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Retrospective spatiotemporal study of antidepressants in Slovenian wastewaters

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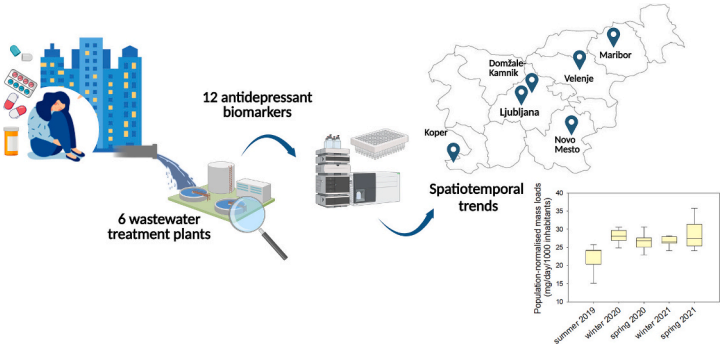
HIGHLIGHTS

- Evaluation of spatiotemporal trends in antidepressant use during the COVID-19 pandemic
- *O*-desmethylvenlafaxine had the highest population-normalised mass loads.
- Antidepressants use increased during the pandemic.
- No clear pandemic-related spatial patterns found.
- The WBE results align with the annual prescription data in Slovenia.

GRAPHICAL ABSTRACT



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ABSTRACT

This study utilizes wastewater-based epidemiology (WBE) to evaluate spatiotemporal changes in the consumption of antidepressants before and during the COVID-19 pandemic in Slovenia. Composite 24-h influent wastewater samples (n = 210) were collected from six wastewater treatment plants between summer 2019 and spring 2021. The samples were extracted using 96-well solid-phase extraction and analysed by liquid chromatography-tandem mass spectrometry. The measured concentrations of target antidepressant biomarkers were then converted to population-normalised mass loads (PNMLs), taking into account flow rate and catchment population. Ten biomarkers, including amitriptyline, bupropion, bupropion-OH, citalopram, norcitalopram, normirtazapine, venlafaxine, *O*-desmethylvenlafaxine, trazodone, and moclobemide, were above the lower limit of quantification and were included in the spatiotemporal temporal assessment. The highest PNMLs were detected for *O*-desmethylvenlafaxine (mean ± SD: 82.1 ± 21.2 mg/day/1000 inhabitants) and venlafaxine (38.0 ± 10.6 mg/day/1000 inhabitants), followed by citalopram (27.0 ± 10.7 mg/day/1000 inhabitants). In addition, the mean metabolite/parent compound ratios were comparable with other WBE

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studies indicating consumption rather than direct disposal. Overall, the results indicated significant spatiotemporal variations depending on the location, and the PNMLs of most biomarkers increased during the first wave of the COVID-19 pandemic (spring of 2020). However, no clear spatial patterns were revealed related to the pandemic.

1. Introduction

Since the outbreak of the COVID-19 pandemic, there has been a growing interest in examining the pandemic's impact on people's mental health. Several epidemiological studies worldwide have reported an increase in the consumption of psychoactive pharmaceuticals, such as antidepressants, anxiolytics, sedatives, and hypnotics, during the pandemic due to depression, higher rates of acute psychological distress, insomnia, anxiety, post-traumatic stress disorder, and addiction problems that originated in the general population (Diaz-Camal et al., 2022; Melchor-Martínez et al., 2021; Pazzagli et al., 2022; Tiger et al., 2023; Winkler et al., 2021; Xiong et al., 2020). For these reasons, understanding the mental well-being of the population is vital since mental illness creates a substantial health, social, and economic burden (WHO, 2022). In Slovenia, approximately 85,000 people (4.3 % of the population) suffered from depression in 2019 (GBDS, 2019). In fact, according to OECD data for 2021, Slovenia ranked 13th (63.6 defined daily doses/1000 people) worldwide in antidepressant consumption (Statista, 2021). More recent studies also suggest that the COVID-19 pandemic has been responsible for increased stress, anxiety, and depression, especially among the younger population (Kozina et al., 2022; Rus Prelog et al., 2022), which is reflected in a 10 % increase in antidepressant prescriptions between 2019 and 2020, i.e., when the COVID-19 pandemic began (ZZZS, 2022).

Wastewater-based epidemiology (WBE) is a well-established approach that offers objective, complementary, and anonymous information on consumption and exposure to different xenobiotics in defined populations by analysing human metabolic excretion products (biomarkers) in influent wastewater (Baker et al., 2014). At present, besides the numerous WBE studies on illicit drugs, alcohol, tobacco, and new psychoactive substance consumption (Bade et al., 2021; Estévez-Danta et al., 2022; Gent and Paul, 2021; Gracia-Lor et al., 2020; Huizer et al., 2021; López-García et al., 2020), the application of WBE also extends to a small number of studies addressing psychoactive pharmaceutical consumption (Boogaerts et al., 2021, 2019; Escolà Casas et al., 2021; Laimou-Geraniou et al., 2023; Quireyns et al., 2022; Rice et al., 2020; Riva et al., 2020). Studies have shown that WBE provides valuable information on the consumption patterns of psychoactive pharmaceuticals over time and has the benefit of high temporal resolution. This knowledge holds significance as weekly patterns can shed light on potential recreational use. Additionally, seasonal patterns can highlight temporal changes within months of the year or between the same months in different years. Long-term trends can also provide insights into the stability or fluctuations in the use of antidepressants over time (Tscharke et al., 2016). The WBE approach also provides good spatial resolution sufficient for identifying variations in the usage of psychoactive pharmaceuticals at different locations, including within countries at the city/village level or monitor consumption trends at the scale of regions and suburbs within a metropolitan area (Laimou-Geraniou et al., 2023). This advanced spatial resolution offers the potential to conduct area-based evaluations that would otherwise be unattainable solely through nationally compiled prescription data.

