24.8.	445	296	46	26	720	337	443	17	195	294
25.8.	409	196	40	31	798	400	265	23	186	287
26.8.	442	184	47	26	995	493	268	4.7	330	262
27.8.	530	188	43	20	1080	555	351	7.9	391	257
28.8.	469	180	40	28	957	367	262	14	378	249
29.8.	493	116	40	24	734	297	435	4.3	286	294
30.8.	477	96	37	27	565	257	477	29	176	291
9.11.	286	370	50	16	468	274	160	3.1	32	235
10.11.	443	129	36	31	675	313	224	9.9	50	405
11.11.	251	68	20	20	339	164	200	3.3	34	300
12.11.	311	396	62	17	774	392	291	2.5	102	218
13.11.	395	232	30	16	909	312	343	4.7	301	325
14.11.	448	198	34	16	633	292	268	2.1	192	291
15.11.	523	130	28	25	592	331	275	2.2	92	339

Table S4. Concentrations of new psychoactive substance biomarkers in raw wastewater collected at two wastewater collectors in the city of Split in 2016.

Date	c / ng L ⁻¹									
	Stupe					Katalinica Brig				
	PEPH	KET	norKET	4-FA	MDA	PEPH	KET	norKET	4-FA	MDA
13.7.	161	22	<1.5	<0.4	<15	194	25	16	<0.4	<15
14.7.	157	36	14	<0.4	<15	256	39	16	<0.4	<15
15.7.	180	44	18	<0.4	<15	376	69	17	5.5	<15
16.7.	208	26	15	<0.4	<15	340	92	18	8.6	<15
17.7.	353	83	41	39	270	331	89	26	17	305
18.7.	277	112	47	68	215	239	86	22	11	197
19.7.	205	56	23	<0.4	65	236	65	17	6.0	89
24.8.	280	4.0	6.8	<0.4	<15	313	11	4.3	<0.4	<15
25.8.	327	<0.9	5.2	<0.4	<15	435	8.1	4.1	<0.4	<15
26.8.	288	<0.9	8.6	<0.4	<15	400	7.0	1.8	<0.4	<15
27.8.	267	4.3	5.7	<0.4	<15	320	5.3	4.4	<0.4	<15
28.8.	239	4.4	11	<0.4	<15	325	2.7	4.5	<0.4	<15
29.8.	278	4.0	11	<0.4	<15	380	8.2	5.3	<0.4	<15
30.8.	344	4.8	11	<0.4	<15	324	7.7	5.7	<0.4	<15
9.11.	635	<0.9	3.5	<0.4	<15	206	<0.9	<1.5	<0.4	<15
10.11.	209	<0.9	<1.5	<0.4	<15	487	<0.9	<1.5	<0.4	<15
11.11.	370	<0.9	<1.5	<0.4	<15	157	<0.9	<1.5	<0.4	<15
12.11.	377	<0.9	<1.5	<0.4	<15	198	<0.9	<1.5	<0.4	<15
13.11.	394	<0.9	2.0	<0.4	<15	206	<0.9	7.4	<0.4	<15
14.11.	501	<0.9	<1.5	<0.4	<15	242	<0.9	7.8	<0.4	<15
15.11.	525	<0.9	<1.5	<0.4	<15	308	<0.9	7.2	<0.4	<15

Note: The concentrations of the other target compounds were below the detection limit in all samples.

Table S5. Concentrations of therapeutic opioid biomarkers in raw wastewater collected at Stupe wastewater collector in the city of Split in 2016.

