Technische Universiteit Delft

Monitoring of SARS-CoV-2 from wastewater

Factors affecting the representativeness of wastewater-based epidemiology for the monitoring of the SARS-CoV-2 virus

A.B. Stikkers

TUDelft

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by

A.B. Stikkers

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Preface

This report was written as part of the thesis work to graduate with a Master's degree in Water Management at TU Delft. This report is the result of my research done by TU Delft in collaboration with **RIVM** and **Partners4UrbanWater**. During the first lecture on wastewater-based epidemiology concerning COVID-19, I immediately wanted to learn more about this topic. Therefore, I am very happy that I was given the opportunity to work on this topic. While working on this thesis, I had the opportunity to meet multiple experts in the field of water management. This was not only helpful in understanding the topic as well as valuable for the various issues of my research. It created for me a lot of enthusiasm working on this project. Everyone was very helpful and curious about the results of my research. I learned a lot during this project, and I look forward to applying these lessons to my future career as a civil engineer.

First, I would like to express my appreciation to my thesis committee for helping me during this project. **Jeroen, Heike, Rémy** and **Gertjan**, I would like to thank you for all the meetings we had and all the help you have given me. In addition to helping with questions, critical notes and connecting me with experts, you have challenged me in learning from my thesis. Until the last moment, I tried to get everything out of this thesis. I am confident that this will help me in my future career.

Secondly, I would like to thank the experts that helped me, besides my thesis committee. From Partners4UrbanWater **Johan** and **Erik** assisted me with data and information on certain topics. **Imke** and **Judith** have supported me with the topic and data on automated samplers. Imke also connected me to experts for specific questions. **Dorothé, Weiyi** and **Anne-Merel** from RIVM, provided information and data. Also, I was guided through the lab and I got the opportunity to give multiple presentations. IMD helped me with information on samplers and flow meters. **Dennis, Tom** and **Sybe**, thank you for the meetings we had at your office in Apeldoorn and **Simon**, thank you for the visit to sampling locations. Also, **Eke** and **Fabian** from Aquon managed a visit to WWTP Leiden Zuid-West and helped me with data on rejected samples.

In addition, I would like to thank the water authorities **Delfland**, **Hollandse Delta**, **Brabantse Delta** and **Schieland en de Krimpenerwaard** for providing data and information on certain topics, and drinking water companies **Dunea** and **Evides** for providing data on the supply of drinking water.

Lastly, I would like to thank my family, roommates and friends that supported me during this thesis work. You were always interested in my study and the fun things we did together provided distraction and energy. In addition, you helped me with revising my work, programming and knowledge on certain topics. I was fortunate to have a lot of support from you.

A.B. Stikkers
Delft, August 2022

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Abstract

This thesis provides knowledge on factors affecting the representativeness of wastewater-based epidemiology (WBE) for monitoring the SARS-CoV-2 virus. The application of WBE to monitor health parameters has become essential due to the COVID-19 outbreak in March 2020. WBE is a well-known method to track the SARS-CoV-2 virus in wastewater and is used as an early warning system for the spread of the virus. However, the accuracy of monitoring trends through WBE is affected by various methodological challenges. This thesis aims to identify and quantify factors that affect the representativeness of monitoring the SARS-CoV-2 virus through WBE as part of the National Sewage Surveillance (NRS).

Factors that possibly influence the representativeness of WBE for monitoring the SARS-CoV-2 virus from loo to lab are identified. To what extent these factors potentially affect the representativeness is discussed in a literature review. The results are presented in a dendrogram, showing the relationship between different factors. Based on the results of the literature review, the seven most important factors affecting the accuracy of monitoring SARS-CoV-2 through WBE were determined to investigate their effect in more detail. To create an overview of the effect of the various factors and to ensure comparison, the results of the different factors are synthesized based on four criteria.

The effect on the representativeness is quantified and synthesized for the following factors: location of shedding, temporary in-sewer storage, RNA decay, deviant flow values, loss of information by overflow events, automated samplers and flow rate measurements. The effect of each factor is quantified individually. Different methodologies were used for the quantification of the different factors. The results were based on data analysis and expert consultations.

It was observed that the effect on the load of SARS-CoV-2 monitored by temporary in-sewer storage is negligible, as well as the effect by the decay of SARS-CoV-2 RNA by temperature and residence time. For the following factors, the influence on representativeness may be significant: The location of shedding, deviant flow values, loss of information by overflow events, automated samplers and flow rate measurements. Based on the results of the synthesis an advice is given on which factors should be included in the methodology of monitoring the virus through WBE and which samples should be excluded.