Several studies have already utilized the WBE approach to identify the effects of the COVID-19 pandemic on spatiotemporal trends in psychoactive pharmaceutical consumption patterns (Alygizakis et al., 2021; Boogaerts et al., 2023a; Galani et al., 2021; Reinstadler et al., 2021; Tomsone et al., 2022; Yavuz-Guzel et al., 2022). However, only a limited number of WBE studies include countries in Central Europe, i.e. Austria (Reinstadler et al., 2021), Slovakia (Fáberová et al., 2017; Mackuľak et al., 2016) and the Czech Republic (Baker et al., 2014). Accordingly, this study aims to fill this gap by monitoring the spatiotemporal use of antidepressants at a higher degree of granularity in Slovenia using WBE before and during the pandemic. Fortunately, a comprehensive sampling campaign conducted from the summer of 2019 to the spring of 2021 (Verovšek et al., 2023) provided a valuable opportunity to retrospectively evaluate the influence of the pandemic on the usage patterns of specific antidepressant medications in Slovenia.

2. Materials and methods

2.1. Sampling

Six wastewater treatment plants (WWTPs) from across Slovenia (Ljubljana, Maribor, Domžale-Kamnik, Velenje, Koper and Novo Mesto), covering approximately 30 % of the Slovenian population, were included in the study (Table S1). Influent wastewater samples (24-h composites) were collected for seven consecutive days, representing "normal" conditions at the WWTPs; these sampling periods excluded any special events such as festivals, maintenance activities, or extreme weather events. The samples ($n = 210$) were collected during the summer of 2019, winter of 2020, spring of 2020, winter of 2021 and spring of 2021. All samples were stored at −20 °C until analysis. It is noteworthy that the first pandemic wave hit Slovenia in the spring of 2020 (March 2020), the second wave started in the autumn of 2020 (October 2020), and the third in the spring of 2021 (March 2021).

2.2. Target biomarkers

Target biomarkers of antidepressants were selected based on sales, prescription frequency, preliminary WBE concentrations (Laimou-Geraniou et al., 2022) in Slovenia and available validated analytical methods (Boogaerts et al., 2023b). In addition, all the investigated compounds have high in-sample stability (Boogaerts et al., 2019; Choi et al., 2020). The final list of biomarkers (Table S2) included amitriptyline (AMIT), bupropion (BUP), bupropion-OH (BUP-OH), citalopram (CIT), norcitalopram (nor-CIT), melitracen (MEL), moclobemide (MOC), normirtazapine (nor-MIRT), trazodone (TZD), *m*-chlorophenylpiperazine (mCPP), venlafaxine (VEN), and *O*-desmethylvenlafaxine (ODVEN). Even though sertraline is among the most prescribed antidepressants in Slovenia, it was excluded from the bioanalytical assay because it did not pass the requirements for method validation. All details regarding reagents, standards, materials and suppliers can be found in the Supplementary material (SM: Materials and methods).

2.3. Sample preparation and analysis

A previously validated method, as described by Boogaerts et al. (2023b), was employed for sample preparation and analysis. The procedure involved the following steps. Initially, 2 mL of influent wastewater was spiked with 6 μL of formic acid and 20 μL of an internal standard mixture. Centrifugation (10,000 ×*g*, 5 min) was performed to separate the solid particles. Subsequently, extraction was carried out using 96-well Oasis MCX solid phase extraction plates (SPE, Oasis MCX 10 mg). The sorbents were first conditioned under vacuum using 500 μL of methanol and 500 μL ultrapure water, with 0.3 % *v*/v formic acid. Then, the supernatant (1.8 mL) was loaded under vacuum, followed by a series of washing steps using (i) 500 μL ultrapure water +0.3 % formic acid, (ii) 500 μL methanol +0.3 % formic acid, and finally, (iii) 500 μL

30/70 (*v*/v) methanol/water. The sorbent was dried under vacuum for 5 min, after which the analytes of interest were eluted with 200 μL of 5 % (v/v) ammonia in a methanol solution. The eluent was then evaporated to dryness on a Biotage SPE Dry 96 device with heated nitrogen (37 °C, 25 L/min bottom, 50 L/min top). The resulting residues were reconstituted by adding 120 μL of 95/5 (v/v) ultrapure water/methanol containing 0.1 % formic acid. The samples were then vortexed (2.5 min at 1500 ×*g*) and filtered (Agilent 0.2 μm polypropylene, 96 well, 1 mL). Finally, the samples were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Chromatographic separation was carried out on a Waters Atlantis T3 Column (150 × 2.1 mm, 3 μm) maintained at 30 °C with Mobile Phase A consisted of 0.1 % v/v formic acid in ultrapure water, and Mobile Phase B consisted of 0.1 % v/v formic acid in methanol. Compounds were eluted using gradient elution (total run time 25 min). The gradient was as follows: 0–0.5 min: 5 % B; 0.5–5 min: increase to 25 % B; 5–16 min: increase to 30 % B; 16–21 min: increase to 95 % B; 21–21.1 min decrease to 5 % B; and equilibration at 5 % B up to 25 min. The flow rate was 0.275 mL/min. The injection volume of 4 μL was used based on the sensitivity and peak shape of the analytes of interest.

Complete details can be found in the supplementary information (see SM: Materials and methods, Measured compounds, and Table S3).