Date	c / ng L ⁻¹											
	DMOR	COD	OC	norHC	TRA	O-TRA	N-TRA	SFNT	BUP	MTHD	EDDP	PP
13.7.	6.5	314	22	5.3	843	1044	267	3.1	17	47	187	3.9
14.7.	<0.3	339	16	3.3	768	825	273	2.5	11	54	158	3.3
15.7.	4.6	238	11	3.1	732	804	223	2.2	24	47	166	2.2
16.7.	4.4	356	12	2.1	792	832	279	1.9	11	44	204	2.1
17.7.	4.4	408	15	3.5	937	951	349	2.1	10	44	171	2.2
18.7.	6.0	352	14	3.3	977	927	374	1.3	5.5	45	217	2.0
19.7.	4.4	267	<0.1	<0.3	797	763	296	<0.2	8.9	46	156	2.0
24.8.	<0.3	327	<0.1	<0.3	921	922	257	3.2	14	127	318	7.7
25.8.	<0.3	344	<0.1	<0.3	933	913	255	2.3	16	96	256	2.4
26.8.	<0.3	318	<0.1	<0.3	783	773	232	1.9	16	88	182	2.2
27.8.	<0.3	375	<0.1	<0.3	891	872	254	2.0	14	83	146	2.2
28.8.	5.1	367	<0.1	<0.3	771	789	237	1.8	15	69	173	<0.2
29.8.	4.4	357	<0.1	<0.3	810	794	225	1.4	13	80	132	1.8
30.8.	4.3	380	<0.1	<0.3	831	896	236	<0.2	15	70	122	1.9
9.11.	<0.3	271	<0.1	<0.3	696	433	213	5.8	17	86	152	4.0
10.11.	<0.3	190	<0.1	<0.3	507	330	136	1.8	6.7	68	120	<0.2
11.11.	<0.3	178	<0.1	<0.3	651	634	200	<0.2	17	64	114	<0.2
12.11.	<0.3	183	<0.1	<0.3	706	487	200	<0.2	12	74	137	<0.2
13.11.	<0.3	258	<0.1	<0.3	679	473	226	3.2	11	77	149	2.3
14.11.	<0.3	293	<0.1	<0.3	655	531	192	<0.2	13	47	147	2.4
15.11.	<0.3	269	<0.1	<0.3	841	704	269	<0.2	8.0	54	130	4.2

Note: The concentrations of the other target compounds were below the detection limit in all samples.

Table S6. Concentrations of therapeutic opioid biomarkers in raw wastewater collected at Katalinica Brig wastewater collector in the city of Split in 2016.

Date	c / ng L ⁻¹											
	DMOR	COD	OC	norHC	TRA	O-TRA	N-TRA	SFNT	BUP	MTHD	EDDP	PP
13.7.	5.4	223	15.0	3.6	582	588	167	1.4	6.4	37	122	2.7
14.7.	5.0	237	10.1	2.7	592	541	176	1.2	8.7	34	181	1.8
15.7.	4.4	232	15.0	1.8	565	521	170	1.6	5.7	40	159	1.8
16.7.	5.5	226	15.0	2.3	606	573	178	1.4	3.0	37	212	1.8
17.7.	5.6	266	17.5	2.6	602	584	190	1.7	3.5	44	119	1.8
18.7.	4.8	240	<0.1	3.2	634	625	195	2.0	3.0	32	110	<0.2
19.7.	4.9	252	<0.1	2.9	575	550	173	1.8	5.6	38	170	1.7
24.8.	6.2	237	<0.1	<0.3	718	671	189	1.5	11	94	175	2.2
25.8.	5.0	195	<0.1	<0.3	618	594	172	1.2	6.3	75	136	<0.2
26.8.	4.8	236	<0.1	<0.3	625	618	180	1.4	<0.1	77	186	<0.2
27.8.	4.5	261	<0.1	<0.3	644	587	179	1.4	6.4	81	150	<0.2
28.8.	4.5	244	<0.1	<0.3	637	626	180	1.6	6.9	78	156	<0.2
29.8.	5.5	245	<0.1	<0.3	625	639	198	1.7	8.0	104	176	<0.2
30.8.	5.5	221	<0.1	<0.3	617	627	188	1.5	<0.1	135	163	1.8
9.11.	<0.3	143	<0.1	<0.3	586	298	135	1.5	2.8	73	115	1.8
10.11.	<0.3	262	<0.1	<0.3	765	625	232	6.1	7.8	87	158	<0.2
11.11.	<0.3	138	<0.1	<0.3	424	287	104	<0.2	7.2	50	98	<0.2
12.11.	<0.3	146	<0.1	<0.3	903	269	138	<0.2	4.7	70	109	<0.2
13.11.	<0.3	185	<0.1	<0.3	515	313	133	<0.2	6.1	64	130	<0.2
14.11.	<0.3	225	<0.1	<0.3	563	357	153	4.9	7.8	80	136	<0.2
15.11.	<0.3	253	<0.1	<0.3	588	430	153	3.9	8.0	89	161	<0.2

Note: The concentrations of the other target compounds were below the detection limit in all samples.

