Reflection of Population Chemical Use by Wastewater-Based Epidemiology: Temporal and Spatial Comparison of Artificial Sweeteners During COVID-19 Waves

---

Master Thesis in Environmental Science (30 Credits)

Swedish University of Agricultural Sciences (SLU)

Department of Aquatic Sciences and Assessment

Programme:
Soil, Water & Environment

Supervisor:
Foon Yin Lai

Submitted by:
Inga Haalck

Uppsala, September 4, 2022

Reflection of Population Chemical Use by Wastewater-Based Epidemiology: Temporal and Spatial Comparison of Artificial Sweeteners During COVID-19 Waves

Author	Inga Haalck
Supervisor	Foon Yin Lai, SLU, Department of Aquatic Sciences and Assessment
Co-Supervisors	Elin Eriksson, SLU, Department of Aquatic Sciences and Assessment Anna Székely. SLU, Department of Aquatic Sciences and Assessment
Examiner	Lutz Ahrens, SLU, Department of Aquatic Sciences and Assessment

Credits	30 Credits
Level	A2E
Course Title	Master Thesis in Environmental Science
Course Code	EX0897
Programme	Soil, Water and Environment
Place of Publication	Uppsala
Year of Publication	2022

Copyright	All featured images are used with permission from the copyright owner.

Keywords	Wastewater-based Epidemiology; Artificial Sweeteners; Population Estimation, COVID-19 pandemic

Abstract

The COVID-19 pandemic and the related measures have highly impacted our daily lives through social distancing and the general restriction of our mobility. This resulted in distress for the population and led to behavioral changes, for instance in our eating behavior.

A wastewater-based epidemiology (WBE) study was performed to study changes in the eating behavior during the pandemic by analyzing artificial sweeteners in the wastewater of Uppsala (Sweden), using direct injection and high performance liquid chromatography coupled to a tandem mass-spectrometer (HPLC-MS/MS). The results showed that no changes in the consumption pattern of artificial sweeteners were relatable to the COVID-19 pandemic and the national regulations. The temporal comparison revealed however an increased trend of artificial sweetener loads in Uppsala over time, showing an annual increase rate of 8.6% for saccharin, 12.3% for acesulfame and 20% for sucralose between 2019 and 2022. A spatial comparison showed that different locations in Sweden have a similar usage pattern, but that the amount of consumed artificial sweeteners varies spatially.

In a second part of the thesis, one of the biggest uncertainties of WBE studies were addressed: the correct estimation of the population size. The second part focused on finding suitable population biomarkers for Sweden. 23 candidates were analyzed in the wastewater and their mass loads were correlated to varying population sizes in Sweden in order to assess their suitability as population markers. Seven out of these 23 candidates were assessed for the first time in the context of population estimation: saccharin, surcralose, metformin, theophylline, citalopram, venlafaxine and its metabolite O-desmethylvenlafaxine. The results showed that 21 compounds, including the seven new propositions, showed a good correlation to the population size and were therefore considered suitable population markers. This knowledge will be helpful to develop a population model that can be used to dynamically estimate the population size for future WBE studies.

Contents

List of Illustrations **VI**
Figures . . . VI
Tables . . . VI

1. Introduction **1**
1.1. Relevance of the Topic . . . 1
1.2. State of Research . . . 2
1.3. Aim of the Study . . . 4

2. Methods **5**
2.1. Target Compounds . . . 5
2.2. Wastewater Samples . . . 7
2.3. Chemical Analyses . . . 8
2.3.1. Sample Preparation & Analysis . . . 8
2.3.2. Quality Assurance & Control (QA/QC) . . . 9
2.4. Data Analysis . . . 9

3. Results **12**
3.1. Part I: Suitability as Population Markers . . . 12
3.1.1. Detectablility in Swedish Wastewater . . . 12
3.1.2. Correlation to the Population Size . . . 12
3.2. Part II: Temporal & Spatial Comparison of Artificial Sweetener Consumption During COVID-19 Waves . . . 13
3.2.1. Detectability & Usage of Artificial Sweeteners in Uppsala . . . 16
3.2.2. General Time Trend in Uppsala . . . 17
3.2.3. Patterns During COVID-19 Waves in Uppsala . . . 18
3.2.4. Spatial Comparison to Other Locations . . . 21

4. Discussion **24**
4.1. Population Correlation . . . 24

4.2. Artificial Sweetener Consumption in Sweden 25
4.3. Uncertainties 27

5. Conclusion **29**

6. Popular Science Summary **36**

7. Acknowledgments **38**

A. Appendix **39**

List of Illustrations

Figures

2.1. Sampling Campaign and COVID-19 Cases in Sweden 8

3.1. Correlation Between Population Size and Mass Loads - Part I 14

3.1. Correlation Between Population Size and Mass Loads - Part II 15

3.2. Total Daily Consumption (kg/day) in Uppsala (on average) 17

3.3. Sucrose Equivalent Doses (SED) over Time 18

3.4. Artificial Sweetener Mass Loads Plotted Against Covid Cases in Sweden 20

3.5. Spatial Comparsion of AS Loads 23

3.6. Sucrose Equivalent Doses (SED) Across Locations 23

A.1. Map of the Sampling Sites 39

Tables

2.1. Target Compounds and Corresponding Internal Standards Used in the Analysis . . . 6

2.2. Overview Table for the Sampling Campaign 7

3.1. Substances Grouped Based on the Correlation to the Population Size 13

3.2. Per Capita Mass Loads (g/day/1000 people) Across Locations 16

A.1. Calibration Curve 39
A.2. MRM Parameters for Positive Ionization 40
A.3. MRM Parameters for Positive Ionization - Part II 41
A.4. MRM Parameters for Negative Ionization 42
A.5. Limit of Detection for the Target Compounds 43
A.6. Summary Data Table - Average, Standard Deviation (SD), Detection Frequency (DF%), Minimum and Maximum 44
A.7. Relative Standard Deviation (RSD%) of the Population and the Flow Rate 44
A.8. Relative Standard Deviation (RSD%) of AS Loads 45

Abbreviations

5-HIAA	5-Hydroxyindoleacetic Acid
AS	Artificial Sweetener
HPLC	High Performance Liquid Chromatography
HTZ	Hydrocholorthiazide
IS	Internal Standard
MRM	Multiple Reaction Monitoring
MS/MS	Tandem Mass Spectrometry
NESS	Non-Extracted Solvent Sample
PCP	Personal Care Product
SED	Sucrose Equivalent Dose
WBE	Wastewater-Based Epidemiology
WWTP	Wastewater Treatment Plant

1 Introduction

1.1. Relevance of the Topic

The COVID-19 pandemic has for now (2022) more than two years affected our lives. Social distancing, the closure of schools and most recreational facilities as well as the regulation to work from home have greatly impacted our daily lives and restricted our normal habits, leading to severe distress for the population (Xiong et al., 2020).

These unprecedented circumstances provided the unique opportunity to study and understand human behavior under particular societal conditions. Studies from different disciplines were performed to reveal changes in behavioral patterns during the pandemic. For instance, it was found that people temporarily changed their eating behavior. Food choices were shifted by increasing the quantity of food and the number of meals that were consumed per day (Janssen et al., 2021). Additionally, unhealthier food choices were made by increasingly consuming sodas and pastries (Giacalone et al., 2020) and alcoholic beverages (Biddle et al., 2020). As people were advised to avoid crowding, supermarkets were less frequently visited, leading to an increase in the consumption of canned and frozen food. Bemanian et al. (2020) observed an increase in emotional eating in Norway, leading to weight gain and a general health deterioration due to the COVID-19 restrictions.

The strategy in regards to COVID-19 varied from country to country. Even in the European Union, each member state had a different strategy in the attempt to reduce the spread of the virus. The Swedish strategy was unique compared to the rest of the world as a fundamental part was the individual responsibility of the people. Instead of law-enforced restrictions like in other countries, most measures were formulated as recommendations, leaving the decisions and the responsibility to the citizens. Most schools were not closed, gym and gastronomy were open throughout the pandemic and no lock-down or curfew were implemented as in other European countries. However, some regulations were formulated concerning the maximum allowed number of people in restaurants and at gatherings. In addition, students of the upper secondary schools as well as university students were attending online classes and home office was recommended if possible for all employees for most time of the pandemic (Olofsson et al., 2021).

According to the Swedish government, the population has a lot of trust in the governmental agencies, resulting in the Swedish citizens behaving as they were recommended and following social distancing and self-isolation (The Government of Sweden, 2020). As a consequence, epidemiology studies showed that people in Sweden were generally more stressed and less happy during the pandemic, which may lead to an unexpected behavioral change and altered consumption patterns in their daily lives (Elvén et al., 2022; Y. Chen et al., 2022).

1.2. State of Research

The above mentioned epidemiology studies were based on self-reporting and surveying, but changes in consumption patterns can also be detected chemically through wastewater-based epidemiology.

The analysis of influent and effluent wastewater can provide a wide range of information, such as the removal efficiency of wastewater treatment plants (WWTPs) or the identification of point sources for contamination (Choi et al., 2018). Additionally, influent wastewater analysis can produce objective and near-real-time estimates on the consumption of a variety of compounds, such as pharmaceuticals and illicit drugs for a given community. The consumption can then be normalized to the population size, which enables the comparison of per capita consumption across different communities over time (Daughton, 2001). This approach is called wastewater-based epidemiology (WBE).

WBE was first implemented in 2001 by Daughton et al. to analyze pharmaceuticals in wastewater and was then also applied on illicit drugs in 2005 (Zucatto et al., 2005). Since then, it has been widely applied. For example in Sweden, WBE was applied to study the differences in illicit drug consumption between Uppsala and Stockholm communities in the spring and autumn seasons (Haalck et al., 2021). WBE application was further extended to a wider range of compounds including personal care products (PCPs), pesticides and other industrial markers (Choi et al., 2018). Biological markers were also studied, for instance mycotoxins and very recently SARS-CoV-2 RNA (Lundy et al., 2021).

In the context of COVID-19, several WBE studies were performed to reveal changes in the intake of pharmaceuticals, illicit drugs and the consumption of alcohol (Estévez-Danta et al., 2022; Montgomery et al., 2021; Reinstadler et al., 2021), but none related to changes in the eating behavior. Outside of the COVID-19 context, artificial sweeteners (AS) were used as an indicator of changing eating habits in wastewater in a long-term study from Li et al. (2021). AS are approved by the European Chemical Agency as food additives for products such as canned and bottled fruits, jams, candy, beverages and personal care products (PCPs) like toothpaste (European Commission - DG Health & Food Safety, 2022). They are usually found in products that are energy reduced, for instance in diet sodas.

Compared to the conventional sugar sucrose, AS are significantly sweeter while having no calories, which has increased their popularity over the last couple of years on the global food market (Sylvetsky et al., 2016). The literature review did not yield any studies focusing on changing eating habits during the pandemic indicated by AS consumption through WBE.

One of the main advantages of WBE is that it provides non-invasive and quantitative information about the consumption of the studied compounds on a community-level (Choi et al., 2018). WBE is thus a valuable complement to surveys for epidemiology studies. There are however some acknowledged weaknesses of the approach, such as the correct estimation of the population size (Castiglioni et al., 2013). This uncertainty relates to the discrepancy between the *de facto* and the *de jure* population (Daughton, 2018). The *de jure* population describes the number of households that lies within a given sewer catchment. This information is usually obtained from the census data and is thus often used for WBE back-calculations. On the other hand, the *de facto* population describes the actual number of people contributing to the wastewater, taking fluctuations in the population into account, e.g. due to commuting or during holidays (Daughton, 2018). Yet, reliable numbers on the *de facto* population can only be obtained via census data if fluctuation in population is considered and recorded on a census day, or by using markers that reflect population activity - so called population markers (C. Chen et al., 2014). Census data is often not updated or too costly to obtain, so an increasing amount of studies can be found that attempt to find suitable population markers.

Simple approaches include the use of the influent flow rate, ammonium concentrations or the biological and/or chemical oxygen demand to estimate the population size (Zessner et al., 2005; van Nuijs et al., 2011; Been et al., 2014). These parameters are however not specific to human metabolism and can be influenced by other processes such as industrial activity. They are thus not considered to be reliable for population estimations (Daughton, 2012).

Another possibility suggested in the literature is to use specific chemicals to estimate the population size (Lai et al., 2015; O'Brien et al., 2014). The idea is to find compounds that show a good correlation between their mass loads in wastewater and the population size. With this correlation, a model can be developed that is able to estimate the de facto population in the given community for future WBE studies just by measuring the mass loads of the population biomarkers. O'Brien et al. (2014) were able to establish such a model and successfully validate several population markers in the Australian context. The difficulty is that in order to develop such a model, one still needs accurate data on the population size at least once for the model validation. In the case of O'Brien et al. (2014), they had the rare opportunity to take wastewater samples on a census day in Australia, meaning they knew how many people were in the catchment that exact day. Other studies used mobile device data to estimate the population size, e.g. in Norway (Thomas et al., 2017; Baz-Lomba et al., 2019). Such data can however be very costly and difficult to obtain. So far, no attempt has been performed to estimate

the population of a Swedish community by using chemical mass loads or mobile device data.

1.3. Aim of the Study

This thesis aimed to find suitable population markers for Sweden. Through literature review, a candidate list of already established population markers was compiled. Their applicability on the Swedish population was assessed by analyzing them in wastewater and correlating their mass loads to varying population sizes in Sweden. In addition, seven compounds were newly proposed and for the first time assessed in the context of population estimation.

In a second part, the thesis aimed to study changing eating habits during the COVID-19 pandemic through WBE. As indicated by the surveys mentioned in Chapter 1.1, people shifted their food choices to more comfort and processed food, which often contain artificial sweeteners (AS). This is the reason why the second part of the thesis will focus on the consumption of the three AS acesulfame, sucralose and saccharin in Uppsala over time, especially with regards to the COVID-19 pandemic. The hypothesis was that especially in times when the Swedish government advised the citizens to work from home, people were more stressed out and thus consumed higher amounts of sodas and comfort food, which would result in increased mass loads of artificial sweeteners in the wastewater. Annual increase rates were calculated and temporal comparisons to pre-covid conditions were drawn. In addition, a spatial comparison to the other four studied locations in Sweden were made with the aim to reveal possible usage differences across locations.

2 Methods

2.1. Target Compounds

The target compounds for this study were chosen based on their previous proposition and anticipated suitability as population biomarkers. Through literature review, a preliminary candidate list was drafted, that included compounds that were proposed and/or validated by other studies as suitable population markers.