2.4. Back-calculations

In the present study, daily mass loads of antidepressant biomarkers were calculated by multiplying their measured values with the corresponding daily wastewater flow rates. Mass loads were then normalised to the number of people contributing to the WWTPs to back-calculate population-normalised mass loads (PNMLs) expressed as mg biomarker/day/1000 inhabitants. The WWTPs provided data for the daily flow rates (L/day) and population equivalents. The equation for the estimation of the PNMLs is given in Eq. (1):

Back-calculation to population-normalised mass loads

$$\text{PNMLs (mg/day/1000 inhabitants)} = \frac{\textit{concentration}\left(\frac{ng}{L}\right) \times \textit{wastewater flow rate}\left(\frac{L}{day}\right)}{\textit{population equivalent}} \quad (1)$$

2.5. Statistical analysis

A statistical evaluation was conducted using SigmaPlot 14.0 and R Studio. The normality and equality of variance of the data were tested using the Shapiro-Wilk test and Brown-Forsythe test, respectively, at a significance level of 5 %. To compare differences between two groups, we employed either a Student *t*-test (data normality and equality of variance were assumed), a Welch t-test (data normality but equality of variance was not assumed), or a Mann-Whitney Rank Sum Test (data normality was not assumed) at the 5 % significance level. To assess spatiotemporal differences in the use of psychoactive pharmaceuticals, either One-way ANOVA or Kruskal-Wallis test (for non-parametric analysis), followed by Tukey's post-hoc test, was used. In addition, to evaluate temporal variations in psychoactive pharmaceutical use, the calculated PNMLs (mg/day/1000 inhabitants) were scaled to daily proportions by dividing the mass loads by the sum of the mass loads within seven days of sampling. In this manner, locations could be combined to examine overall temporal patterns in psychoactive pharmaceutical use while facilitating comparisons between different periods within a single location. A Mann-Kendall trend analysis was applied to detect any monotonic increase (positive S value) or decrease (negative S value) in psychoactive pharmaceutical use across the 7-day sampling periods at all sampling locations.

3. Results and discussion

3.1. Method application

Out of the twelve biomarkers investigated, ten were quantified at least once (Table 1) and were subsequently selected for spatiotemporal analysis. However, melitracen and mCPP were below the lower limit of quantification (LLOQ), which is the minimum concentration of an analyte that can be accurately and precisely measured. Consequently, these two compounds were excluded from the analysis.

The measured PNMLs in all locations during the sampling periods can be found in Table S4. On average, the highest PNMLs were found for the antidepressants *O*-desmethylvenlafaxine (mean ± SD: 82.1 ± 21.2 mg/day/1000 inhabitants), venlafaxine (38.0 ± 10.6 mg/day/1000 inhabitants), citalopram (27.7 ± 11.4 mg/day/1000 inhabitants), and norcitalopram (12.3 ± 7.2 mg/day/1000 inhabitants) across all samples. This observation is consistent with the annual number of prescriptions obtained from the database of the Health Insurance Institute

Table 1
Quantification frequency (percentage of samples >LLOQ) of target compounds during all sampling periods (summer 2019, winter/spring 2020 and winter/spring 2021) in Ljubljana, Maribor, Novo Mesto, Velenje, Domžale-Kamnik and Koper (n = 35 per location).

Compound	LLOQ (ng/L)	Quantification frequency (%)					
		Ljubljana (LJ)	Maribor (MB)	Novo Mesto (NM)	Velenje (VE)	Domžale-Kamnik (DK)	Koper (KP)
Amitriptyline	25	0	0	66	0	0	0
Bupropion	10	6	6	0	0	0	21
Bupropion-OH	10	100	100	100	91	100	100
Citalopram	10	100	100	100	100	100	100
Norcitalopram	20	100	100	100	85.7	100	100
Melitracen	25	0	0	0	0	0	0
Moclobemide	2.5	100	100	100	97	100	94
Normirtazapine	10	91	94	91	6	66	89
Trazodone	5	100	100	100	94	100	100
mCPP	30	0	0	0	0	0	0
Venlafaxine	5	100	100	100	97	100	100
O-desmethylvenlafaxine	50	100	100	100	97	100	100

of Slovenia (ZZZS, 2022). Venlafaxine, citalopram, trazodone, and mirtazapine were the most frequently prescribed antidepressants. Similar findings have been reported in other studies conducted in Belgium, Greece, India, Turkey, and the USA, where the highest PNMLs of antidepressants were observed for venlafaxine/*O*-desmethylvenlafaxine (Boogaerts et al., 2019; Kosma et al., 2019; Papageorgiou et al., 2016; Quireyns et al., 2022; Skees et al., 2018; Subedi et al., 2017; Yavuz-Guzel et al., 2022).