Table S7. Concentrations of alcohol and nicotine biomarkers in raw wastewater collected at two wastewater collectors in the city of Split in 2016.

Date	c / $\mu\text{g L}^{-1}$					
	Stupe			Katalinica Brig		
	EtS	COT	HCOT	EtS	COT	HCOT
13.7.	10.2	4.6	6.7	11	2.6	4.5
14.7.	8.1	3.5	5.4	17	2.8	4.3
15.7.	12	4.1	5.9	23	2.5	3.8
16.7.	11	4.8	7.5	21	2.8	4.6
17.7.	34	5.1	7.6	27	3.1	5.2
18.7.	16	4.3	6.3	18	3.0	5.0
19.7.	13	4.5	6.4	13	2.8	4.3
24.8.	15	4.8	6.4	13	3.0	3.8
25.8.	15	4.4	7.1	14	3.0	4.5
26.8.	14	4.5	7.0	11	3.0	4.4
27.8.	11	4.2	5.6	16	2.8	4.0
28.8.	17	4.0	6.9	14	2.5	3.9
29.8.	6.6	4.4	7.0	7.4	2.9	4.4
30.8.	12	4.2	6.7	10	2.0	4.2
9.11.	8.0	3.7	4.9	8.9	2.2	3.9
10.11.	7.6	4.3	6.5	5.5	2.5	3.9
11.11.	8.5	3.4	4.9	4.1	2.1	2.8
12.11.	9.1	3.5	4.9	8.0	2.4	3.7
13.11.	9.2	3.9	5.6	8.7	2.6	3.9
14.11.	11	4.0	6.5	7.2	2.8	4.1
15.11.	7.1	4.1	6.2	7.2	3.1	4.5

Table S8. Daily mass loads of target biomarkers (g day⁻¹) in two wastewater collectors in the city of Split in 2016.

Biomarker	OFF-TOURIST REFERENCE WEEK			PEAK-TOURIST REFERENCE WEEK			FESTIVAL WEEK		
	Range	Average ± SD	Median	Range	Average ± SD	Median	Range	Average ± SD	Median
MOR	20 – 34	28 ± 4	28	32 – 42	35 ± 3	34	28 – 37	31 ± 4	30
M3G	5.4 – 25	13 ± 8	13	4.5 – 12	7.3 ± 2.4	7.1	0.78 – 1.4	1.1 ± 0.2	1.1
M6G	1.5 – 4.6	2.5 ± 1.2	2.2	1.8 – 2.5	2.1 ± 0.2	2.1	0.83 – 2.1	1.5 ± 0.5	1.8
norMOR	0.98 – 2.1	1.6 ± 0.4	1.6	1.7 – 2.3	2.0 ± 0.3	2.1	1.5 – 1.8	1.6 ± 0.1	1.6
BE	33 – 73	50 ± 16	41	56 – 79	64 ± 8	62	41 – 147	92 ± 40	88
COC	15 – 32	22 ± 6	18	22 – 36	27 ± 5	25	16 – 42	28 ± 10	30
AMP	13 – 24	17 ± 4	14	17 – 36	23 ± 6	23	18 – 43	30 ± 9	28
MAMP	0.20 – 0.64	0.40 ± 0.17	0.38	0.30 – 1.4	0.72 ± 0.42	0.52	0.34 – 3.0	0.93 ± 0.93	0.71
MDMA	3.0 – 22	7.7 ± 6.6	4.9	13 – 21	17 ± 4	17	29 – 610	242 ± 246	129
THC-COOH	19 – 25	22 ± 2	22	15 – 22	19 ± 2	20	11 – 18	15 ± 2	16
PEPH	18 – 36	24 ± 6	24	17 – 25	21 ± 3	22	11 – 19	15 ± 3	16
KET	BLD*	–	–	0.21 – 0.50	0.33 ± 0.11	0.31	1.4 – 6.1	3.6 ± 1.5	3.5
norKET	BLD* – 0.29	0.21 ± 0.06	0.19	0.32 – 0.62	0.44 ± 0.12	0.39	0.50 – 2.1	1.3 ± 0.6	1.1
4-FA	BLD*	–	–	BLD*	–	–	BLD* – 2.4	0.91 ± 1.02	0.26
MDA	BLD*	–	–	BLD*	–	–	BLD* – 16	11 ± 6	13
DMOR	BLD*	–	–	0.14 – 0.33	0.23 ± 0.09	0.19	0.19 – 0.36	0.29 ± 0.05	0.29
COD	11 – 18	15 ± 2	15	18 – 21	20 ± 1	20	15 – 18	17 ± 1	17
OC	BLD*	–	–	BLD*	–	–	BLD* – 1.1	0.81 ± 0.22	0.82
norHC	BLD*	–	–	BLD*	–	–	0.13 – 0.27	0.19 ± 0.04	0.19
TRA	33 – 69	46 ± 13	41	42 – 57	50 ± 5	50	41 – 50	43 ± 3	43
O-TRA	24 – 33	30 ± 3	32	42 – 56	50 ± 5	50	41 – 49	44 ± 4	42
N-TRA	9.3 – 15	12 ± 2	12	12 – 16	14 ± 1	15	12 – 18	14 ± 2	14
SFNT	BLD* – 0.31	0.19 ± 0.10	0.12	0.05 – 0.17	0.11 ± 0.03	0.11	0.06 – 0.13	0.11 ± 0.02	0.10
BUP	0.45 – 0.85	0.65 ± 0.16	0.60	0.56 – 0.85	0.70 ± 0.10	0.70	0.26 – 0.97	0.54 ± 0.24	0.45
MTHD	4.0 – 6.9	5.2 ± 1.0	5.0	4.4 – 7.7	6.0 ± 1.1	5.8	2.4 – 2.8	2.6 ± 0.2	2.5
EDDP	7.6 – 12	9.2 ± 1.5	9.3	9.6 – 17	12 ± 3	10	7.8 – 12	10 ± 1	10
PP	BLD* – 0.25	0.13 ± 0.08	0.11	BLD* – 0.36	0.14 ± 0.11	0.09	0.06 – 0.20	0.13 ± 0.04	0.11
EtS	403 – 734	544 ± 139	479	478 – 955	824 ± 168	855	652 – 1654	1005 ± 325	958
COT	182 – 252	218 ± 28	219	192 – 275	244 ± 27	254	202 – 228	219 ± 9	221
HCOT	267 – 368	324 ± 40	321	312 – 400	367 ± 38	385	308 – 352	334 ± 18	340