Several compounds on the candidate list were chosen based on the study of O'Brien et al. (2014). They studied several pharmaceuticals and personal care products (PPCPs) and found 14 chemicals for which the mass loads showed a good linear relationship with the population size. In a follow-up study from 2017, they then tested the stability of these markers to ensure that degradation processes would not interfere with their ability to predict the population size. On that basis, they proposed best-practice criteria to consider when choosing population markers. In addition to a high correlation to the population size, population markers should be easily detectable, stable in the sewer system and should show a consistent excretion throughout different populations to enable comparisons (O'Brien et al., 2017). Compounds that fulfill these criteria and were therefore added to the candidate list were acesulfame, atenolol, carbamazepine, gabapentin, ibuprofen and hydrochlorothiazide (HTZ). With doubts about their stability but a great correlation to the population size, caffeine, naproxen, codeine, acetaminophen and salicylic acid were added as well.

Senta et al. (2015) investigated the suitability of nicotine and its major metabolites cotinine and trans-3'-hydroxycotinine. The nicotine metabolites were confirmed as suitable biomarkers, while the parent compound was doubted for its unspecific sources and high variability (Senta et al., 2015). Caffeine and its major metabolite paraxanthine (1,7-dimethylxanthine) were also confirmed in the study and were therefore added to the candidate list. Based on the metabolism of caffeine, the decision was made to also include its other urinary metabolite theophylline (1,3-dimethylxanthine) (Heckman et al., 2010). C. Chen et al. (2014) additionally proposed 5-Hydroxyindoleacetic acid (5-HIAA), a urinary metabolite of serotonin, as a promising candidate for a population biomarker.

The above mentioned best-practice criteria proposed by O'Brien et al. (2017) were helpful to choose other high-consumption chemicals, that were not proposed as population markers yet but are frequently detected in significant concentrations in the Swedish wastewater and environment (Golovko et al., 2021). The preliminary candidate list was thus completed by venlafaxine and its metabolite desmethylvenlafaxine, citalopram, and metformin. Based on the confirmation of acesulfame as suitable population biomarker, two other artificial sweeteners (AS) sucralose and saccharin were added to the candidate list as well.

Ultimately, a total of 23 compounds were selected for the candidate list as possible population markers. The substances can be classified in different groups, ranging from artificial sweeteners, to pharmaceuticals and antidepressants. A list of all compounds including the corresponding internal standards for the analysis can be found in Table 2.1.

Table 2.1.: Target Compounds and Corresponding Internal Standards Used in the Analysis

Substance	Group	Internal Standard
Acesulfame K	Artifical Sweetener	Acesulfame-d4
Saccharine	Artifical Sweetener	Hydrochlorothiazide-13C6
Sucralose	Artifical Sweetener	Sucralose-d6
Atenolol	Pharmaceutical	Atenolol-d6
Carbamazepine	Pharmaceutical	Carbamazepine-d10
Codeine	Pharmaceutical	Codeine-d3
Gabapentin	Pharmaceutical	Gabapentin-d10
Hydrocholorthiazide (HTZ)	Pharmaceutical	Hydrochlorothiazide-13C6
Ibuprofen	Pharmaceutical	Ibuprofen-d3
Naproxen	Pharmaceutical	Atenolol-d6
Metformin	Pharmaceutical	Metformin-d6
Acetaminophen	Pharmaceutical	Acetaminophen-13C3
Salicylic Acid	Pharmaceutical	Ibuprofen-d3
Caffeine	Food Additive	Caffeine-13C3
Paraxanthine	Metabolite (Caffeine)	Caffeine-13C3
Theophylline	Metabolite (Caffeine)	Caffeine-13C
Nicotine	Licit Drug	Nicotine-d4
Cotinine	Metabolite (Nicotine)	Cotinine-d3
trans-3'-hydroxycotinine	Metabolite (Nicotine)	Cotinine-d3
Citalopram	Antidepressant	Venlafaxine-d6
Venlafaxine	Antidepressant	Venlafaxine-d6
O-desmethylvenlafaxine	Metabolite (Venlafaxine)	Venlafaxine-d6
5-Hydroxyindoleacetic Acid (5-HIAA)	Metabolite (Serotinin)	5-HIAA-d5

2.2. Wastewater Samples

The wastewater samples were taken in connection to the project "Swedish Environmental Epdemiology Center (SEEC)" as part of the Pandemic Laboratory Preparedness (PLP) program for which WBE was performed in order to monitor the COVID-19 prevalence in Sweden (SciLifeLab Data Centre, n.d.). For that purpose, wastewater samples were collected during the pandemic in different communities all over Sweden. Out of the 14 analyzed locations, five locations were selected for this project that reflect a variety of population sizes. Table 2.2 summarizes which locations were chosen, how many samples were available and the size of each de jure population. The estimations of inhabitants were provide by the respective WWTP staff. A geographical map of the locations can be found in Appendix A.1.

A total of 183 samples taken during the pandemic were included in this study. In addition, 14 samples taken in spring and autumn 2019 from Uppsala were included in the analysis in order to provide a comparison between the samples taken during the pandemic and pre-covid conditions.

Table 2.2.: Overview Table for the Sampling Campaign

	Location	De Jure Population	Number of Samples
1	Östhammar	4 500	31
2	Knivsta	11 000	11
3	Enköping	22 000	34
4	Kalmar	65 000	16
5	Uppsala	180 000	91
			Total: 183

Samples from Uppsala were weekly composite samples, meaning that during a whole week, everyday a sample was taken and then later composited according to the flow rate into one weekly sample. At the other locations, the samples were also taken flow-dependent but on only one day of the corresponding week.

Figure 2.1 shows the COVID-19 waves in Sweden and gives an overview about measures implemented during the pandemic. In addition, the figure shows the coverage of the sampling campaign. Most samples were provided for Uppsala and almost the whole course of the pandemic could be captured. For the other locations, less samples were available and at a later stage of the pandemic. Additionally, samples from Knivsta were only provided every second week and in Östhammar no samples were taken during the summer. All samples were stored at -20°C and were defrosted the day of analysis.

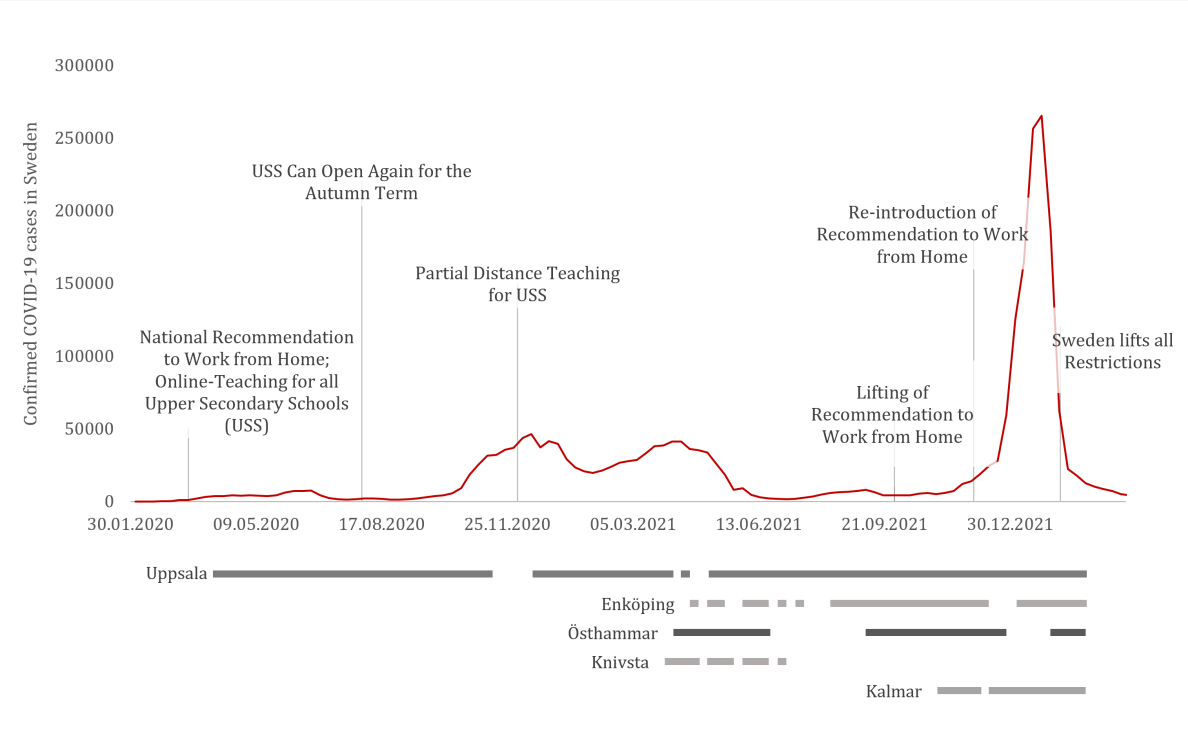


Figure 2.1.: Sampling Campaign and COVID-19 Cases in Sweden

2.3. Chemical Analyses

2.3.1. Sample Preparation & Analysis

The samples were first defrosted and centrifuged. In order to obtain a 20/80 ratio between methanol and water, 20 µL of methanol were added to 160 µL of sample. The samples were then spiked with 20 µL of the corresponding internal standard (IS) (100 µg/L) to a final level of 10 µg/L.

All target compounds were then analyzed with direct injection, using the HPLC system ExionLC™ coupled to the mass spectrometer SCIEX Triple Quad™ 3500. For the positive ionization, mobile phase A contained 0.1% formic acid in MilliQ water and mobile B was composed of 0.1% formic acid in methanol. For the negative ionization, the composition of mobile phase A was 0.1% acetic acid in MilliQ water and of mobile phase B was 0.1% acetic acid in methanol. The coloumn used for the analysis was Phenomenex® Kinetex® Biphenyl Column (100x2.1mm, 2.6 µm).

A data acquisition method was set up after compiling the instrumental parameters, i.e., multiple reaction monitoring (MRM) parameters, for the target candidates and IS chemicals.

More information about the MRM parameters as well as the achieved limits of detection (LOD) can be found in the Appendix (A.2, A.3, A.4, A.5).

2.3.2. Quality Assurance & Control (QA/QC)

The quantification was performed using a nine-point calibration curve covering 0 µg/L until 100 µg/L (compare Appendix A.1). The standard solutions were additionally injected five times during the sample analysis as quality control of the instrumental performance.

Blank and non-extracted solvent samples (NESS) were prepared and analyzed together with the samples to monitor possible contamination and the inter-day variation. NESS samples contained MilliQ water spiked with native and internal standard (20/80 methanol and water ratio) .

Every 20th wastewater sample was alternately either duplicated or spiked (matrix spike). Spiked samples consisted of 160 µL sample, spiked with 20 µL internal and native standard mix each.

Good recoveries (78-124%) were achieved for all compounds, expect 5-HIAA. The within-run precision indicated by the relative standard deviation (RSD%) varied between 2-8%. The instrumental RSD% ranged between 0.5 and 16%. The inter-day variation ranged between 3-17%. The blanks showed that no contamination was present in the analysis.

2.4. Data Analysis

For the quantitative evaluation of the samples, calibration curves were used. Calibration curves are generated by measuring a serial dilution of a reference native standard (compare Appendix A.1). In each calibration standard point, a known and constant concentration of the mass-labeled internal standard (IS) is added for a correction of the instrumental variation and matrix effect during the analysis. The curve is then built as the native-to-IS ratio in the area against that in the concentration with 1/x weighting. The resulting equation then allows to quantify the native compound concentration in a sample.

The measured concentrations were normalized to flow rate in order to obtain mass loads. These were then normalized to the population size to receive per capita mass loads following Equation 2.1.

$$Per\ Capita\ Mass\ Load\ (g/day/1000\ people) = \frac{Concentration\ (mg/L) * Flow\ Rate(L/day)}{Population\ Size\ (in\ Thousands)} \quad (2.1)$$

Linear regressions were calculated using the R software (*ggplot package*). The data input used were the varying population sizes from the studied locations and the mass loads that were not normalized to the population size.

For the data processing of the artificial sweetener (AS) results, Li et al. (2020) and Li et al. (2021) were used as a reference. To talk about the consumption of a given compound, the mass loads usually need to by normalized by the excretion rate. As acesulfame, saccharin and sucralose are however not metabolized in the human body, the excretion rates were set to 1 which made this normalization unnecessary (Magnuson et al., 2016; Li et al., 2020).

The relative sweetness or sucrose equivalent dose was calculated following Equation 2.2. Artificial sweeteners have varying levels of sweetness. For instance, acesulfame and saccharin are 200 and 300 times sweeter than the conventional sugar sucrose, respectively (Luo et al., 2019). In order to obtain the same level of sweetness, you would therefore only need 1/200 of the amount of acesulfame compared to amount of sucrose. For sucralose the equivalent would be 1/600. A lower concentration of sucralose in the wastewater can therefore still indicate a higher number of consumed doses and hence a higher usage. This is the reason why the sucrose equivalent dose (SED) is a good indicator for the actual usage of a compound.

$$SED\ (kg/day/1000people) = \frac{Per\ Capita\ Load\ (g/day/1000people) * Sweetening\ Intensity}{1000} \tag{2.2}$$

Secondly, the total daily consumption was calculated. For that purpose, the average per capita consumption from all samples taken during the pandemic (March 2020-February 2022, n=91) were multiplied with the population of Uppsala (180 000).

$$Daily\ Consumption\ (kg/day) = \frac{Per\ Capita\ Consumption\ (g/day/10000\ people) * Population}{1000} \tag{2.3}$$

Lastly, the annual increase rates for acesulfame, saccharin and sucralose consumption in Uppsala were calculated following Equation 2.4. Y_t in this case is average of the baseline samples from 2019 and Y_{t+n} the average from February 2021 to February 2022 (n=51).

$$Increase\ Rate = \exp\left[\ln\left(Y_{t+n}\right) - \ln\left(Y_t\right)/n\right] - 1 \tag{2.4}$$

To be able to distinguish between an increase in the AS consumption and the influence of a growing population, the relative standard deviation of the mass loads were compared with the relative standard deviation of the population size (taken from the Swedish Tax Agency) and the flow rate. The later is based on the simplified assumption that the flow rate correlates with the population size.

One-sided ANOVA with subsequent Tukey post hoc tests were performed to check for significance during the temporal and spatial comparison (significance level <0.05).