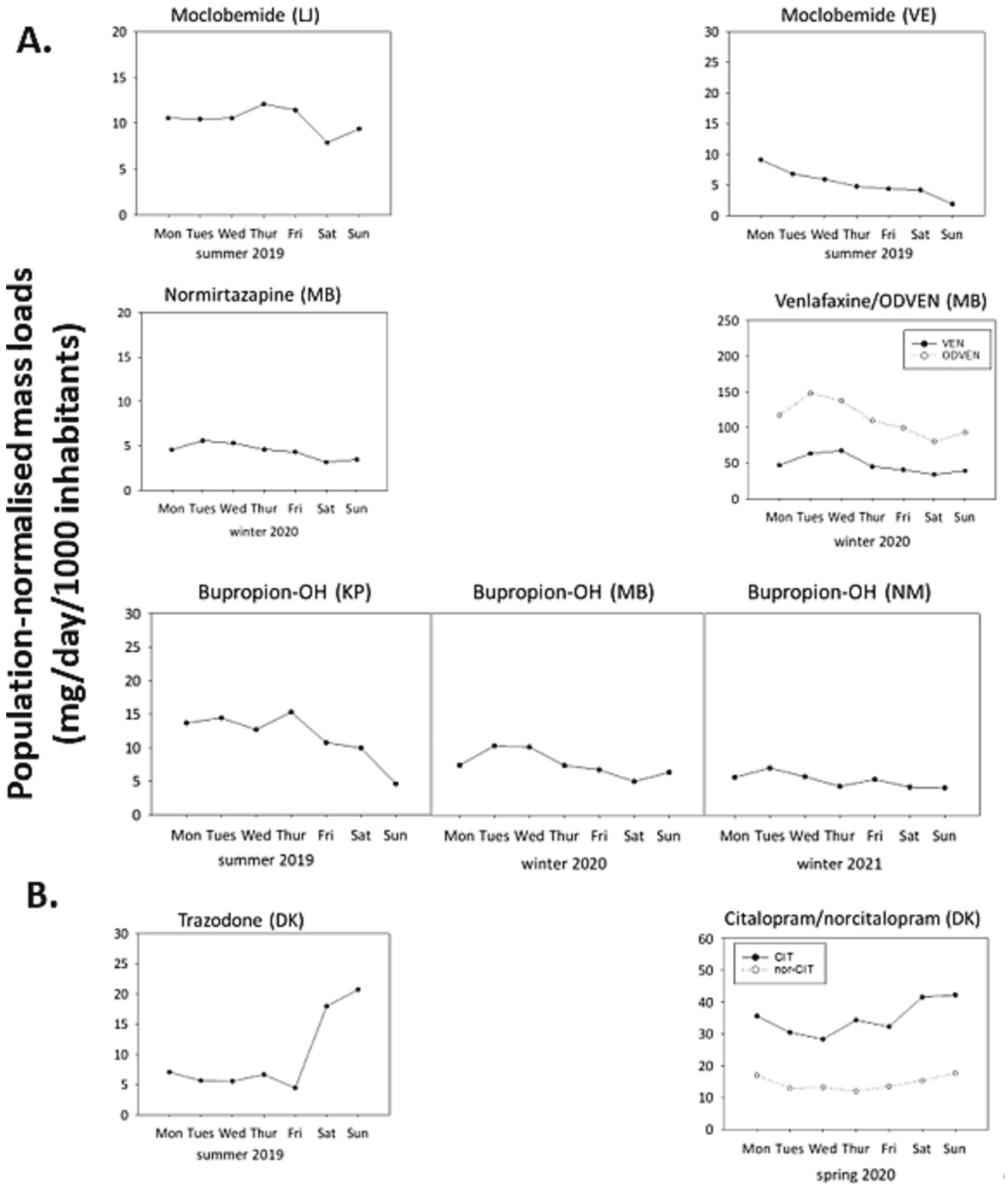

Fig. 1. Plots showing population-normalised mass loads (PNMLs) in mg/day/1000 inhabitants of biomarkers of antidepressants. Data shows statistically significant lower PNMLs and a decreasing trend during weekends (A) and higher PNMLs and an increasing trend at weekends (B).

3.2. Temporal differences in antidepressant use

3.2.1. Weekly trends (weekdays vs. weekends)

In general, the consumption of antidepressants is expected to be stable since patients are required to take their medication continuously and at strict time intervals. Initiating or discontinuing treatment is usually done by gradually adjusting the dosage. When a trend analysis was conducted to examine weekday-weekend (Mon-Fri vs Sat-Sun) antidepressant use, a few minor trends were identified; however, no clear overall pattern in the PNMLs emerged. Most statistically significant differences were observed prior to the pandemic, with a decrease ($p < 0.05$) in the PNMLs of moclobemide in Ljubljana (summer 2019), normirtazapine in Maribor (winter 2020), and bupropion-OH in Koper (summer 2019) during the weekend (Fig. 1A). Conversely, a statistically significant increase ($p < 0.05$) in the PNMLs of trazodone in Domžale-Kamnik (summer 2019) was observed during the weekend (Fig. 1B). Furthermore, the negative S-value of the Mann-Kendall trend indicated a decreasing trend over the sampling week (Fig. 1A) for moclobemide ($S = -21$) in Velenje (summer 2019), venlafaxine/*O*-desmethylvenlafaxine ($S = -13/-15$), and bupropion-OH ($S = -15$) in Maribor (winter 2020).

The only discernible effect of the pandemic, as indicated by a comparison between weekdays and weekends, was observed for citalopram/norcitalopram in Domžale-Kamnik during the first wave in spring 2020 and for bupropion-OH in Novo Mesto during the second wave in winter 2021. The data demonstrate a statistically significant increase in the PNMLs of citalopram and norcitalopram during the weekend ($p < 0.05$), whereas the PNMLs of bupropion-OH decreased ($p < 0.05$). The Mann-Kendall trend analysis revealed similar outcomes, indicating a monotonic decrease in the PNMLs of bupropion-OH in Novo Mesto ($S = -15$) during the sampling week of winter 2021. Conversely, the PNMLs of the other antidepressants exhibited independent distributions throughout the sampling periods, suggesting consistent use throughout the week (Fig. S1-S6). A trend in the PNMLs of antidepressants between weekdays and weekends was also observed in Belgium during the pre-pandemic period and second lockdown (October 2020–May 2021), with the highest PNMLs mainly observed during the weekends (Boogaerts et al., 2023a). However, in contrast to the findings of Boogaerts et al. (2023a) and this study, other studies by Yavuz-Guzel et al. (2022) and Reinstadler et al. (2021) did not reveal a statistically significant difference in antidepressant consumption between weekdays and weekends. Consequently, it is challenging to draw definite conclusions regarding whether the differences observed in our data are related to the pandemic or influenced by other factors, such as population movement.

During the pandemic and the associated social restrictions, including lockdown measures and remote working, the reduced disparity in the PNMLs between workdays and weekends could be attributed to changes in the socio-demographic characteristics of the population within the catchment area. To test this, Boogaerts et al. (2023a) employed a dynamic population proxy, specifically mobile phone data, to attribute the observed weekly pattern to demographic shifts induced by the pandemic measures within the catchment population. These shifts included reduced weekday commuting, increased weekend visitors, and the impact of reduced tourism. However, in our study, we relied on a fixed population size provided by the WWTPs, as accessing mobile phone data was not feasible.