* Mass loads in samples where the biomarker concentration was below the detection limit are not reported.

Table S9. Estimation of consumption of illicit drugs, therapeutic opioids, nicotine, and alcohol in the city of Split in 2016.

Drug	Unit	OFF-TOURIST REFERENCE WEEK			PEAK-TOURIST REFERENCE WEEK		
		Range	Average \pm SD	Median	Range	Average \pm SD	Median
Heroin	mg day ⁻¹ 1000 inhabitants 15 – 64 years ⁻¹	430–945	710 \pm 170	697	633–789	696 \pm 52	697
Cocaine		827–1822	1250 \pm 398	1022	1319–1852	1515 \pm 194	1464
Amphetamine		260–457	324 \pm 78	274	300–656	420 \pm 115	416
Methamphetamine		6.0–20	12 \pm 5	12	8.5–41	21 \pm 12	15
MDMA		91–661	235 \pm 201	149	365–612	475 \pm 103	492
Ketamine		–	–	–	6.9–16	11 \pm 4	10
Methadone		118–180	143 \pm 24	146	140–256	173 \pm 43	152
Pseudoephedrine		mg day ⁻¹ 1000 inhabitants >15 years ⁻¹	114–229	152 \pm 38	152	100–151	127 \pm 17
Codeine	215–344		281 \pm 38	284	326–386	357 \pm 23	355
Tramadol	601–1277		840 \pm 232	752	720–984	866 \pm 81	868
Cannabis	g day ⁻¹ 1000 inhabitants 15 – 64 years ⁻¹	24–31	28 \pm 2	28	18–26	23 \pm 3	23
Nicotine	g day ⁻¹ 1000 inhabitants >15 years ⁻¹	3.2–4.3	3.8 \pm 0.5	3.8	3.3–4.3	4.0 \pm 0.4	4.2
Alcohol	kg day ⁻¹ 1000 inhabitants >15 years ⁻¹	6.8–12	9.2 \pm 2.4	8.1	7.6–15	13 \pm 3	14

Note: Ketamine consumption in the off-season reference week was not estimated because concentrations in all samples were below the detection limit.