3 Results

3.1. Part I: Suitability as Population Markers

3.1.1. Detectablility in Swedish Wastewater

All 23 compounds were detectable in the Swedish influent wastewater. The lowest detection frequency (DF%) was observed for 5-HIAA with only 16-63%, which is the reason why 5-HIAA was excluded from further data exploration. Salicylic acid was detected with a frequency of 90-100% and carbamazepine of 97-100%. All the remaining compounds were detected with 100% frequency.

The average mass loads varied significantly between compounds, although there was a certain regularity observable across locations. For instance, acetaminophen showed consistently high mass loads across all locations. The average mass load ranged from 17 g/day/1000 people in Knivsta to 52 g/day/1000 people in Enköping. Metformin and acesulfame also showed high mass loads across all locations and the average ranged between 17-35 g/day/1000 people and 16-24 g/day/1000 people, respectively.

Consistently low values were observed for carbamazepine with only 0.034-0.067 g/day/1000 people across all locations. Citalopram showed similar results with only 0.031-0.082 g/day/1000 people average mass loads.

Nicotine was the only compound that showed very inconsistent mass loads with an average of 31.7 g/day/1000 people in Enköping but only around 2 g/day/1000 people in the other four locations. A summary data table can be found in Appendix A.6.

3.1.2. Correlation to the Population Size

Figure 3.1 shows the linear relationship for each individual compound, indicated by the R^2-value. With an R^2-value higher than 0.8, the correlation was considered high and the compound suitable to reflect the population size.

For almost all compounds the correlation between mass load and population size was very high. Several compounds reached R^2-values of 0.99 and higher (compare Table 3.1). Theopylline, paraxanthine, venlafaxine and saccharin reached even 0.999 indicating an almost perfect fit. Nicotine was the only compound that showed a very low R^2-value (-0.275) and is therefore considered unfit.

The newly proposed compounds (underlined in Table 3.1) showed very good correlation values with six out of seven propositions reaching higher than 0.99 and citalopram reaching over 0.90. This result therefore indicates a good suitability of the proposed compounds as population biomarkers.

Table 3.1.: Substances Grouped Based on the Correlation to the Population Size

R^2 value	Substances
>0.99	Caffeine, Theophylline, Paraxanthine, Venlafaxine, Desmethylvenlafaxine, Saccharin, Sucralose, Acesulfame K, Metformin, Atenolol, Cotinine,
>0.95	Carbamazepine, Codeine, Naproxen, Trans-3'-Hydroxycotinine, HTZ
>0.90	Citalopram, Salicylic Acid
>0.80	Acetaminophen, Gabapentin
<0.80	Nicotine (R^2 value -0.275)

Newly Proposed Compounds are Underlined

3.2. Part II: Temporal & Spatial Comparison of Artificial Sweetener Consumption During COVID-19 Waves

For this study, samples from five Swedish communities were included. As indicated in Figure 2.1, most samples were available for Uppsala which were taken over a span of two years (March 2020 - February 2022). Additionally, samples from spring and autumn 2019 were included that serve as a pre-covid reference to the samples taken during the pandemic. This long span of analyzed samples together with the baseline allow us to identify trends and changing consumption patterns in Uppsala, that we cannot identify with the lower number of samples that are available from the other locations. This is the reason why the following paragraphs will firstly focus on Uppsala, before a spatial comparison will be drawn in chapter 3.2.4.

Average mass loads mentioned in the following paragraphs always refer to the samples taken during the pandemic (March 2020 - February 2022) and do not include the baseline from 2019. The baseline is only taken into account when specifically stated.

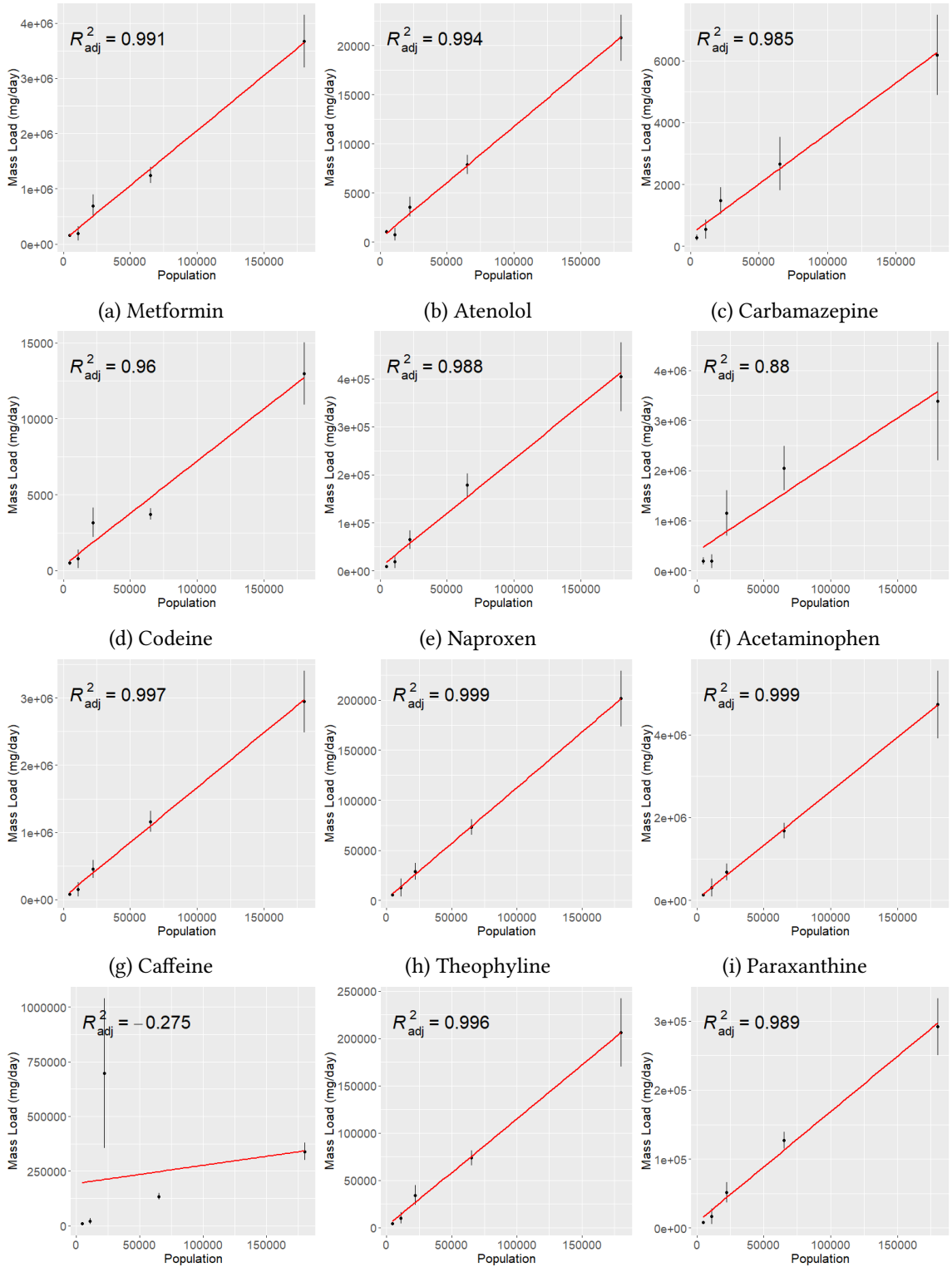


Figure 3.1.: Correlation Between Population Size and Mass Loads - Part I

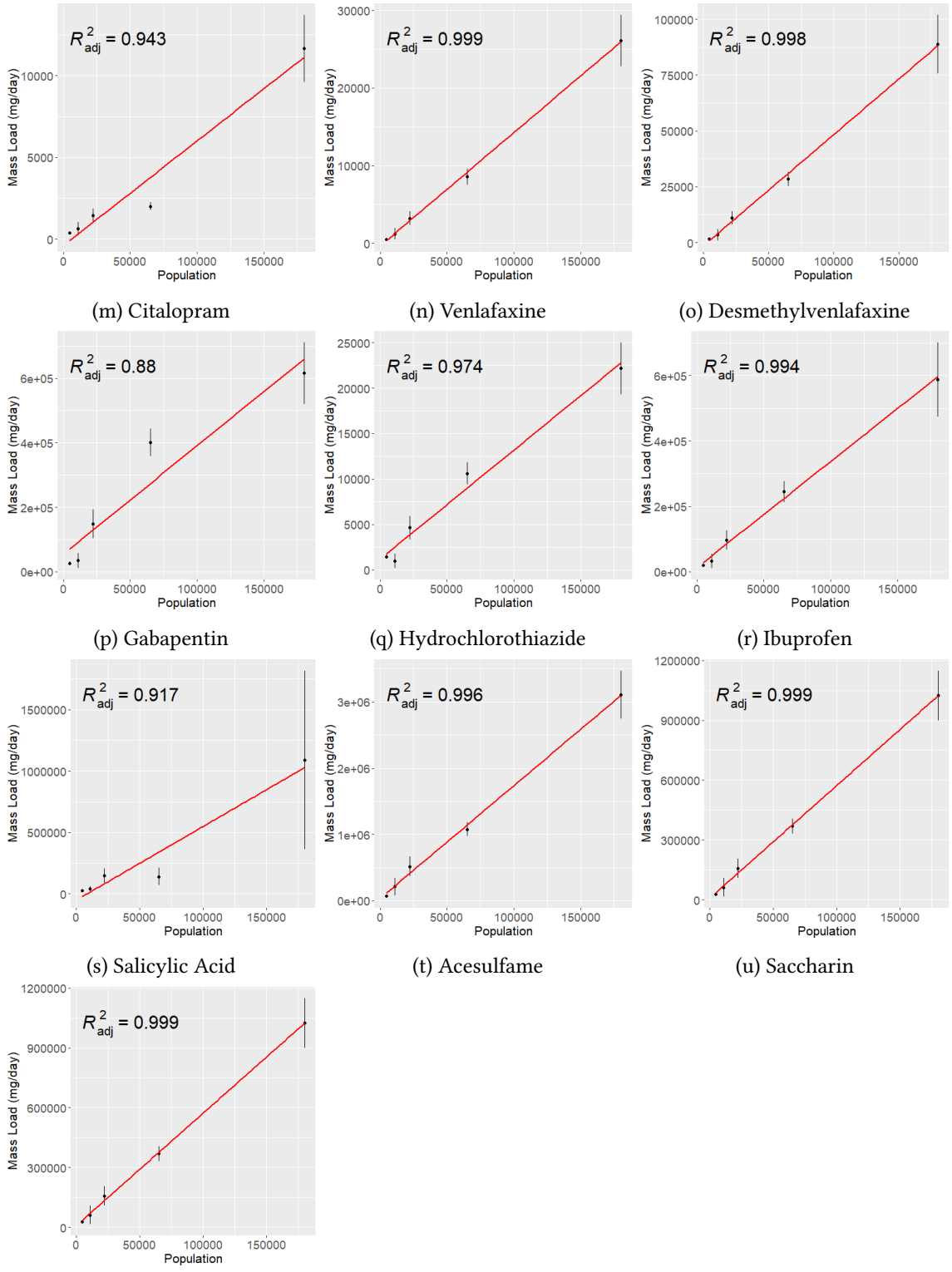


Figure 3.1.: Correlation Between Population Size and Mass Loads - Part II

3.2.1. Detectability & Usage of Artificial Sweeteners in Uppsala

In Uppsala, all three artificial sweeteners (AS) were detected with a frequency of 100% over the two year span. The highest average mass loads were observed for acesulfame with 17.3 g/day/1000 inhabitants, followed by sucralose with 6.71 g/day/1000 inhabitants. Saccharin showed the lowest average mass load with 5.69 g/day/1000 inhabitants (compare Table 3.2).

The total daily consumption was calculated by multiplying the average mass load with the population. In Uppsala, 5.34 kg artificial sweetener are consumed per day, of which more than half trace back to acesulfame (3.11 kg). Sucralose and saccharin account for 1.21 kg and 1.02 kg per day, respectively (compare Figure 3.2).

Table 3.2.: Per Capita Mass Loads (g/day/1000 people) Across Locations

		Acesulfame	Saccharin	Sucralose
Uppsala	Mean (SD)	17.3 (2.29)	5.69 (0.728)	6.71 (1.32)
	Range	10.7 - 21.5	3.68 - 7.13	3.47 - 9.62
	DF%	100	100	100
Kalmar	Mean (SD)	16.6 (1.66)	5.67 (0.616)	7.20 (0.806)
	Range	13.6 - 20.4	4.27 - 6.65	6.08 - 8.86
	DF%	100	100	100
Enköping	Mean (SD)	23.5 (6.94)	7.09 (2.22)	8.46 (2.62)
	Range	1.47 - 33.8	0.42 - 10.4	0.614 - 13.2
	DF%	100	100	100
Knivsta	Mean (SD)	19.5 (12.6)	5.66 (4.46)	7.489 (5.03)
	Range	11.1 - 57.2	3.75 - 19.1	3.78 - 22.3
	DF%	100	100	100
Östhammar	Mean (SD)	16.16 (2.42)	6.16 (0.769)	5.85 (1.32)
	Range	10.2 - 20.9	4.23 - 7.2	3.05 - 8.16
	DF%	100	100	100

SD = Standard Deviation; DF% = Detection Frequency

To account for the varying sweetness level of the compounds, the sucrose equivalent doses (SED) were calculated. For that purpose, the mass load was multiplied with the sweetness level. As sucralose is 2 or 3 times sweeter than saccharin and acesulfame, plotting the relative sweetness shows that sucralose is the compound that is consumed most in SED (4.03 kg/day/1000 people). Average SED that are consumed from acesulfame and saccharin amount to 3.45 and 1.71 kg/day/1000 people.