In conclusion, WBE is a valuable method to use for pandemic preparedness and monitoring health indicators from wastewater. There are many factors that influence the representativeness of samples for the monitoring of the SARS-CoV-2 virus. This research adds knowledge to the understanding of factors influencing the accuracy of WBE. The results elaborated by this study hope to contribute to the early detection of, not only, the SARS-CoV-2 virus, but also for other epidemic viruses, as well as other health indicators.

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Contents

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Acronyms

- **ALOD** Assay Limit Of detection
- **CBS** Centraal Bureau Statistiek
- **COVID-19** Coronavirus Disease 2019
- **CSO** Combined Sewer Overflow
- **DO** Dissolved Oxygen
- **DWF** Dry Weather Flow
- **IQR** Interquartile Range
- **KNMI** Koninklijk Nederlands Meteorologisch Instituut
- **LOD** Limit of Detection
- **LOQ** Limit of Quantification
- **MST** Microbial Source Tracking
- **NRS** Nationale Rioolwater Surveillance
- **N** Nitrogen
- **P** Phosphorous
- **pppd** Per person Per Day
- **QA/QC** Quality Assurance and Quality Control
- **Q1** 25th percentile
- **Q3** 75th percentile
- **RIVM** Rijksinstituut voor Volksgezondheid en Milieu
- **RWA** Regenwater Aanvoer
- **SARS-CoV-2** Severe Acute Respiratory Syndrome Coronavirus 2
- **SSO** Storm Sewer Outfall
- **TKN** Total Kjeldahl Nitrogen
- **TSS** Total Suspended Solids
- **UASB** Upflow Anaerobic Sludge Blanket
- **WBE** Wastewater-Based Epidemiology
- **WWF** Wet Weather Flow
- **WWTP** Wastewater Treatment Plant

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1

Introduction

1.1. Background information

March 2020, the World Health Organization (WHO) assessed the coronavirus disease 2019 (COVID-19) outbreak as a pandemic (Ghebreyesus, 2020). A new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused an outbreak in Wuhan in 2019 (Q. Li et al., 2020; Sanche et al., 2020). COVID-19 patients could suffer from fever, cough, shortness of breath, headaches and diarrhea (Ciotti et al., 2020). Patients can also suffer from long term effects, like long-covid, mental health problems or learning delay. COVID-19 turned out to be exceptionally infectious and a number of new variants have been even more infectious. Infections lead to pressure on the hospital system and medical staff, due to severe symptoms of patients who needed to be treated in the hospital. The virus continues to cause excess mortality around the world, with over 6.4 million COVID-19 deaths recorded by August 2022 according to the WHO Coronavirus (COVID-19) Dashboard.

1.2. Problem statement

The COVID-19 virus poses a threat to human health, health systems and socio-economic systems. The public health authorities aim to minimize the number of infections to decrease the impact of the SARS-CoV-2 virus. To reduce transmission in an early stage is essential to avoid exponentially growing levels of COVID-19 infections. Monitoring and early localization of outbreaks of the virus help to know how the virus is spreading and at what pace. Monitoring the spread of the virus can be based on results of tests in symptomatic persons, such as PCR-tests results. The accuracy of this method for monitoring the virus is limited due to a changing willingness to test. People test when having symptoms or when they have been in contact with an infected person. Furthermore, the proportion of asymptomatic among infected persons may rise as a result of vaccination. The PCR-test based surveillance is believed to systematically underestimate the proportion of infected people, as diagnostic capacity is limited and it is only applied to part of the population (Polo et al., 2020).

Wastewater-based epidemiology (WBE) is a well-known method to track the spread of SARS-CoV-2 virus in wastewater (Bibby et al., 2021). WBE can be used as an early warning system of an increasing spread of the virus in cities (Medema, Heijnen, et al., 2020). WBE is a relatively new field which has been advancing over the past two decades (Daughton, 2020). To use WBE for tracking the virus it is essential to know how the SARS-CoV-2 virus is behaving from loo to lab. WBE relies on the assumption that the measured concentration in a wastewater sample of any substance excreted by humans is representative for the catchment (Polo et al., 2020).