3.2.2. Long-term trends (2019–2021)

Diverse temporal patterns in psychoactive pharmaceutical use were identified across the six locations in Slovenia (Fig. 2), with Domžale-Kamnik exhibiting the highest degree of variation while Velenje and Koper showed the least variability. In Domžale-Kamnik, we observed a significant increase ($p < 0.05$) in the PNMLs of bupropion-OH, citalopram, norcitalopram, moclobemide and normirtazapine in both winter and spring 2020 compared to winter and spring 2021 (Fig. 2 and Fig. S7). Conversely, in Ljubljana, there was relatively less temporal variation in the usage of antidepressants, suggesting a consistent pattern in the PNMLs of these medications, except for moclobemide which exhibited a 50 % decrease in consumption from 10.3 ± 1.4 mg/day/1000 inhabitants in summer 2019 to 5.1 ± 1.7 mg/day/1000 inhabitants during lockdown in spring 2021 (Fig. 2 and Fig. S8).

A similar trend for moclobemide was also observed in Novo Mesto, with a 36 % decrease in PNMLs from summer 2019 to spring 2021 (Fig. 2 and Fig. S9). This decline was also reflected in the number of annual prescriptions for moclobemide, which decreased by 16 % between 2019 and 2021. Moclobemide belongs to a class of antidepressants that, despite being among the most effective for treating unipolar major depression, is not commonly prescribed due to dietary restrictions, side effects and safety concerns (Sub Laban and Saadabadi, 2023). It is worth noting that there were no significant fluctuations in the mass loads of trazodone, except during winter 2021 when the PNMLs reached 27.9 mg/day/1000 inhabitants on Wednesday, compared to the average of 7.4 ± 1.1 mg/day/1000 inhabitants during the rest of the sampling periods. Additionally, in Novo Mesto, only a few temporal variations were observed. Except for moclobemide, there was a general increase in the PNMLs of several antidepressants during the COVID-19 pandemic (Fig. 2 and Fig. S9). For instance, the PNMLs of trazodone increased by 35 % between the pre-pandemic and lockdown periods. Moreover, an increase in the PNMLs of amitriptyline, normirtazapine, venlafaxine, and *O*-desmethylvenlafaxine, was also observed, especially during the spring 2021 lockdown (7.9, 5.2, 35.7, and 67.6 mg/day/1000 inhabitants, respectively), compared to winter 2020 (3.1, 2.8, 22.2, 44.2 mg/day/ 1000 inhabitants, respectively).

The variations in PNMLs may be attributed to changes in the population dynamics during the pandemic, such as the implementation of work-from-home policies, restrictions on movement between municipalities, and the absence of tourism. According to the available Mobility Report for Slovenia, the percentage of time spent in the municipality of residence and working from home increased to 25 % and 55 %, respectively, during the spring 2020 and winter 2021 lockdown periods compared to the pre-pandemic period (Mathieu et al., 2020). Another possible explanation could be the sharp decline in international tourist arrivals in Slovenia, mainly in Ljubljana, during the pandemic. Annual reports indicate an average 60–70 % decrease in tourist numbers between 2019 and 2021 (Tourism in numbers/Turizem v številkah, 2020,2021). Boogaerts et al. (2023a) also observed increased PNMLs of antidepressants during the initial lockdown phase (March–June 2020), likely resulting from the demographic shifts occurring during the COVID-19 pandemic rather than consumption habits.

In Velenje, there was an increasing trend in the consumption of trazodone during both winter and spring 2021, with a 60 % increase in the PNMLs observed in spring 2021 (18.8 ± 10.5 mg/day/1000 inhabitants) compared to spring 2020 (7.5 ± 0.9 mg/day/1000 inhabitants). The data also indicated slightly increased PNMLs for citalopram, norcitalopram, venlafaxine, and *O*-desmethylvenlafaxine from summer 2019 to spring 2021 (Fig. 2 and Fig. S10). However, it is important to note that the PNMLs for citalopram during one day (Tuesday) in the sampling week of spring 2020 were exceptionally high (351.3 mg/day/1000 inhabitants) compared to the rest of the week (average 30.1 ± 13.4 mg/day/1000 inhabitants). This fluctuation was not observed in the PNMLs of its metabolite, norcitalopram (11.6 mg/day/1000 inhabitants on that day and 11.0 ± 1.7 mg/day/1000 inhabitants on average). This discrepancy suggests direct disposal of citalopram rather than consumption (Fig. 2 and Fig. S10). In Koper (Fig. 2 and Fig. S11), there were no significant variations observed in the consumption of antidepressants, except for bupropion, which showed an increase in consumption between 2020 (<LLOQ) and 2021 (4 ± 1.7 mg/day/1000 inhabitants). Similar studies have reported different findings; for instance, some show a significant increase in PNMLs (Boogaerts et al., 2023a; Yavuz-Guzel et al., 2022), while others found no statistically significant difference or a slight increase, except for venlafaxine, which exhibited a notable increase (Alygizakis et al., 2021; Galani et al., 2021; Reinstadler et al., 2021).