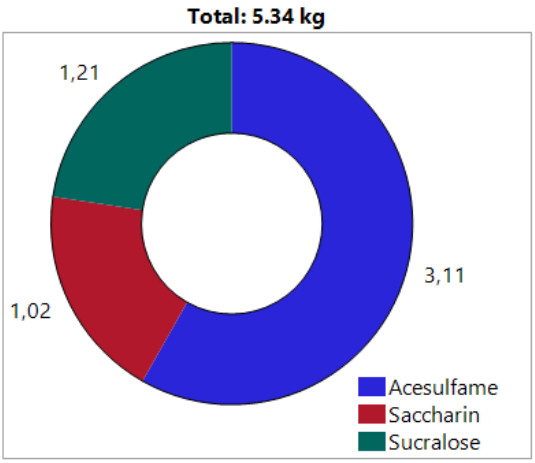


Figure 3.2.: Total Daily Consumption (kg/day) in Uppsala (on average)

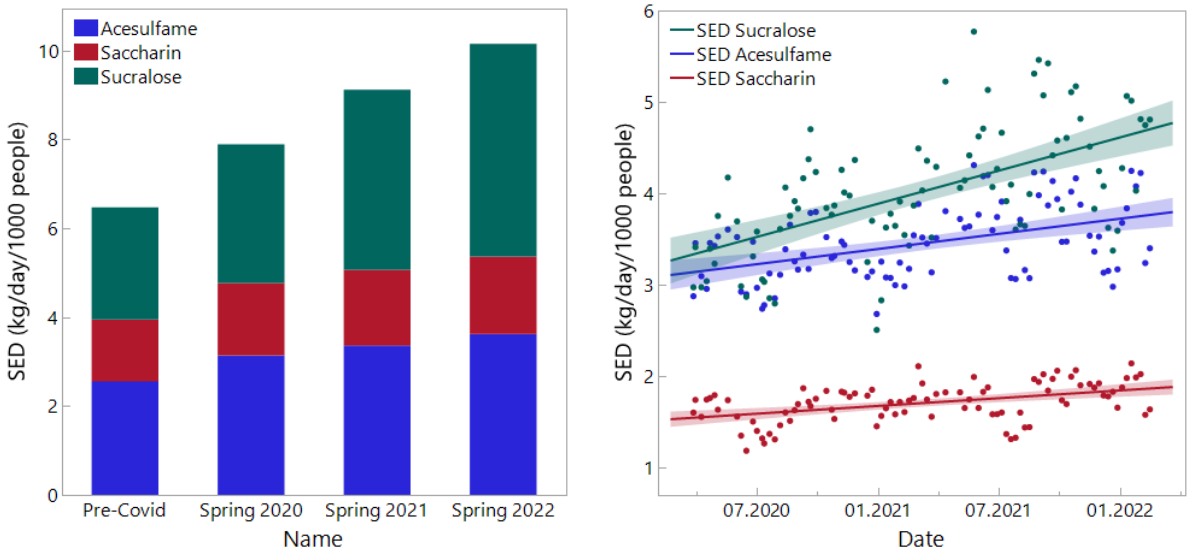
3.2.2. General Time Trend in Uppsala

In order to identify temporal trends in the AS consumption, three weeks from each March 2019, 2020 and 2021 together with samples from February 2022 were averaged and compared with each other. Figure 3.3a shows the sucrose equivalent doses (SED) for acesulfame, sucralose and saccharin over the years shown in a bar chart.

The chart shows that the general artificial sweetener consumption increased over time. From an average of 6.47 kg/day/1000 people in spring 2019 to 10.2 kg/day/1000 people in spring 2022. Individually, it can be seen that the SED of acesulfame increased over the years, from 2.56 kg/day/1000 people in spring 2019 to 3.62 kg/day/1000 people in spring 2022. For saccharin, the trend is not as evident, but here too the SED increases from 1.39 to 1.74 kg/day/1000 people between in three years. Sucralose shows the highest increase in SED. In spring 2019 the average SED was 2.53 kg/day/1000 people and it almost doubled to 4.79 kg/day/100 people in 2022. These individual trends are illustrated in Figure 3.3b and are reflected in the annual increase rate. Corresponding to the above mentioned trends, the consumption of acesulfame increased with an annual rate of 12.3% from 2019 to 2021/2022. For saccharin the increase rate amounted to 8.6% and for sucralose to 20%.

In order to ensure the time trend that we observed for the AS mass loads traces back to an increased consumption and not only an increased population size, the relative variation of population in Uppsala (indicated by the official registration at Skatteverket) was compared to the variation of the flow rate and then lastly to the average mass load of each year that we measured.

Over the study period (2019-2022), the variation (relative standard deviation %) of the population in Uppsala was about 2%. Similar variation, 4%, was also observed for the influent wastewater flow rate at the Uppsala WWTP (compare Appendix A.7). While the factor of increase in population cannot be excluded when it comes to explaining the increase in the AS usage, the variation (2-4%) of the population or wastewater flow rate was much lower than that (18%) of the AS. Especially, the variation of sucralose over the study period was 25% (compare Appendix A.8).



(a) SED in Spring 2020-2022 as Compared to Pre-Covid

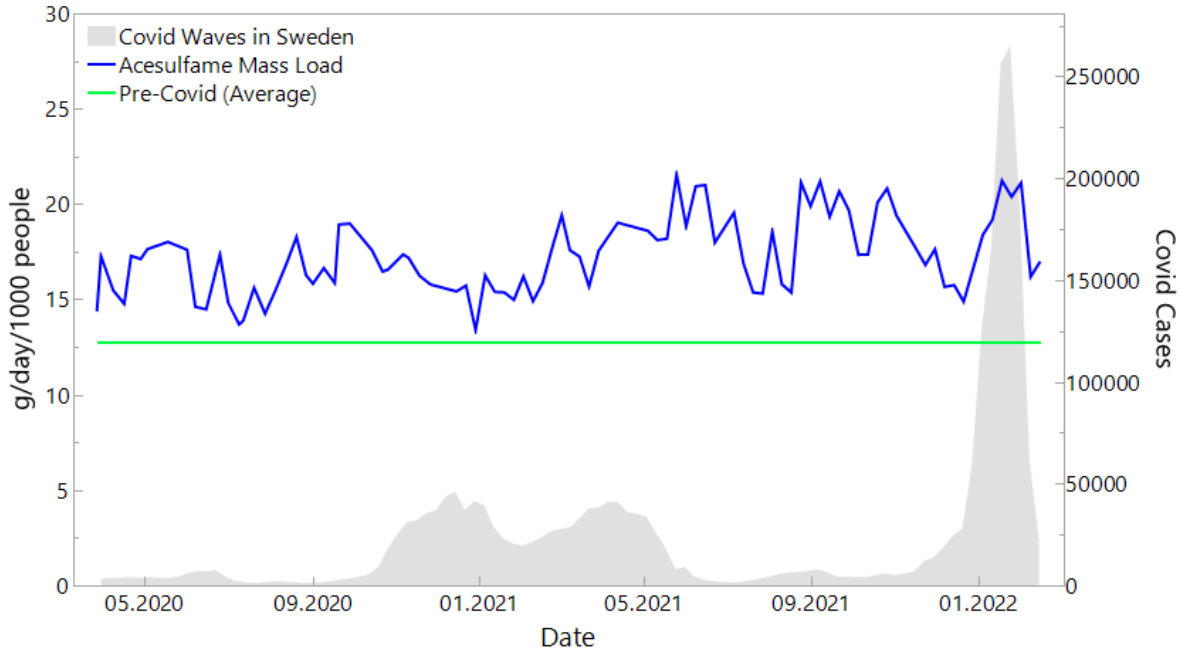
(b) Trend Line of SED for Acesulfame, Saccharin and Sucralose - Shadow Indicates the Confidence Interval

Figure 3.3.: Sucrose Equivalent Doses (SED) over Time

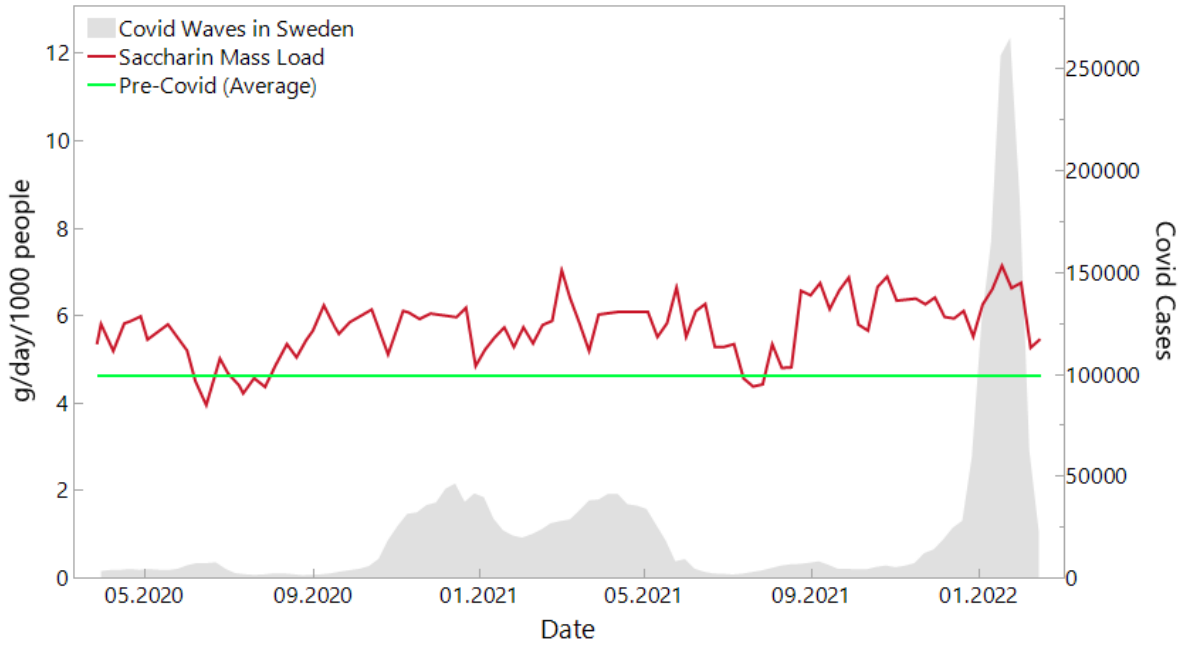
3.2.3. Patterns During COVID-19 Waves in Uppsala

Patterns during the COVID-19 waves were evaluated by plotting the AS mass loads against the COVID-19 cases (compare Figure 3.4).

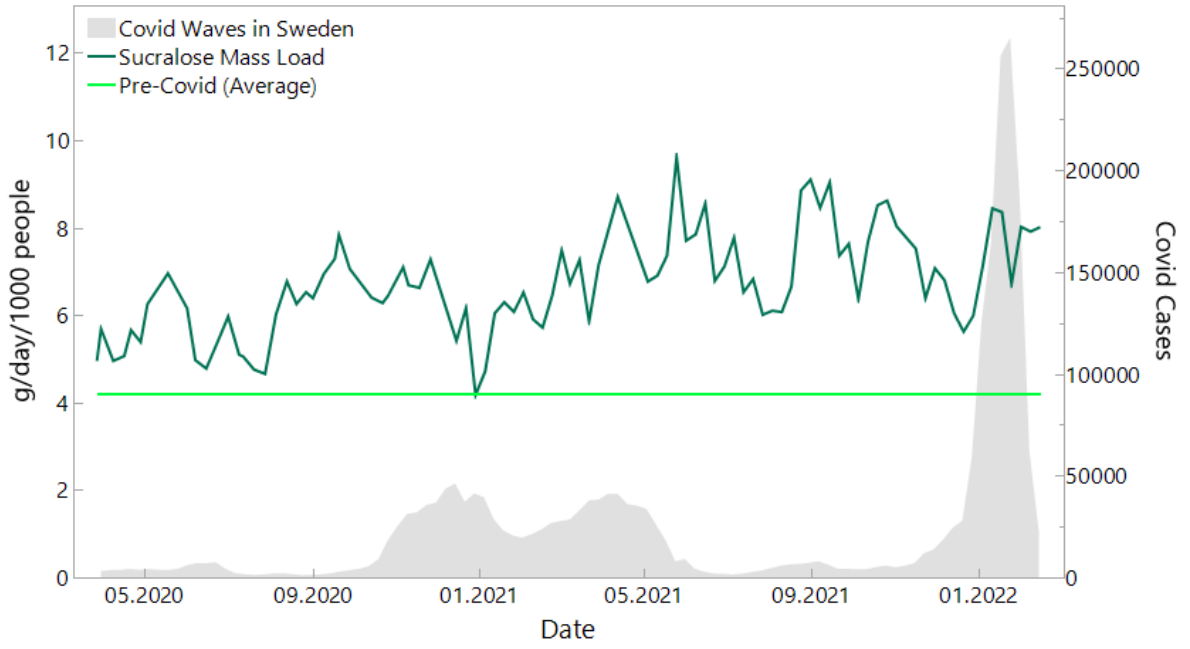
Over the course of the pandemic, the mass loads of the artificial sweeteners fluctuated without a distinct pattern. It is however observable that the mass loads increase over time, reaching new peaks at the end of May 2021 for both acesulfame (21.5 g/day/1000 people) and sucralose (9.62 g/day/1000 people). Saccharin reaches its highest point in February 2021 (7.13 g/day/1000 people). The lowest mass loads were observed at the end of December for acesulfame and sucralose simultaneously (13.4 and 4.18 mg/day/1000 people), while saccharin showed its lowest mass loads during the summer season of 2020 (3.94 mg/day/1000 people).



(a) Acesulfame



(b) Saccharin



(c) Sucralose

Figure 3.4.: Artificial Sweetener Mass Loads Plotted Against Covid Cases in Sweden

No maximum or minimum value coincided with a change of COVID-19 measures.

On the 29th of September 2021, all recommendation concerning COVID-19 were lifted for the first time, until they were re-introduced at the beginning of December 2021. At the beginning of February 2022, the regulations were lifted again. This was the only period with sudden changes in COVID-19 measures in Sweden.

It appeared that the usage patterns of AS was not highly influenced by these changes in the covid-infection measures for people in Uppsala. No particular peak of usage could be correlated to the introduction or lifting of the COVID-19 measures.

The indicated increase of AS usage in Chapter 3.2.2, can also be seen in Figure 3.4. For acesulfame, all measured mass loads during the pandemic lied above the pre-covid average (indicated by the bright green line). For sucralose, only on one occasion (end of December 2020), a value was below the pre-covid average. Mass loads of saccharin were closest to the pre-covid average and occasionally below.