Currently many countries use WBE for the monitoring and analysis of the SARS-CoV-2 virus, mainly on small scale (X. Li et al., 2021). The Dutch Rijksinstituut voor Volksgezondheid en Milieu (RIVM) monitors and analyzes the concentration of SARS-CoV-2 in wastewater in The Netherlands through WBE as part of the National Sewage Surveillance (NRS). The RIVM analyzes samples from all 315 wastewater treatment plants (WWTPs) in The Netherlands. The measured concentration RNA in the samples is normalized to 100,000 inhabitants using equation 1.1. The load of the virus per 100,000 inhabitants is recorded per geographic area and disclosed on the national government website. The NRS aims to monitor the load of the SARS-CoV-2 virus over time, allowing trend analysis.

$$Load\ (per\ 100,000\ inhabitants) = conc.\ (virus\ gene\ copies) * volume\ (24h) * \frac{100,000}{number\ of\ inhabitants} \quad (1.1)$$

The accuracy of monitoring trends by WBE is affected by various methodological challenges. For WBE the total uncertainty is largely unknown, as well as the impact of this uncertainty of each step on the prevalence estimation (X. Li et al., 2021). Therefore, research on the identification of the factors that affect the accuracy of WBE is necessary, as well as quantifying their impact. This gives understanding of what the viral load measured from the samples represents, how the load is affected from loo to lab and how the load changes over time. This ensures that WBE can be applied for giving a reliable trend, resulting in tracking the spread of the SARS-CoV-2 virus accurately. By increasing our understanding and insight into how the various steps impact the prevalence measurements, will also help to monitor the virus correctly and ensure the comparability of data between WWTPs over time. This will enable an early-warning surveillance and increases the possibility to respond more promptly with the right measures to limit the spread of the virus when prevalence rises. Therefore, to ensure the goal of the NRS, this research on the factors affecting the accuracy of monitoring SARS-CoV-2 is necessary.

Many aspects from loo to lab affect the viral load measured from wastewater samples, and therefore the focus of this thesis is defined. This research identifies the factors influencing the accuracy of WBE for the monitoring of the SARS-CoV-2 virus for the following steps: 1) virus shedding, 2) in-sewer transportation, 3) sampling and storage 4) analysis of the SARS-CoV-2 virus and 5) back-estimation (X. Li et al., 2021). In figure 1.1 an overview on these stages from loo to lab is presented. For determination of the effect on the representativeness by different factors this research focused on the factors in step 1, 2 and 3 of the steps described above, due to the scope of this thesis.

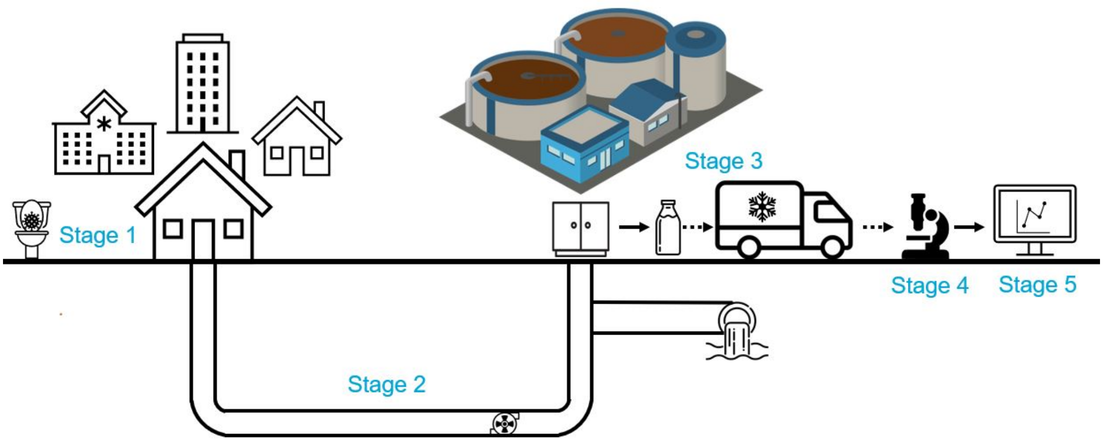

Figure 1.1: Schematic overview of the stages from loo to lab.

1.3. Research question

This research focused on the identification of factors that possibly influence the representativeness of monitoring the SARS-CoV-2 virus through WBE and quantification of important factors based on the results of a literature review. The focus of this research is the monitoring and analysis of the SARS-CoV-2 virus as part of the NRS with WBE. This study gives more insight in the types and causes of the factors affecting the representativeness. Consequently, a better understanding of the SARS-CoV-2 virus measurements from wastewater is provided.

The research question of this research is:

"What is the effect of factors influencing the representativeness of the wastewater-based epidemiology for monitoring the SARS-CoV-2 virus in wastewater as part of the National Sewage Surveillance?"

Sub-questions helped answering this research question. The sub-questions are given below. Sub-questions 1 and 2 are researched for the five different stages (virus shedding, in-sewer transportation, sampling and storage, analysis of the SARS-CoV-2 virus and back-estimation). From the results of sub-questions 1 and 2, a decision was made on which factors are researched for the effect on the representativeness of WBE. Sub-questions 3, 4 and 5 are researched for these important factors. These factors are in stage 1, 2 and 3 of the defined stages, presented in figure 1.1.