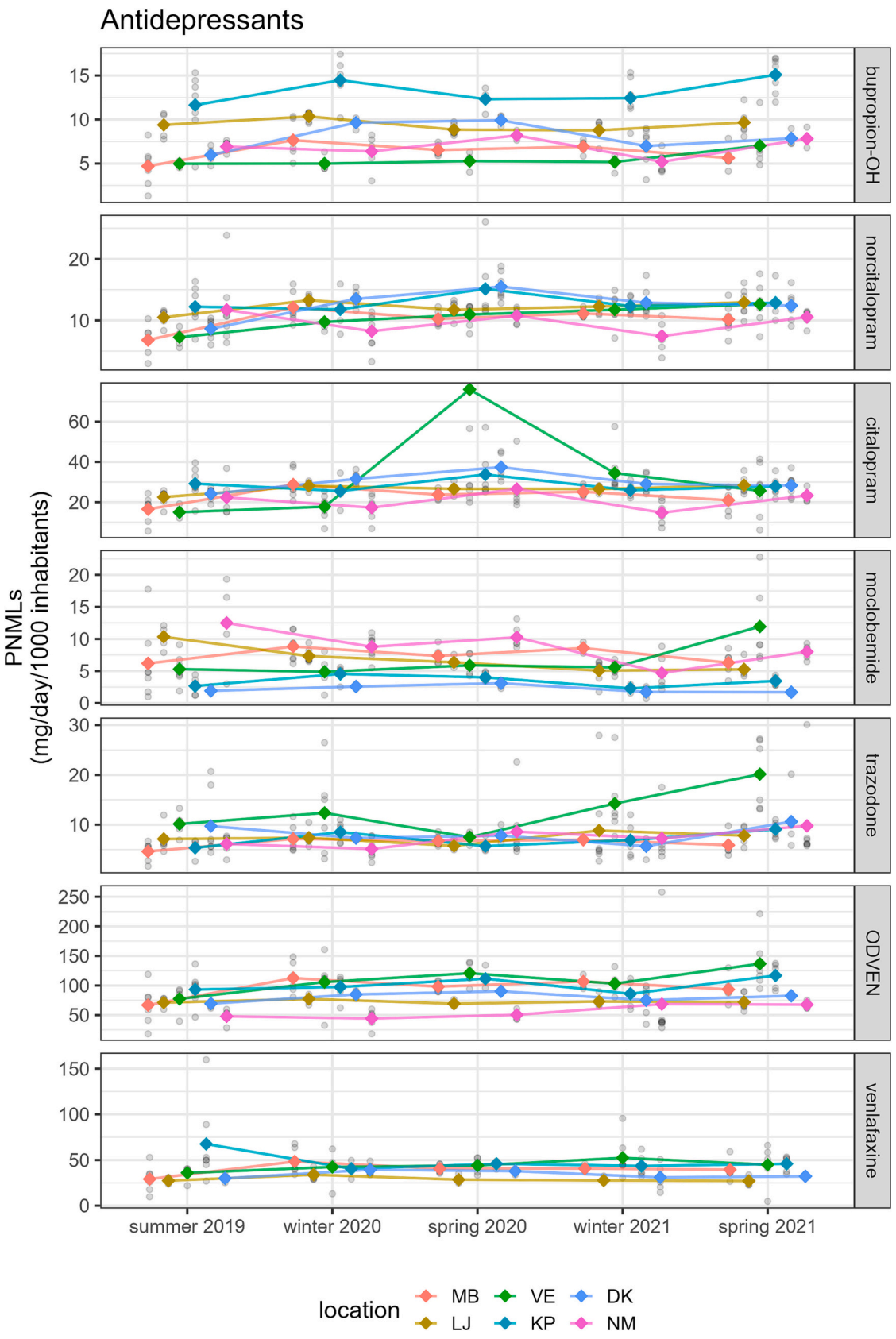

Fig. 2. Graphs showing the long-term trends in the population-normalised mass loads (PNMLs) of the different antidepressant biomarkers in all WWTPs. Only compounds with detection frequency > 85 % in each location are shown. Coloured dots represent the average of 7-day values of PNMLs which are shown in grey. For citalopram, the outlier due to dumping has been removed from the figure (but not the mean calculation).

The overall increase in the PNMLs of venlafaxine, normirtazapine, and trazodone from summer 2019 to winter 2021 aligns with the rise in their annual number of prescriptions, specifically a 4 % increase in venlafaxine, a 5 % increase for the parent drug mirtazapine, and a 10 % increase for trazodone ("ZZZS", 2022). However, there was a 7 % increase in mirtazapine annual prescriptions in 2020, followed by a slight 2 % decrease compared to 2021. Similarly, bupropion and citalopram exhibited a comparable trend, with a 5 % and 7 % increase in the annual number of prescriptions between 2019 and 2020, respectively, returning to similar levels as 2019 in 2021. It is important to note that sales and prescription data are compiled nationally, while WBE operates at different spatial resolutions, such as city-level or national-level assessments, and this may account for the discrepancies between the number of prescriptions and WBE data. Furthermore, such differences could arise from (i) prescriptions may not be filled; (ii) filled prescriptions may not be consumed; (iii) pharmaceuticals may not be taken at the recommended dose and (iv) specific insurance modalities are not included.

3.2.3. Seasonal trends

This study compared seasons across different years, which means that longitudinal trends could have influenced the observed variations. However, verifying intra-year seasonal patterns is challenging due to the limited coverage of each season in the sample collection. Also, since the samples were collected as a part of a previous study (Verovšek et al., 2023), i.e., not specifically for studying the effects of the pandemic, the sample size was limited in terms of the seasons and periods within each season. Nevertheless, longitudinal trends across all locations, resulting from ANOVA and Kruskal-Wallis test with Tukey's post-hoc test, are visualised in Fig. S13-S18. The results demonstrate comparable patterns in Ljubljana, Maribor, and Domžale-Kamnik. In winter 2020, the PNMLs for four (citalopram, norcitalopram, venlafaxine, and *O*-desmethylvenlafaxine), five (citalopram, norcitalopram, venlafaxine, *O*-desmethylvenlafaxine, and normirtazapine) and six (bupropion-OH, citalopram, norcitalopram, venlafaxine, *O*-desmethylvenlafaxine, and moclobemide) antidepressant biomarkers, respectively, were statistically higher compared to summer 2019. This observation could be attributed to seasonal variation, as both sampling campaigns occurred before the pandemic. Similar findings of higher mass loads during winter have been reported in long-term monitoring studies for antidepressants (Golovko et al., 2014), such as citalopram, venlafaxine and tramadol (Mackuľak et al., 2016).

This seasonal increase could be associated with seasonal affective disorder (SAD), a mood disorder where individuals with normal mental health throughout most of the year exhibit depressive symptoms at the same time each year, commonly but not always in winter months when sunlight is reduced. In Maribor, seasonal variation is more apparent, as the PNMLs of citalopram, norcitalopram, *O*-desmethylvenlafaxine and normirtazapine in both winter 2020 and winter 2021 were higher compared to summer 2019 ($p < 0.05$) (Fig. 2 and Fig. S12).