3.2.4. Spatial Comparison to Other Locations

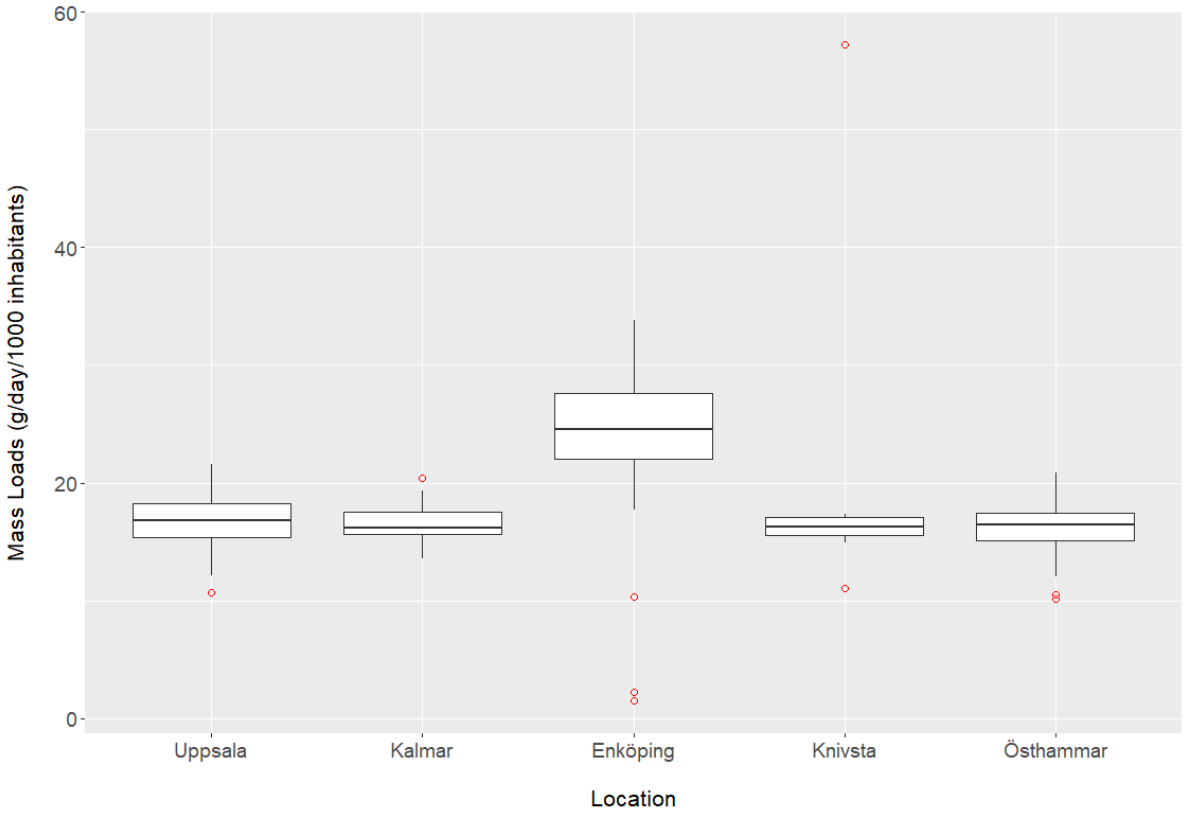
Per-capita mass loads of AS were compared across five locations (compare Figure 3.5). Enköping showed higher mass loads for all the three AS compared to the other locations.

Acesulfame mass loads showed an average of 23.5 g/day/1000 people in Enköping, which is the highest average from all locations and compounds. Knivsta showed the second highest average mass load for acesulfame (19.5 g/day/1000 people). Levels in Uppsala, Kalmar and Östhammar were significantly lower (p-value <0.0001) than in Enköping with averages of around 17 g/day/1000 people.

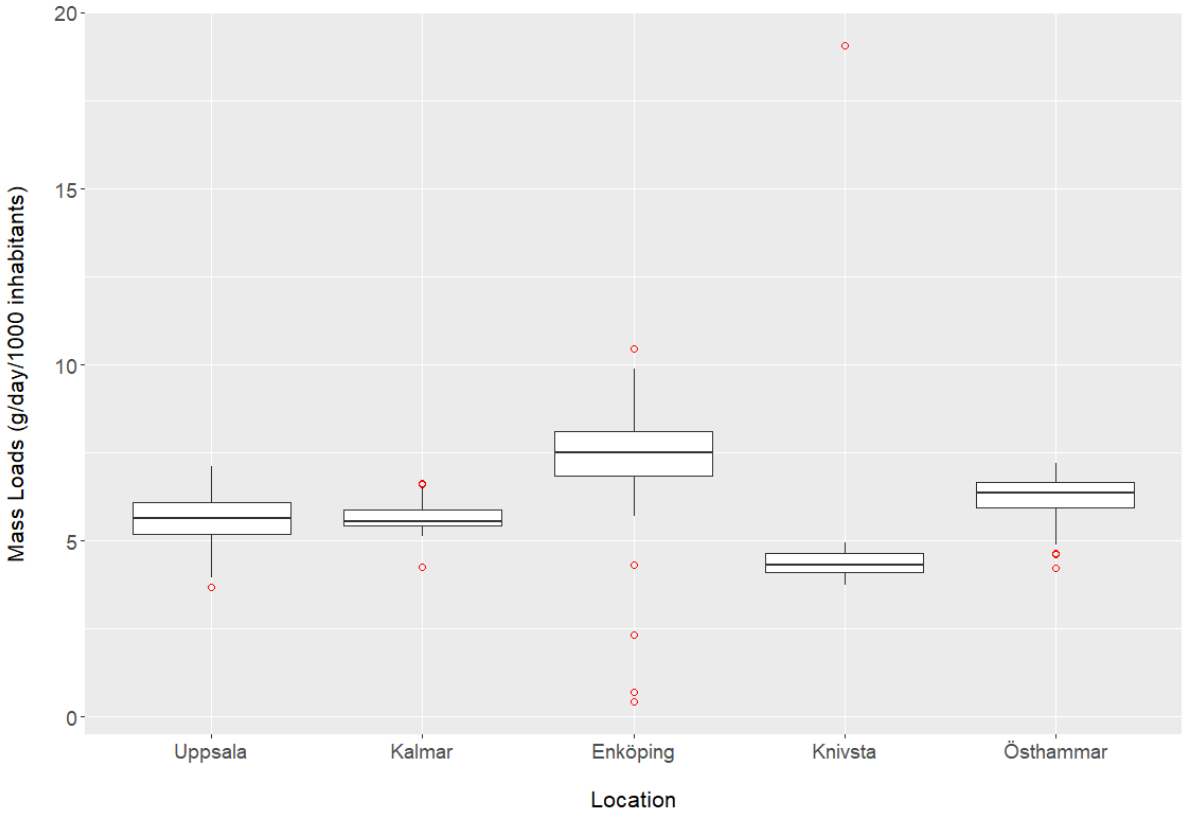
For saccharin, the highest average mass loads were measured in Enköping (7.09 g/day/1000 people). It was least used in Knivsta (5.66 g/day/1000 people), shortly followed by Kalmar (5.67 g/day/1000 people) and Uppsala (5.69 g/day/1000 people). In Östhammar the average mass load was 6.16 g/day/1000 people.

Sucralose was again mostly used in Enköping, followed by Knivsta and Kalmar (average: 8.46, 7.49, 7.2 g/day/1000 people, respectively). In Östhammar and Uppsala the levels were significantly lower (p-value <0.0001) than in Enköping with average mass loads of 5.85 and 6.71 g/day/1000 people, respectively.

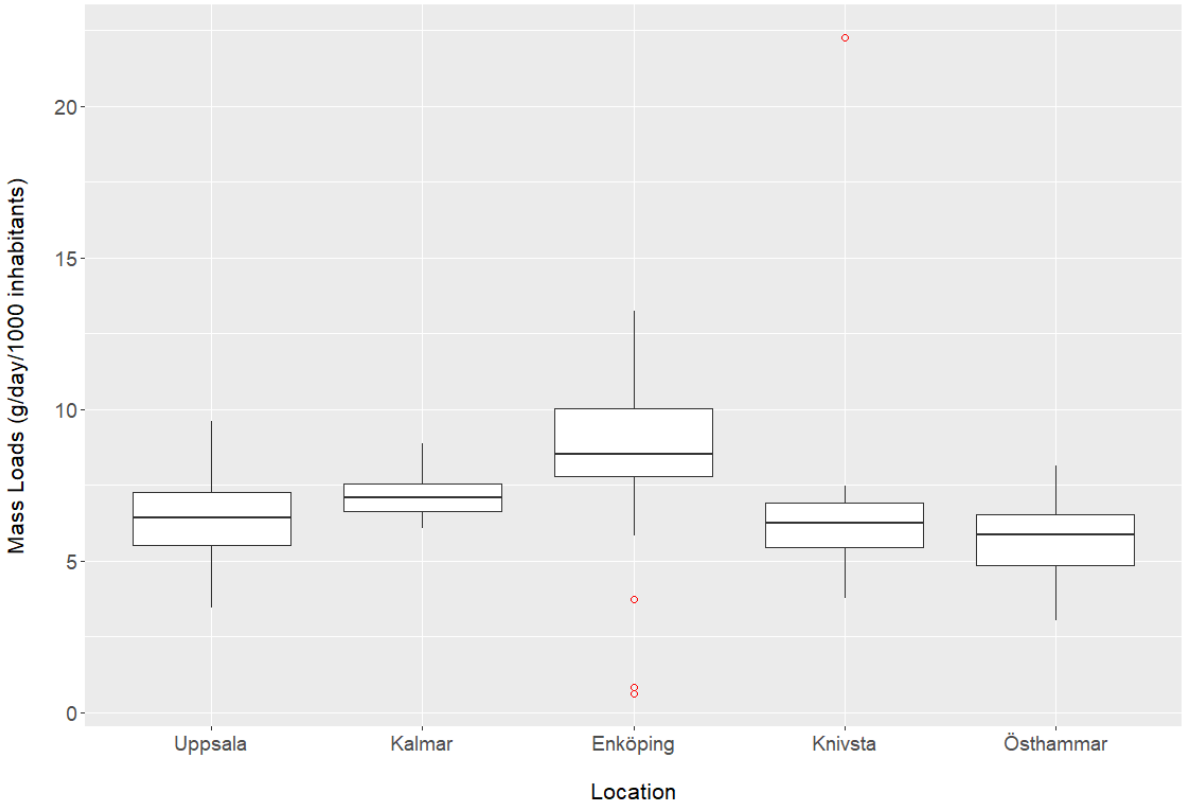
Figure 3.6 shows the composition of sucrose equivalent doses, similar to Figure 3.3. It appears that although the amount of consumed AS varies across locations, the composition follows the same pattern. At all locations, sucralose was the most used AS, followed by acesulfame and lastly saccharin.



(a) Acesulfame



(b) Saccharin



(c) Sucralose

Figure 3.5.: Spatial Comparsion of AS Loads

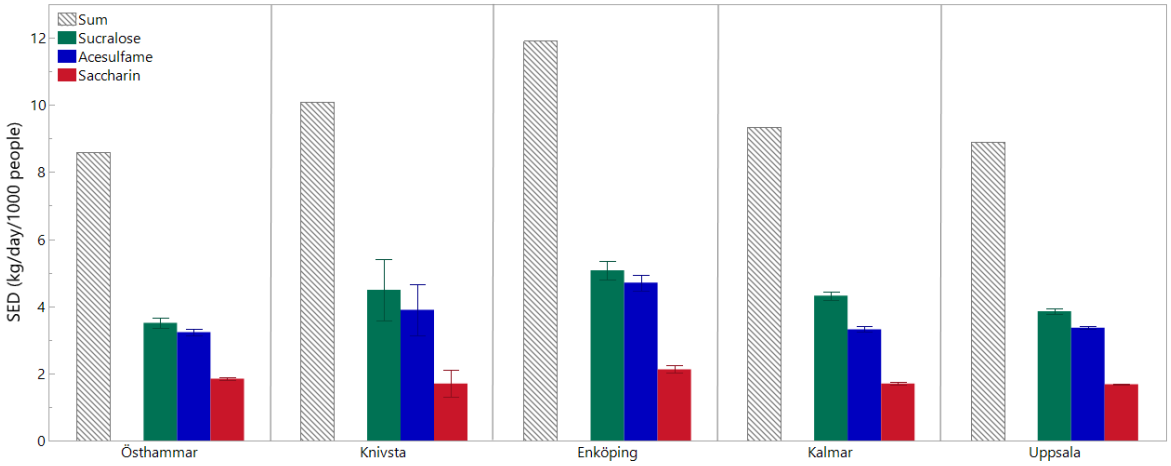


Figure 3.6.: Sucrose Equivalent Doses (SED) Across Locations

4 Discussion

4.1. Population Correlation

Due to potential different preferences and consumption patterns across countries, the correlation between chemical mass load and population size may vary and therefore needs validation before applying in a another country.

As stated in Chapter 2.1, most of the candidates chosen for this study were based on the literature (O'Brien et al., 2014; Senta et al., 2015; C. Chen et al., 2014). For example, the study by O'Brien et al. (2014) validated the correlation with certain chemicals that are highly used in the Australian population. This thesis therefore attempted to verify the suitability of the suggested compounds in the Swedish population.

The results showed that most of the compounds validated in Australia are also suitable for the Swedish population. Compounds that showed better results in Sweden than in Australia were atenolol, caffeine and codeine. For atenolol an R^2-value of 0.994 was achieved in Sweden as compared to 0.823 in Australia. Caffeine achieved 0.997 in Sweden and only 0.869 in Australia. Codeine varied between 0.96 in Sweden and 0.849 in Australia. In contrast, gabapentin represented the Australian population better than the Swedish with R^2-values of 0.968 and 0.88, respectively.

Nicotine showed no correlation to the population size which is in accordance with other studies that point out the general unsuitability of nicotine as a consumption biomarker. The disposal of cigarette butts on the street and resulting run-off that ends up in the WWTP, cause unspecificity of the nicotine source and therefore make it a generally unfit to indicate nicotine consumption (Castiglioni et al., 2015).

The detection frequency of 5-HIAA was very low and variable, which led to the decision to exclude the compound from further analysis. Difficulties in the analysis and detection of 5-HIAA were reported by other studies already and were related to its unstable nature (C. Chen et al., 2014). To overcome this problem C. Chen et al. (2014) proposed to acidify the samples to pH 2.

In a study from Thai et al. (2019), the stability of 5-HIAA was investigated and they found out that for direct injection, an acidification actually promotes the degradation of 5-HIAA in the sample and that higher detection frequencies were achieved at a higher pH. But even then, it could not consistently be detected (Thai et al., 2019).

Venlafaxine, its metabolite desmethylvenlafaxine, citalopram, metformin, theophylline, as well as sucralose and saccharin were tested for the first time as possible population biomarkers. All of them showed a very good correlation to the population size and are thus a great fit in the Swedish context.