1. Which factors affect the representativeness of samples for the monitoring of the SARS-CoV-2 virus from wastewater?
2. To what extent will the factors influence the representativeness of samples?
3. How can the effect of factors possibly affecting the representativeness of samples be quantified?
4. What is the quantified effect of the different factors affecting the representativeness of samples for monitoring of SARS-CoV-2 virus?
5. What recommendations can be made on the basis of the quantified factors to improve the accuracy of monitoring the SARS-CoV-2 virus through WBE?

1.4. Reading guide

Firstly, a literature review has been conducted on the factors that possibly influence the representativeness of monitoring the SARS-CoV-2 virus. A dendrogram is used to give the relations between the different factors. The results are given per factor for the different stages in chapter 2. Based on the results from the literature review, seven important factors with respect to the representativeness are determined. For these seven factors quantitative knowledge is elaborated.

The quantitative results are synthesized to create an overview on the effect of the various factors and to ensure comparison of the factors. The criteria for this synthesis are explained in chapter 3.

Then the results for the quantified factors were discussed in chapters 4 to 10. The results on these factors are given in a logical order with respect to the path of the virus from loo to lab. Each chapter gives an introduction, the methodology used, results, points of discussion and a conclusion and input for synthesis. The results of the different factors are synthesized in chapter 11.

In chapter 12 the general limitations, as well as limitations on the literature review and synthesis are discussed. Chapter 13 draws the overall conclusions of this thesis. Furthermore, chapter 14 gives recommendations based on the findings and points of discussion of this thesis. In addition, the list of acronyms elaborated before this chapter on the introduction contains a list of abbreviations used in this research.

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2
Literature review

This chapter describes the factors that possibly affect the representativeness of monitoring the SARS-CoV-2 virus from wastewater through WBE. The factors are explained for five different stages: 1) virus shedding, 2) in-sewer transportation, 3) sampling and storage 4) analysis of the SARS-CoV-2 virus and 5) back-estimation (X. Li et al., 2021). The hierarchical relation between the different factors is presented in a dendrogram. This dendrogram is presented in figure 2.1 on the next page. The structure from the dendrogram is used to describe the different factors in the following sub-sections. For each stage the effect of the possible factors are described, as well as to what extent these factor will influence the representativeness of the monitoring of the SARS-CoV-2 virus.

Based on the results of the literature review, it was determined which topics will be further investigated in this study. The various factors are assessed for degree of influence on the representativeness of the samples for monitoring SARS-CoV-2 virus, focused on the purpose of the NRS. Several factors are relevant and potentially influence representativeness, but are beyond the scope of this study. These factors will have less quantitative influence on representativeness based on the literature read. Also, some factors are outside the study background, and therefore beyond the scope of this thesis. In the conclusion of this literature review, the various factors were classified using the following classification:

- Important factors interesting to research
- Important factors but beyond the scope of this research
- Negligible factors

For the negligible factors, it applies that they are not relevant, as the application of WBE in The Netherlands already properly implemented these factors, or the influence on representativeness is negligible. For other countries or a different purpose of WBE, these factors could play a role. For other applications, the classification given in the conclusion of this chapter should be revised.

The conclusion of this chapter gives an overview of the relevant factors in the dendrogram with different colors. In addition, an overview of the factors that appear in the literature to have most influence on the representativeness of virus monitoring is provided. These factors were examined quantitatively later in this study.