3.3. Spatial differences

Spatial differences, determined by ANOVA or Kruskal-Wallis test with Tukey's post-hoc test, are visualised in Fig. S19. The data reveals spatial trends for all compounds in almost all sampling periods (Fig. 3). These differences could be attributed to variations in the demographic characteristics of the catchment areas, such as average age, the proportion of university students, and the tourist numbers. On average, the highest PNMLs for *O*-desmethylvenlafaxine were obtained in Velenje (106.9 ± 19.6 mg/day/1000 inhabitants), displaying a statistically significant difference ($p < 0.05$) compared to Novo Mesto, which had the lowest PNMLs (55.7 ± 11.5 mg/day/1000 inhabitants). Both venlafaxine and *O*-desmethylvenlafaxine exhibited similar spatial variations across all sampling periods, with the highest variation observed in spring 2020 during the first wave of the pandemic. Notably, they show identical spatial variations in winter and spring 2020 (Fig. 3. and Fig. S20).

Spatial variations were observed for citalopram and its metabolite norcitalopram in winter 2020, while no spatial variations were observed in spring 2021. The highest PNMLs (Fig. 3 and Fig. S20) for trazodone (12.3 ± 4.4 mg/day/1000 inhabitants) were found in Velenje (12.3 ± 4.4 mg/day/1000 inhabitants). Variations in PNMLs of trazodone were mainly observed during the winter and spring of 2021. Amitriptyline was only detected above LLOQ in Novo Mesto. On the other hand, bupropion was detected and quantified primarily in Koper (sampling periods summer 2019 and winter and spring 2021), as well as in Ljubljana and Maribor, while it was <LLOQ in Velenje, Domžale-Kamnik and Novo Mesto (Fig. 3 and Fig. S20). Koper exhibited the highest PNMLs for bupropion-OH (13.2 mg/day/1000 inhabitants), consistent with the higher PNMLs of bupropion as the parent compound in the same location. Moclobemide had significantly lower PNMLs in Domžale-Kamnik than the other WWTPs, with most spatial variations observed in spring 2020 during the first lockdown. However, for normirtazapine, which was measured in all WWTPs except for Velenje (only in spring 2021 was >LLOQ), the greatest spatial variation was in spring 2021 (Fig. 3 and Fig. S20). Overall, the results show that spatial variations were mainly evident for bupropion, venlafaxine, moclobemide and tramadol during spring 2020, coinciding with the first wave of the pandemic in Slovenia.

3.4. Parent compound/metabolite ratios

In the present study, both the parent compound and the metabolite were included for the following substances: bupropion, citalopram, trazodone, and venlafaxine. The metabolite/parent compound ratio (M/PC ratio) was not calculated for trazodone/mCPP since the concentration of mCPP was below the LLOQ. The M/PC ratios for citalopram/norcitalopram and venlafaxine/*O*-desmethylvenlafaxine remained relatively stable across all the sampling periods and locations, with a relative standard deviation (% RSD) of the measured M/PC ratio below 25 %. The measured M/PC ratios in influent wastewater are consistent with other studies (Table 2). However, the average M/PC ratios of bupropion/bupropion-OH were slightly lower than in other WBE studies (Boogaerts et al., 2019). Notably, the bupropion/bupropion-OH ratios were only above the LLOQ in a limited number of samples. The relatively stable spatiotemporal pattern in the M/PC ratios suggests that the mass loads of the biomarkers likely derive from consumption rather than direct disposal of the parent compound. However, for citalopram, there was a notable change in the M/PC (0.03 compared to the average of 0.44) on a specific sampling day during spring 2020 in Velenje, indicating potential dumping (Section 3.2.2).

3.5. Limitations of the study

The sampling campaign involved six WWTPs in urban and rural areas, accounting for just 30 % of the Slovenian population; however, they cover the main geographic regions, and are used in the SCORE monitoring. Due to logistical considerations and the involvement of multiple sampling locations and periods, the start of sampling collection varied by location, resulting in different orders of data points across sampling periods and locations. Therefore, the data was arranged from Monday to Sunday to facilitate statistical comparisons and evaluate spatiotemporal trends. Another limitation is the inclusion of only one week of sampling within each period (summer 2019, winter 2020, spring 2020, winter 2021 and spring 2021) for the spatiotemporal comparisons. Consequently, consumption rates may not fully represent the rest of the year. However, since all sampling periods were conducted in a "typical" week, which should reflect baseline consumption, we assume that weekdays and weekends represent baseline use within a regular working week. Furthermore, the fact that we did not consistently sample in the same season adds complexity to the interpretation of any seasonal trends.