4.2. Artificial Sweetener Consumption in Sweden

Studies that measured artificial sweeteners in Swedish influent wastewater are very limited. Most studies focus on the removal efficiency and the environmental fate of artificial sweeteners and sucralose in particular (Gago-Ferrero et al., 2017; Zou et al., 2015). In the following paragraphs, all comparisons are drawn to the reference location Uppsala as the longest period of time could be captured for this location.

There was one detailed screening study from the Swedish Environmental Institute in 2008, that measured sucralose in different matrices including influent wastewater (Swedish Environmental Research Institute, 2008). They detected sucralose at levels of 7 600 - 7 900 ng/L in Stockholm, which Lange et al. (2012) calculated into an average mass load of 0.8 g/day/1000 people. This lies 8 magnitudes below the average of Uppsala (6.71 g/day/1000 people). Comparable studies on acesulfame and saccharin concentrations in Swedish influent wastewater could not be found.

However, studies from other parts in Europe and around the world included more AS than just sucralose. Two recent studies from Australia examined the patterns and the long-term (7-year) trend of the artificial sweetener consumption (Li et al., 2020; Li et al., 2021). The usage pattern for the three AS was the same in Australia as the one observed in Uppsala during this study. The highest mass loads were observed for acesulfame with 6.9 g/day/1000 people, followed by sucralose (3.7 g/day/1000 people) and lastly saccharin (2 g/day/1000 people) (Li et al., 2020). These measured levels are around half as high as the ones observed in this study, but still, in Australia they could also observe an increase of AS consumption over time. Within a span of 7 years, the consumption increased with an annual rate of 10% for sucralose and 6% for saccharin. The consumption of acesulfame fluctuated over time, which is why no increase or decrease rate could be concluded (Li et al., 2021).

To give an European example, Scheurer et al. (2009) measured AS in German wastewater. Acesulfame was measured at levels around half as high (9.46 g/day/1000 people) as the results obtained in this study.

Saccharin loads, on the other hand, were almost twice as high as compared to our results (9.26 g/day/1000 people).

Sucralose levels were very low in Germany with 0.22 g/day/1000 people and were thus 30 times lower than the average measured in Uppsala. This result indicates a different usage pattern of AS in Germany as compared to Sweden. In Germany, acesulfame and saccharin appear to be similar in usage while sucralose is much less common. Loos et al. (2009) studied the abundance of sucrose within Europe by monitoring river water. They noticed that sucralose is more common in western European countries (e.g. Belgium, France, Switzerland) and Scandinavia than it is in Germany and Eastern Europe. This may explain these discrepancies in the AS usage.

As indicated by Loos et al. (2009), Switzerland is supposed to have a more comparable usage of sucralose to Sweden. In a study from Buerge et al. (2009), acesulfame, saccharin and sucralose were measured in wastewater from 10 municipalities in the Zurich area, Switzerland. Acesulfame and saccharin loads were detected at an average of 10 and 3.9 g/day/1000 people, respectively. This corresponds to only half of what was measured in Uppsala. Sucralose was measured at an average of 1.5 g/day/1000 people in the Swiss population, which is almost seven times higher what was measured in Germany but still a four order magnitude lower than the levels measured in Uppsala.

Overall, the measured levels of AS in this study appeared to be higher than in other countries. Especially the loads of sucralose were much higher, also than previously observed in Sweden.

One explanation is the global AS market that grew with an annual rate of 5.1% between 2008 and 2015 (Sylvetsky et al., 2016). Increasing problems with obesity and the general trend towards a healthier diet increased the demand for non-caloric sweeteners. A report from the Swedish Board of Agriculture (*Jordbruksverket*) stated that the sugar consumption (including AS) in Sweden reduced between 2000 and 2009. However, as sugar and AS are not measured individually, they assume that while the sugar consumption decreased, the non-caloric sweeteners replaced the sugar deficit and the AS consumption increased unaccounted (Jordbruksverket, 2015).

One other explanation is the growing population in Uppsala. In Chapter 3.1.2, a very good correlation between the population size and the AS loads was established. This suggests that an increase in AS loads results from an increased population size and not necessarily from an increased consumption. To differentiate this two processes from each other, a comparison between the variability of the population and the AS loads was compared over time (2019-2022) (compare Chapter 3.2.2). In that way, it was shown that at least part of the trend resulted from an actual increase in usage. As census data is missing, it is however not possible to quantify how much of the increase results from an a growing population size.

A third possible reason is an increased usage induced by the pandemic. During the pandemic, certain behavioral changes were observed in the population via wastewater analysis. Most WBE studies performed in this context focused on the consumption of pharmaceuticals, illicit and licit drugs (Estévez-Danta et al., 2022; Reinstadler et al., 2021; Galani et al., 2021).

In the context of shifting eating habits, most studies were implemented through surveys with two contradicting trends: on the one hand, people had more time to cook at home and therefore ate less processed food; on the other hand, more comfort food such as sodas, pastries and ice-cream were consumed while staying and working from home (Janssen et al., 2021; Bracale et al., 2020). Generally, people tended to consume more food, even though they might not have shifted their food choices (Janssen et al., 2021). These findings indicate that we would expect an increase in the artificial sweetener consumption. As mentioned in the introduction chapter, the Swedish regulations were quite steady with a long period of recommended home office but without lock-downs and curfews. This is why we cannot observe a lot of sudden changes.

Even though we cannot derive specific patterns related to the COVID-19 measures in this data set, we recognize a clear trend with increasing AS loads over time. This time trend could be due to different factors, such as the growing market, changed eating habits, and/or an influence of the pandemic measures.

4.3. Uncertainties

An uncertainty analysis from Castiglioni et al. (2013) concluded that the highest uncertainties in WBE studies arise from the sampling, the (in-sewer and storage) stability of the target compounds, the analytical measurements and the performance of back-calculations, especially considering the estimation of the population size. This is the reason why best-practice criteria were proposed to minimize these uncertainties.

Sampling should be performed in a flow-proportional manner and preferably in consecutive instead of scattered days. The samples from Uppsala were weekly composite samples taken proportionally to the flow. They therefore fulfill the criteria. The samples from Kalmar, Enköping, Knivsta and Östhammar were taken flow-proportionally but they were daily composite samples, taken on one day of the studied week. These sample may therefore not be a good representation of the weekly average as a limitation of our results.

Analytical uncertainties were minimal with the within-run precision and instrument variability in the acceptable ranges (Castiglioni et al., 2013).

One of the main uncertainties of WBE studies is the correct estimation of the population size. The population in Uppsala or other locations may fluctuate due to the mobility of residences during holiday times, especially the summer vacation time. It is very difficult to access de facto population in each of the locations in Sweden for this study. In Chapter 1.2, it was already mentioned that researchers have been attempting to overcome this uncertainty by different ways in order to obtain the availability of accurate population data.

Due to the lack of time and resources, it was unfortunately not possible to obtain such data for this study. This is the reason why the conventional approach of using the number of residents connected to the sewage facility was used as an population size approximation. This could be a limitation in our temporal and spatial comparison.

5 Conclusion

The temporal comparison of the AS consumption showed no specific pattern relatable to the COVID-19 waves and national regulations, but showed an increased trend in AS loads over time. The spatial comparison showed that the studied locations have a similar usage pattern but that the amount of consumed AS varies spatially.

The results also showed that all 23 compounds from the candidate list were measurable in Swedish wastewater and expect for 5-HIAA, all of them with an acceptable detection frequency. Compounds that showed good correlations to the population size in other countries, were also a good fit for Sweden. Venlafaxine, desmethylvenlafaxine, citalopram, metformin, theophylline, sucralose and saccharin were proposed and confirmed as possible population biomarkers for the first time.

By including samples from populations with varying sizes, it was possible to analyze the correlation between mass loads and the population size and thereby receive a first assessment about the suitability of these compounds as population biomarkers. Yet, without census data, no further application of this knowledge is possible. The next step would be to develop a population model, that enables the prediction of the population size, similar to what was done in O'Brien et al. (2014).

Bibliography

Baz-Lomba, Jose Antonio, Francesco Di Ruscio, Arturo Amador, Malcolm Reid, et al. (2019). "Assessing Alternative Population Size Proxies in a Wastewater Catchment Area Using Mobile Device Data". In: *Environmental science & technology* 53.4, pp. 1994–2001. DOI: 10.1021/acs.est.8b05389 (cit. on p. 3).

Been, Frederic, Luca Rossi, Christoph Ort, Serge Rudaz, et al. (2014). "Population normalization with ammonium in wastewater-based epidemiology: application to illicit drug monitoring". In: *Environmental science & technology* 48.14, pp. 8162–8169. DOI: 10.1021/es5008388 (cit. on p. 3).

Bemanian, Mitra, Silje Mæland, Rune Blomhoff, Åsgeir Kjetland Rabben, et al. (2020). "Emotional Eating in Relation to Worries and Psychological Distress Amid the COVID-19 Pandemic: A Population-Based Survey on Adults in Norway". In: *International journal of environmental research and public health* 18.1. DOI: 10.3390/ijerph18010130 (cit. on p. 1).

Biddle, N., B. Edwards, and Gray, M., Sollis, K. (2020). "Alcohol consumption during the COVID- 19 period". In: *ANU Centre for Social Research and Methods* (cit. on p. 1).

Bracale, Renata and Concetta M. Vaccaro (2020). "Changes in food choice following restrictive measures due to Covid-19". In: *Nutrition, metabolism, and cardiovascular diseases : NMCD* 30.9, pp. 1423–1426. DOI: 10.1016/j.numecd.2020.05.027 (cit. on p. 27).

Buerge, Ignaz J., Hans-Rudolf Buser, Maren Kahle, Markus D. Müller, et al. (2009). "Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: an ideal chemical marker of domestic wastewater in groundwater". In: *Environmental science & technology* 43.12, pp. 4381–4385. DOI: 10.1021/es900126x (cit. on p. 26).

Castiglioni, Sara, Lubertus Bijlsma, Adrian Covaci, Erik Emke, et al. (2013). "Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers". In: *Environmental science & technology* 47.3, pp. 1452–1460. DOI: 10.1021/es302722f (cit. on pp. 3, 27).

Castiglioni, Sara, Ivan Senta, Andrea Borsotti, Enrico Davoli, et al. (2015). "A novel approach for monitoring tobacco use in local communities by wastewater analysis". In: *Tobacco control* 24.1, pp. 38–42. DOI: 10.1136/tobaccocontrol-2014-051553 (cit. on p. 24).

Chen, Chang, Chris Kostakis, Jacobus P. Gerber, Ben J. Tscharke, et al. (2014). "Towards finding a population biomarker for wastewater epidemiology studies". In: *The Science of the total environment* 487, pp. 621–628. DOI: 10.1016/j.scitotenv.2013.11.075 (cit. on pp. 3, 5, 24).

Chen, Yun, Walter Osika, Göran Henriksson, Johan Dahlstrand, et al. (2022). "Impact of COVID-19 pandemic on mental health and health behaviors in Swedish adolescents". In: *Scandinavian journal of public health* 50.1, pp. 26–32. DOI: 10.1177/14034948211021724 (cit. on p. 2).

Choi, Phil M., Ben J. Tscharke, Erica Donner, Jake W. O'Brien, et al. (2018). "Wastewater-based epidemiology biomarkers: Past, present and future". In: *TrAC Trends in Analytical Chemistry* 105, pp. 453–469. ISSN: 01659936. DOI: 10.1016/j.trac.2018.06.004 (cit. on pp. 2, 3).

Daughton, Christian G. (2001). "Illicit Drugs in Municipal Sewage". In: *Pharmaceuticals and Care Products in the Environment*. Ed. by Christian G. Daughton and Tammy L. Jones-Lepp. Vol. 791. ACS Symposium Series. Washington, DC: American Chemical Society, pp. 348–364. ISBN: 9780841237391. DOI: 10.1021/bk-2001-0791.ch020 (cit. on p. 2).

– (2012). "Real-time estimation of small-area populations with human biomarkers in sewage". In: *The Science of the total environment* 414, pp. 6–21. DOI: 10.1016/j.scitotenv.2011.11.015 (cit. on p. 3).

– (2018). "Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM)". In: *The Science of the total environment* 619-620, pp. 748–764. DOI: 10.1016/j.scitotenv.2017.11.102 (cit. on p. 3).

Elvén, Maria, Birgitta Kerstis, Jonas Stier, Charlotta Hellström, et al. (2022). "Changes in Physical Activity and Sedentary Behavior before and during the COVID-19 Pandemic: A Swedish Population Study". In: *International journal of environmental research and public health* 19.5. DOI: 10.3390/ijerph19052558 (cit. on p. 2).

Estévez-Danta, Andrea, Lubertus Bijlsma, Ricardo Capela, Rafael Cela, et al. (2022). "Use of illicit drugs, alcohol and tobacco in Spain and Portugal during the COVID-19 crisis in 2020 as measured by wastewater-based epidemiology". In: *The Science of the total environment* 836, p. 155697. DOI: 10.1016/j.scitotenv.2022.155697 (cit. on pp. 2, 27).

European Commission - DG Health & Food Safety (2022). *Database on Food Additives*. URL: https://webgate.ec.europa.eu/foods_system/main/?event=display (cit. on p. 2).

Gago-Ferrero, Pablo, Meritxell Gros, Lutz Ahrens, and Karin Wiberg (2017). "Impact of on-site, small and large scale wastewater treatment facilities on levels and fate of pharmaceuticals, personal care products, artificial sweeteners, pesticides, and perfluoroalkyl substances in recipient waters". In: *The Science of the total environment* 601-602, pp. 1289–1297. DOI: 10.1016/j.scitotenv.2017.05.258 (cit. on p. 25).

Galani, Aikaterini, Nikiforos Alygizakis, Reza Aalizadeh, Efstathios Kastritis, et al. (2021). "Patterns of pharmaceuticals use during the first wave of COVID-19 pandemic in Athens, Greece as revealed

by wastewater-based epidemiology". In: *The Science of the total environment* 798, p. 149014. DOI: 10.1016/j.scitotenv.2021.149014 (cit. on p. 27).

Giacalone, Davide, Michael Bom Frøst, and Celia Rodríguez-Pérez (2020). "Reported Changes in Dietary Habits During the COVID-19 Lockdown in the Danish Population: The Danish COVIDiet Study". In: *Frontiers in nutrition* 7, p. 592112. ISSN: 2296-861X. DOI: 10.3389/fnut.2020.592112 (cit. on p. 1).

Golovko, Oksana, Stefan Örn, Mattias Sörengård, Kim Frieberg, et al. (2021). "Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems". In: *The Science of the total environment* 754, p. 142122. DOI: 10.1016/j.scitotenv.2020.142122 (cit. on p. 6).

Haalck, Inga, Paul Löffler, Christine Baduel, Karin Wiberg, et al. (2021). "Mining chemical information in Swedish wastewaters for simultaneous assessment of population consumption, treatment efficiency and environmental discharge of illicit drugs". In: *Scientific reports* 11.1, p. 13510. DOI: 10.1038/s41598-021-92915-4 (cit. on p. 2).

Heckman, Melanie A., Jorge Weil, and Elvira Gonzalez de Mejia (2010). "Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters". In: *Journal of food science* 75.3, R77–87. DOI: 10.1111/j.1750-3841.2010.01561.x (cit. on p. 5).

Janssen, Meike, Betty P. I. Chang, Hristo Hristov, Igor Pravst, et al. (2021). "Changes in Food Consumption During the COVID-19 Pandemic: Analysis of Consumer Survey Data From the First Lockdown Period in Denmark, Germany, and Slovenia". In: *Frontiers in nutrition* 8, p. 635859. ISSN: 2296-861X. DOI: 10.3389/fnut.2021.635859 (cit. on pp. 1, 27).

Jordbruksverket (2015). "Livsmedelkonsumtionen i siffror: Hur har konsumtionen utvecklats de senaste femtio åren och varför?" In: (cit. on p. 26).

Lai, Foon Yin, Shalona Anuj, Raimondo Bruno, Steve Carter, et al. (2015). "Systematic and day-to-day effects of chemical-derived population estimates on wastewater-based drug epidemiology". In: *Environmental science & technology* 49.2, pp. 999–1008. DOI: 10.1021/es503474d (cit. on p. 3).

Lange, Frank T., Marco Scheurer, and Heinz-J Brauch (2012). "Artificial sweeteners–a recently recognized class of emerging environmental contaminants: a review". In: *Analytical and bioanalytical chemistry* 403.9, pp. 2503–2518. DOI: 10.1007/s00216-012-5892-z (cit. on p. 25).

Li, Dandan, Jake W. O'Brien, Benjamin J. Tscharke, Phil M. Choi, et al. (2020). "National wastewater reconnaissance of artificial sweetener consumption and emission in Australia". In: *Environment international* 143, p. 105963. DOI: 10.1016/j.envint.2020.105963 (cit. on pp. 10, 25).

Li, Dandan, Jake W. O'Brien, Benjamin J. Tscharke, Phil M. Choi, et al. (2021). "Trends in artificial sweetener consumption: A 7-year wastewater-based epidemiology study in Queensland, Australia". In: *The Science of the total environment* 754, p. 142438. DOI: 10.1016/j.scitotenv.2020.142438 (cit. on pp. 2, 10, 25).

Loos, Robert, Bernd Manfred Gawlik, Kristin Boettcher, Giovanni Locoro, et al. (2009). "Sucralose screening in European surface waters using a solid-phase extraction-liquid chromatography-triple

quadrupole mass spectrometry method". In: *Journal of chromatography. A* 1216.7, pp. 1126–1131. ISSN: 0021-9673. DOI: 10.1016/j.chroma.2008.12.048 (cit. on p. 26).

Lundy, Lian, Despo Fatta-Kassinos, Jaroslav Slobodnik, Popi Karaolia, et al. (2021). "Making Waves: Collaboration in the time of SARS-CoV-2 - rapid development of an international co-operation and wastewater surveillance database to support public health decision-making". In: *Water research* 199, p. 117167. DOI: 10.1016/j.watres.2021.117167 (cit. on p. 2).

Luo, Jingyang, Qin Zhang, Miao Cao, Lijuan Wu, et al. (2019). "Ecotoxicity and environmental fates of newly recognized contaminants-artificial sweeteners: A review". In: *The Science of the total environment* 653, pp. 1149–1160. DOI: 10.1016/j.scitotenv.2018.10.445 (cit. on p. 10).

Magnuson, Bernadene A., Michael C. Carakostas, Nadia H. Moore, Sylvia P. Poulos, et al. (2016). "Biological fate of low-calorie sweeteners". In: *Nutrition reviews* 74.11, pp. 670–689. DOI: 10.1093/nutrit/nuw032 (cit. on p. 10).

Montgomery, Alexander B., Isaac Bowers, and Bikram Subedi (2021). "Trends in Substance Use in Two United States Communities during Early COVID-19 Lockdowns Based on Wastewater Analysis". In: *Environmental Science & Technology Letters* 8.10, pp. 890–896. ISSN: 2328-8930. DOI: 10.1021/acs.estlett.1c00426 (cit. on p. 2).

O'Brien, Jake W., Andrew Phillip William Banks, Andrew Joseph Novic, Jochen F. Mueller, et al. (2017). "Impact of in-Sewer Degradation of Pharmaceutical and Personal Care Products (PPCPs) Population Markers on a Population Model". In: *Environmental science & technology* 51.7, pp. 3816–3823. DOI: 10.1021/acs.est.6b02755 (cit. on pp. 5, 6).

O'Brien, Jake W., Phong K. Thai, Geoff Eaglesham, Christoph Ort, et al. (2014). "A model to estimate the population contributing to the wastewater using samples collected on census day". In: *Environmental science & technology* 48.1, pp. 517–525. DOI: 10.1021/es403251g (cit. on pp. 3, 5, 24, 29).

Olofsson, Tobias and Andreas Vilhelmsson (2021). *Timeline of government interventions and events regarding the COVID-19 pandemic in Sweden January 31, 2019, to June 3, 2021.* DOI: 10.5281/zenodo.5718433 (cit. on p. 1).

Reinstadler, Vera, Verena Ausweger, Anna-Lena Grabher, Marco Kreidl, et al. (2021). "Monitoring drug consumption in Innsbruck during coronavirus disease 2019 (COVID-19) lockdown by wastewater analysis". In: *The Science of the total environment* 757, p. 144006. DOI: 10.1016/j.scitotenv.2020.144006 (cit. on pp. 2, 27).

Scheurer, Marco, Heinz-J Brauch, and Frank T. Lange (2009). "Analysis and occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT)". In: *Analytical and bioanalytical chemistry* 394.6, pp. 1585–1594. DOI: 10.1007/s00216-009-2881-y (cit. on p. 25).

SciLifeLab Data Centre (n.d.). *The Swedish COVID-19 & Pandemic Preparedness Data Portal.* URL: https://www.covid19dataportal.se/ (cit. on p. 7).

Senta, Ivan, Emma Gracia-Lor, Andrea Borsotti, Ettore Zuccato, et al. (2015). "Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment". In: *Water research* 74, pp. 23–33. DOI: 10.1016/j.watres.2015.02.002 (cit. on pp. 5, 24).

Swedish Environmental Research Institute (2008). "Measurements of Sucralose in the Swedish Screening program 2007. PART II; Sucralose in Biota samples and regional STP samples". In: IVL Report B1795. URL: https://www.ivl.se/download/18.343dc99d14e8bb0f58b7535/1445517357748/B1769.pdf (cit. on p. 25).

Sylvetsky, Allison C. and Kristina I. Rother (2016). "Trends in the consumption of low-calorie sweeteners". In: *Physiology & behavior* 164.Pt B, pp. 446–450. DOI: 10.1016/j.physbeh.2016.03.030 (cit. on pp. 3, 26).

Thai, Phong K., Jake W. O'Brien, Andrew P. W. Banks, Guangming Jiang, et al. (2019). "Evaluating the in-sewer stability of three potential population biomarkers for application in wastewater-based epidemiology". In: *The Science of the total environment* 671, pp. 248–253. DOI: 10.1016/j.scitotenv.2019.03.231 (cit. on p. 25).

The Government of Sweden (2020). *Strategy in response to the COVID-19 pandemic*. URL: https://www.government.se/articles/2020/04/strategy-in-response-to-the-covid-19-pandemic/ (cit. on p. 2).

Thomas, Kevin V., Arturo Amador, Jose Antonio Baz-Lomba, and Malcolm Reid (2017). "Use of Mobile Device Data To Better Estimate Dynamic Population Size for Wastewater-Based Epidemiology". In: *Environmental science & technology* 51.19, pp. 11363–11370. DOI: 10.1021/acs.est.7b02538 (cit. on p. 3).

van Nuijs, Alexander L. N., Jean-François Mougel, Isabela Tarcomnicu, Lieven Bervoets, et al. (2011). "Sewage epidemiology–a real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium". In: *Environment international* 37.3, pp. 612–621. DOI: 10.1016/j.envint.2010.12.006 (cit. on p. 3).

Xiong, Jiaqi, Orly Lipsitz, Flora Nasri, Leanna M. W. Lui, et al. (2020). "Impact of COVID-19 pandemic on mental health in the general population: A systematic review". In: *Journal of affective disorders* 277, pp. 55–64. DOI: 10.1016/j.jad.2020.08.001 (cit. on p. 1).

Zessner, M. and S. Lindtner (2005). "Estimations of municipal point source pollution in the context of river basin management". In: *Water Sci Technology* 52.52 // 9, pp. 175–182. DOI: 10.2166/wst.2005.0313 (cit. on p. 3).

Zou, Hongyan, Michael Radke, Amelie Kierkegaard, Matthew MacLeod, et al. (2015). "Using chemical benchmarking to determine the persistence of chemicals in a Swedish lake". In: *Environmental science & technology* 49.3, pp. 1646–1653. DOI: 10.1021/es505548k (cit. on p. 25).

Zucatto, Ettore, Chiara Chiabrando, Sara Castiglioni, Davide Calamari, et al. (2005). “Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse”. In: *Environmental Health: A Global Access Science Source* 2005.4. DOI: 10.1186/1476-069X-4-14 (cit. on p. 2).

6 Popular Science Summary

The COVID-19 pandemic has impacted our daily lives and we needed to adapt to the measures that were implemented by the government. This resulted in distress for the population and caused behavioral changes in several parts of our live. For instance, it was found that people changed their eating habits by consuming more processed food or candy. These changes can also be studied by analyzing the wastewater of a community.

When we consume different compounds, e.g. pharmaceuticals or food additives, these compounds are metabolized in the human body, excreted with urine and are then transported via the wastewater to the local wastewater-treatment plants (WWTP). This is where wastewater samples can be taken. The chemical analysis of these samples can then provide information about the consumption of the studied compounds for the community that is connected to the WWTP. This approach is called wastewater-based epidemiology (WBE).

WBE was used in this thesis to study if people in Uppsala changed their eating behavior during the pandemic, indicated by artificial sweeteners. Artificial sweeteners (AS) are food additives that are used in energy-reduced products such as diet sodas, but also in canned and processed food. The hypothesis was that, in times when home office was recommended by the Swedish government, people were more stressed out and then tended to eat more comfort food in form of processed food, sodas and candy, which would then lead to higher concentrations of artificial sweeteners in the wastewater.

The results showed that no changes in the consumption pattern of AS were relatable to the COVID-19 pandemic and the national regulations. A temporal comparison showed however an increased trend of AS loads in Uppsala over time, indicating an increasing consumption.

When applying WBE, you need to follow certain steps. One step is the back-calculation to the population size of the studied community. For that, you need accurate data on the population size. As people are mobile, commute to other cities to work or go on holidays, the population size fluctuates. In order to account for this fluctuations, researchers tried to find compounds that can be linked to the population size by correlating them.

In a second part of this thesis, 23 different compounds were analyzed in the wastewater of five different locations in Sweden, to assess if their mass loads can be correlated to the varying population sizes of the studied locations. It turned out that 21 out of these 23 compounds show a good correlation, which makes them suitable to indicate population fluctuations. This knowledge will be used in a future project, in which a population model will be developed to dynamically predict the population size of Swedish communities.

7 Acknowledgments

I would like to thank the members of the Department of Aquatic Sciences and Assessment for being so welcoming and providing me with the necessary facilities for my thesis. I would like to thank Anna Székely for providing the wastewater samples that I used for my study. I would also like to thank Elin Eriksson for her help in the lab.

A special thanks goes to my main-supervisor Foon for being a great support and giving me guidance throughout the whole process of the thesis.