Another limitation is the use of fixed population data provided by the

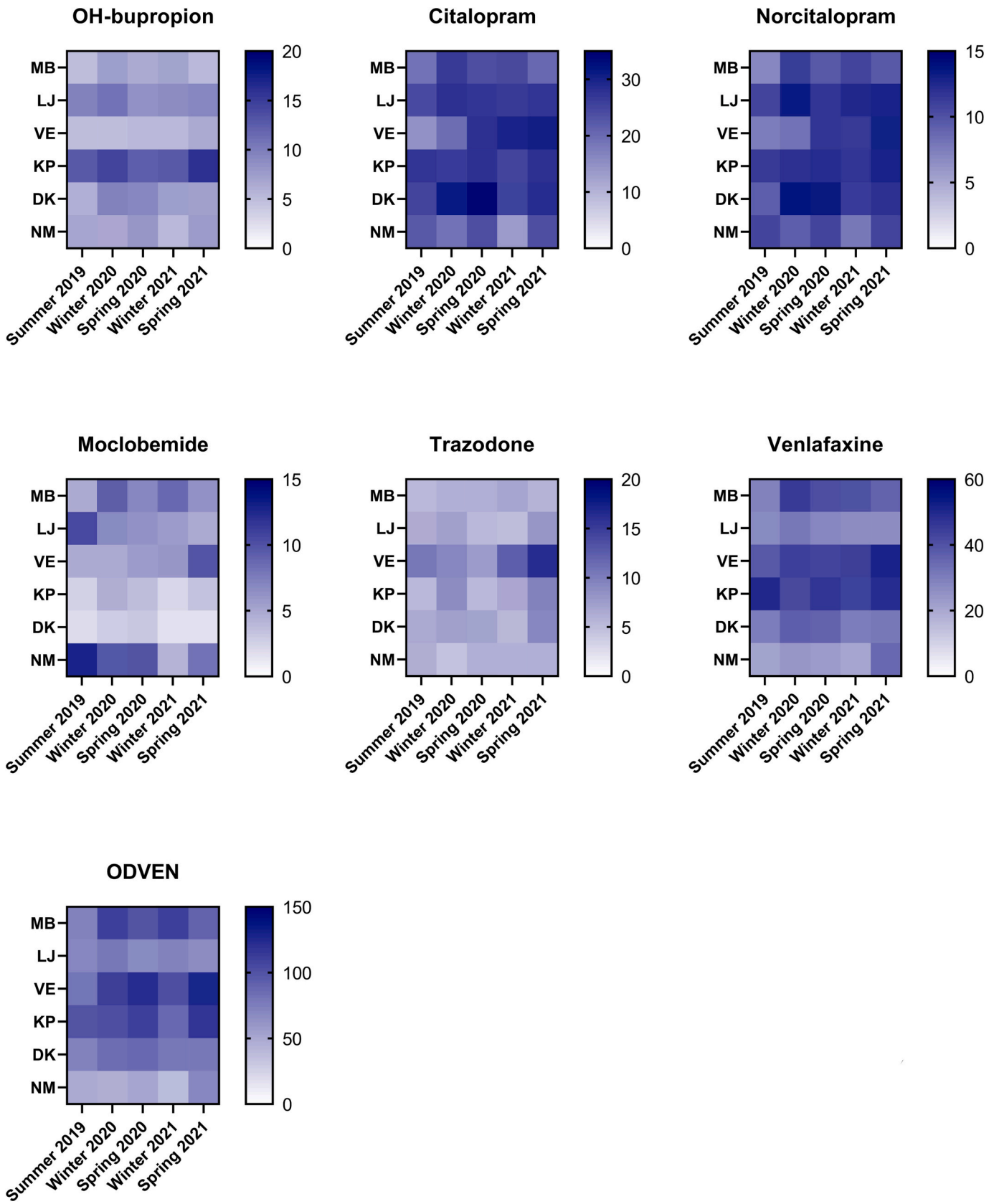

Fig. 3. Heatmaps of the spatiotemporal variations, visualised by heatmaps, in the population-normalised mass loads of the different biomarkers of antidepressant use. The darker the colour, the higher the PNMLs. Only compounds with detection frequency > 85 % in each location are shown.

Table 2
Comparison of the measured metabolite/parent compound ratios with other WBE studies.

Parent compound/ metabolite	Measured M/ PC ratio in this study (average ± SD)	Measured M/ PC ratio in other studies	Ref.
Bupropion/Bupropion-OH	3.98 ± 1.36	4.5	(Boogaerts et al., 2019)
Citalopram/ Norcitalopram	0.44 ± 0.06	0.5–0.8	(Boogaerts et al., 2023a, 2019)
Venlafaxine/*O*-desmethylvenlafaxine	2.33 ± 0.25	2.0–5.8	(Boogaerts et al., 2023a, 2019; Rúa-Gómez and Püttmann, 2012)

WWTPs, in the back-calculations of psychoactive pharmaceutical consumption. It is important to note that variations in population could occur due to pandemic measures resulting in factors such as changes in commuting habits and tourism. An increase in the mass loads could be attributed to a greater number of people consuming psychoactive pharmaceuticals, higher quantities, or a combination of both. To address this limitation, the use of dynamic population markers is recommended. Previous studies have utilised markers such as mobile phone data (Boogaerts et al., 2023a), chemical oxygen demand, biological oxygen demand, phosphorus, nitrogen, and ammonium loads (Di Marcantonio et al., 2022; Reinstadler et al., 2021; Tomsone et al., 2022) to account for variations in the socio-demographics of the catchment population. However, in this study, we attempted to use mobile phone tracking data, but permission to access this data was denied. Additionally, using other dynamic population markers, such as nitrogen, is not feasible due to the inclusion of wastewater from agricultural activities.

4. Conclusions

This study highlights the potential of WBE as a valuable tool for monitoring trends in psychoactive pharmaceutical consumption with a high level of temporal and spatial resolution, surpassing other data sources such as general population surveys, hospital records, and prescription and sales data. The findings of the study revealed numerous temporal and spatial variations. However, no clear patterns related to the pandemic were observed in the analysis of weekdays vs weekends and spatial trends. Incorporating dynamic population numbers in the calculation of PNMLs would be beneficial in elucidating changes in the socio-demographic characteristics of the population within the catchment area. Among the compounds detected, *O*-desmethylvenlafaxine, venlafaxine, citalopram and norcitalopram exhibited the highest PNMLs, while the M/PC ratios of the different biomarkers were consistent with findings from other WBE studies, demonstrating relative stability throughout the entire sampling campaign. This finding suggests that measured PNMLs likely derive from human consumption rather than dumping. Overall, the PNMLs of most psychoactive pharmaceuticals showed an increasing trend during the sampling campaigns, particularly during the first wave of the pandemic in the spring of 2020. These findings align with available data on annual prescriptions, further supporting the validity of the results obtained through WBE.

CRediT authorship contribution statement

Maria Laimou-Geraniou: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Maarten Quireyns:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing – review & editing. **Tim Boogaerts:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing – review & editing. **Natan Van Wichelen:** Methodology, Investigation, Writing – review & editing. **David Heath:** Conceptualization, Writing – review & editing. **Alexander L.N. van Nuijs:** Conceptualization, Writing – review & editing, Supervision, Project administration. **Adrian Covaci:** Conceptualization, Writing – review & editing, Supervision, Project administration. **Ester Heath:** Conceptualization, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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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.2023.166586.

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