A Appendix

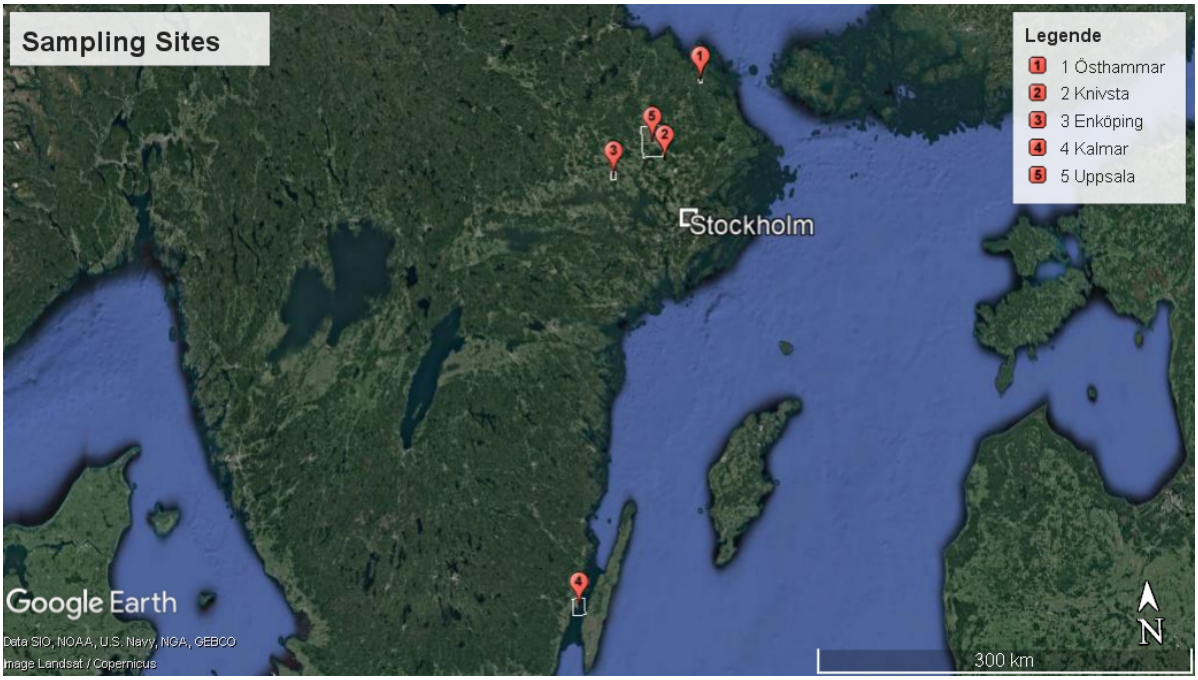


Figure A.1.: Map of the Sampling Sites

Table A.1.: Calibration Curve

Point	1	2	3	4	5	6	7	8	9
Concentration (µg/L)	0	0.5	1	2.5	5	10	25	50	100

Table A.2.: MRM Parameters for Positive Ionization

Q1	Q3	Name	DP	EP	CE	EXP
130	71	Metformin 1	56	10	29	8
130	43.1	Metformin 2	56	10	45	12
267.2	190	Atenolol 1	60	10	27	9
267.2	145	Atenolol 2	60	10	38	9
195.1	138.1	Caffeine 1	71	10	28	8
195.1	110.1	Caffeine 2	71	10	32	8
237.2	194	Carbamazepine 1	96	10	31	16
237.2	193	Carbamazepine 2	96	10	47	12
300.2	215.1	Codeine 1	60	10	37	12
300.2	165.1	Codeine 2	60	10	57	10
231.2	185.1	Naproxen 1	64	10	23	10
231.2	170.1	Naproxen 2	64	10	33	12
152.1	110	Acetaminophen 1	56	10	24	8
152.1	65.1	Acetaminophen 2	56	10	42	6
158.2	116	Acetaminophem-13C6 1	76	10	22	8
158.2	98.9	Acetaminophem-13C6 2	76	10	31	8
198.1	140.1	Caffeine-13C3 1	82	10	27	10
198.1	112.1	Caffeine-13C3 2	82	10	31	9
273	145	Atenolol-d6 1	101	10	35	10
273	91	Atenolol-d6 2	101	10	63	8
303.1	152.1	Codeine-d3 1	110	10	81	10
303.1	165.1	Codeine-d3 2	110	10	53	10
181.1	124	Theophyline 1	80	10	25	10
181.1	96	Theophyline 2	80	10	30	10
181.2	124.2	Paraxanthine_POS 1	60	10	25	8
181.2	96.1	Paraxanthine_POS 2	60	10	32	8
162.9	130.1	Nicotine 1	51	10	27	12
162.9	117	Nicotine 2	51	10	39	18
325.1	109	Citalopram 1	90	10	37	8
325.1	262.1	Citalopram 2	90	10	29	16
278.1	58.1	Venlafaxine 1	60	10	47	10
278.1	121	Venlafaxine 2	60	10	43	8
264	58.2	O-desmethylvenlafaxine 1	70	10	45	6
264	107.1	O-desmethylvenlafaxine 2	70	10	39	8
177.1	80	Cotinine 1	90	10	35	12
177.1	98	Cotinine 2	90	10	30	12

Table A.3.: MRM Parameters for Positive Ionization - Part II

Q1	Q3	Name	DP	EP	CE	EXP
172.2	137.2	Gabapentin 1	51	10	23	6
172.2	154.1	Gabapentin 2	51	10	19	14
193.1	134.1	trans-3'-hydroxycotinine 1	60	10	30	15
193.1	118.1	trans-3'-hydroxycotinine 2	60	10	30	11
192	146	5-HIAA 1	50	10	24	15
192	117	5-HIAA 2	50	10	50	15
247.2	204.1	Carbamazepine-D10 1	65	10	30	8
247.2	202.1	Carbamazepine-D10 2	65	10	51	8
247.1	123.9	Carbamazepine-D10 3	140	10	34	9
247.1	229.1	Carbamazepine-D10 4	140	10	26	8
167	136	Nicotine D4 1	61	10	23	10
167	121.1	Nicotine D4 2	61	10	39	12
182.2	147.3	Gabapentin D10 1	46	10	25	14
182.2	164.3	Gabapentin D10 2	46	10	21	14
284.2	64.2	Venlafaxine D6 1	46	10	37	4
284.2	121.2	Venlafaxine D6 2	46	10	33	10
136.1	60	Metformin D6 1	46	10	21	10
136.1	77	Metformin D6 2	46	10	33	4
180	101	Cotinine D3 1	76	10	37	8
197	151	5-HIAA D5 1	50	10	24	15

Table A.4.: MRM Parameters for Negative Ionization

Q1	Q3	Name	DP	EP	CE	EXP
162	82	Acesulfame K 1	-45	-10	-22	-5
162	78	Acesulfame K 2	-45	-10	-45	-5
296	269	Hydrochlorothiazide 1	-85	-10	-28	-20
296	205	Hydrochlorothiazide 2	-85	-10	-34	-12
205.1	161	Ibuprofen 1	-52	-10	-12	-10
205.1	159	Ibuprofen 2	-52	-10	-11	-10
229.2	185.1	Naproxen 1	-53	-10	-11	-10
229.2	170.1	Naproxen 2	-53	-10	-22	-10
137	93	Salicyclic Acid 1	-45	-10	-24	-6
137	65	Salicyclic Acid 2	-45	-10	-40	-6
182	42.1	Saccharin 1	-70	-10	-57	-12
182	105.9	Saccharin 2	-70	-10	-24	-12
397	35.1	Sucralose 1	-85	-10	-55	-10
397	37.1	Sucralose 2	-85	-10	-55	-10
207.9	164	Ibuprofen-d3 1	-20	-10	-10	-7
301.7	275	Hydrochlorothiazide-13C6 1	-100	-10	-26	-5
301.7	210.9	Hydrochlorothiazide-13C6 2	-100	-10	-32	-13
400.9	365.2	Sucralose-d6 1	-86	-10	-14	-7
400.9	278.8	Sucralose-d6 2	-86	-10	-28	-14
395	359	Sucralose_adductsA 1	-80	-10	-14	-8
397	361	Sucralose_adductsA 2	-80	-10	-14	-8
401	365	Sucralose-d6_adductsA 1	-85	-10	-14	-12
403	367	Sucralose-d6_adductsA 2	-85	-10	-14	-12
441	395	Sucralose_adductsB 1	-50	-10	-16	-12
443	397	Sucralose_adductsB 2	-50	-10	-16	-12
447	401	Sucralose-d6_adductsB 1	-85	-10	-14	-12
449	403	Sucralose-d6_adductsB 2	-85	-10	-14	-12
166	86	Acesulfame D4 1	-45	-10	-22	-5
166	78	Acesulfame D4 2	-45	-10	-45	-7

Table A.5.: Limit of Detection for the Target Compounds

Native Compound	LOQ (µg/L)
Atenolol	0.1
Caffeine	0.2
Carbamazepine	0.05
Codeine	0.1
Naproxen	0.1
Gabapentin	0.1
Acetaminophen	1.5
Nicotine	0.1
Cotinine	0.05
Trans-3'-Hydroxycotinine	1
5-Hydroxyindoleacetic Acid (5-HIAA)	2.5
Metformin	0.5
Citalopram	0.01
Venlafaxine	0.01
O-desmethylvenlafaxine	0.01
Paraxanthine	1
Theophylline	1
Acesulfame	0.5
Hydrocholorthiazide	0.05
Ibuprofen	1
Salicylic acid	1
Saccharine	0.5
Sucralose	2

Table A.6.: Summary Data Table - Average, Standard Deviation (SD), Detection Frequency (DF%), Minimum and Maximum

	Östhammar					Knivsta					Enköping				
	Mean	SD	DF%	Min	Max	Mean	SD	DF%	Min	Max	Mean	SD	DF%	Min	Max
Metformin	34.490	4.936	100	25.749	43.930	17.504	12.025	100	12.334	55.315	31.455	9.236	100	1.999	43.614
Atenolol	0.232	0.046	100	0.152	0.353	0.070	0.057	100	0.044	0.250	0.163	0.046	100	0.007	0.231
Caffeine	16.617	2.855	100	9.315	24.689	13.691	9.831	100	6.466	44.261	20.644	6.118	100	1.377	28.982
Carbamazepine	0.061	0.022	96.77	0.037	0.124	0.050	0.028	100	0.028	0.129	0.067	0.020	100	0.004	0.104
Codeine	0.110	0.029	100	0.051	0.185	0.070	0.056	100	0.033	0.240	0.144	0.043	100	0.008	0.223
Naproxen	2.069	0.346	100	1.316	2.827	1.675	1.215	100	0.918	5.469	2.947	0.889	100	0.193	4.243
Acetaminophen	42.894	15.435	100	12.807	105.542	17.305	12.782	100	2.807	54.237	52.219	20.611	100	3.206	89.153
Theophylline	1.246	0.201	100	0.719	1.607	1.155	0.817	100	0.762	3.729	1.322	0.379	100	0.080	1.772
Paraxanthine	27.894	4.782	100	16.250	40.072	27.554	19.706	100	18.792	89.565	30.983	9.175	100	1.987	41.148
Nicotine	2.029	0.298	100	1.302	2.458	1.906	1.242	100	1.120	5.773	31.712	15.540	100	0.260	58.957
Citalopram	0.082	0.012	100	0.051	0.114	0.057	0.038	100	0.038	0.176	0.066	0.018	100	0.004	0.094
Venlafaxine	0.104	0.016	100	0.072	0.138	0.109	0.067	100	0.067	0.319	0.145	0.041	100	0.009	0.207
O-desmethylvenlafaxine	0.372	0.051	100	0.243	0.469	0.308	0.225	100	0.162	1.011	0.499	0.135	100	0.028	0.672
Cotinine	1.015	0.212	100	0.519	1.367	0.919	0.536	100	0.600	2.587	1.558	0.484	100	0.073	2.413
Gabapentin	5.569	1.300	100	2.323	9.168	3.114	2.143	100	1.706	9.662	6.742	2.003	100	0.383	9.898
trans-3'-hydroxycotinine	1.893	0.361	100	1.025	2.720	1.523	1.032	100	0.951	4.716	2.345	0.672	100	0.142	3.328
5-HIAA	1.513	0.369	16.13	1.055	1.938	1.516	0.535	45.45	0.820	2.364	0.841	0.346	20.59	0.106	1.217
Acesulfame K	16.160	2.382	100	10.179	20.874	19.501	12.046	100	11.083	57.241	23.539	6.841	100	1.474	33.781
Hydrochlorothiazide	0.325	0.046	100	0.208	0.432	0.087	0.071	100	0.055	0.311	0.211	0.058	100	0.014	0.289
Ibuprofen	4.259	0.769	100	2.601	5.867	2.930	1.968	100	1.938	9.083	4.422	1.349	100	0.264	6.190
Salicyclic Acid	5.418	1.410	100	2.275	8.353	3.663	1.841	90.91	2.136	8.861	6.764	2.732	97.06	0.512	11.261
Saccharin	6.164	0.756	100	4.228	7.199	5.664	4.253	100	3.755	19.070	7.091	2.187	100	0.423	10.441
Sucralose	5.846	1.295	100	3.049	8.155	7.487	4.792	100	3.781	22.275	8.459	2.584	100	0.615	13.232

	Kalmar					Uppsala				
	Mean	SD	DF%	Min	Max	Mean	SD	DF%	Min	Max
Metformin	19.174	2.275	100	15.998	23.700	20.393	2.674	100	13.320	26.344
Atenolol	0.121	0.015	100	0.095	0.149	0.116	0.013	100	0.089	0.151
Caffeine	17.875	2.412	100	14.643	22.806	16.375	2.550	100	10.181	21.042
Carbamazepine	0.041	0.013	100	0.025	0.081	0.034	0.007	100	0.024	0.074
Codeine	0.057	0.006	100	0.044	0.068	0.072	0.011	100	0.049	0.108
Naproxen	2.748	0.375	100	2.257	3.464	2.246	0.400	100	1.347	3.067
Acetaminophen	31.506	6.736	100	13.604	42.639	18.801	6.538	100	6.979	32.744
Theophylline	1.124	0.121	100	0.915	1.366	1.118	0.155	100	0.755	1.411
Paraxanthine	25.820	2.842	100	21.055	30.766	26.278	4.534	100	16.688	35.946
Nicotine	2.064	0.224	100	1.598	2.544	1.886	0.219	100	1.428	2.337
Citalopram	0.031	0.004	100	0.024	0.038	0.065	0.011	100	0.038	0.090
Venlafaxine	0.132	0.016	100	0.101	0.159	0.145	0.018	100	0.105	0.190
O-desmethylvenlafaxine	0.438	0.050	100	0.348	0.533	0.494	0.073	100	0.341	0.640
Cotinine	1.132	0.122	100	0.923	1.321	1.145	0.202	100	0.725	1.606
Gabapentin	6.158	0.647	100	5.201	7.676	3.420	0.531	100	2.391	4.526
trans-3'-hydroxycotinine	1.954	0.190	100	1.619	2.344	1.621	0.230	100	1.097	2.090
5-HIAA	1.671	0.545	62.50	1.042	2.705	1.638	0.679	53.85	0.686	3.202
Acesulfame K	16.567	1.603	100	13.611	20.390	17.275	2.007	100	13.401	21.544
Hydrochlorothiazide	0.163	0.019	100	0.132	0.206	0.123	0.016	100	0.084	0.154
Ibuprofen	3.771	0.492	100	2.944	4.771	3.268	0.630	100	1.993	4.955
Salicyclic Acid	2.159	1.086	93.75	0.275	3.673	6.055	4.040	100	1.887	36.391
Saccharin	5.672	0.596	100	4.269	6.649	5.687	0.695	100	3.943	7.130
Sucralose	7.200	0.780	100	6.078	8.864	6.711	1.151	100	4.175	9.617

Table A.7.: Relative Standard Deviation (RSD%) of the Population and the Flow Rate

Population					
Year	2018	2019	2020	2021	RSD% 2019/2021
Population (Skatteverket)	224519	230416	233745	237180	2.33%
Flow Rate					
Year	2019 (n=14)	2020 (n=37)	2021 (n=49)	2022 (n=8)	RSD% 2019/2022
Mean Flow Rate	63187.4286	48320.4445	49673.8776	51907.625	3.63%

Table A.8.: Relative Standard Deviation (RSD%) of AS Loads

	Acesulfame	Saccharin	Sucralose	Total
Mean Baseline 11-2019	12.8	4.62	4.22	21.6
Average 2020 (n=37)	16.2	5.37	5.99	27.6
Average 2021 (n=49)	17.8	5.83	7.095	30.7
Average 2022 (n=8)	19.1	6.29	7.80	33.2
RSD%	17%	13%	25%	18%