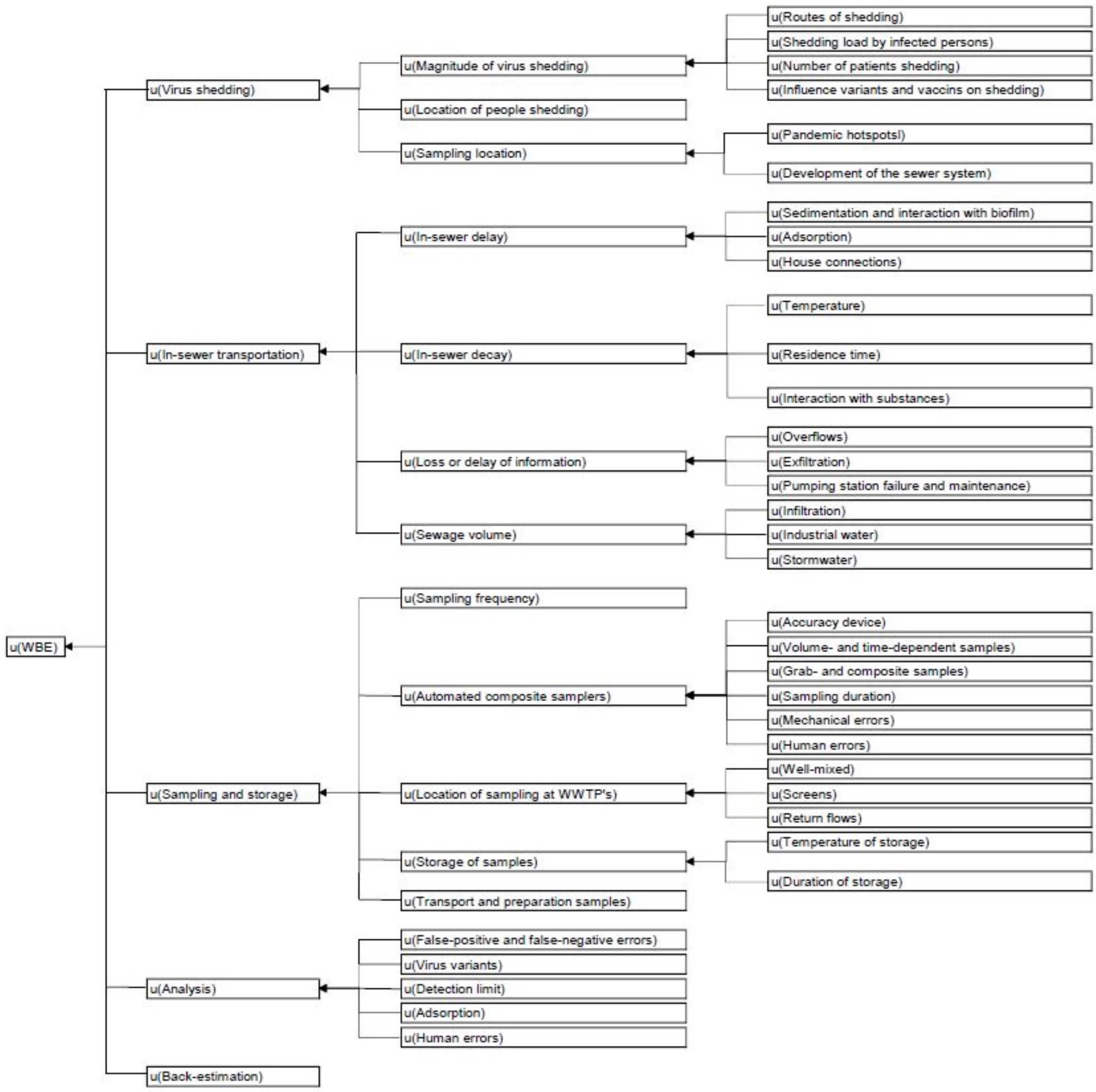

Figure 2.1: Dendrogram showing the hierarchical relation between different factors affecting the representativeness for monitoring the SARS-CoV-2 virus through WBE.

2.1. Virus shedding

At the toilet the SARS-CoV-2 virus is shed to the sewer system. Information regarding the virus shedding is necessary to understand the measured and monitored load of the SARS-CoV-2 virus. Lack of data and varying numbers related to the virus shedding affect the understanding of the monitored load of the virus. This section explains the impact of different factors that influence the representativeness of monitoring the virus in terms of virus shedding. Firstly, the magnitude of virus shedding is discussed. Secondly, the location where people shed the virus, and lastly, the influence of the sampling location.

2.1.1. Magnitude of virus shedding

For monitoring the virus through WBE, understanding of the virus load shed by infected persons is important. Four factors affecting the amount of virus shedding will be discussed in the following paragraphs. These factors are the routes of shedding, the influence of the stage of infection on the amount shed, the number of infected persons that shed the virus, and lastly, the influence of virus variants and vaccination against COVID-19.

Routes of shedding SARS-CoV-2
The SARS-CoV-2 virus and genetic material (RNA) is shed via respiratory fluids, saliva, urine and stool by part of the people infected with COVID-19 (Crank et al., 2022). The contribution to the load of the virus in the sewage varies for the different sources. To understand the source of the virus in the wastewater, knowledge about the routes of shedding is necessary.

The research of X. Li et al. (2021) concluded that the mean probability of shedding SARS-CoV-2 in stool is 20 times higher compared to urine. Also, the amount of copies in a urine sample was 10 - 10^4 copies/ml, compared to 10 - 10^8 copies/ml for stool. Therefore, the study by X. Li et al. (2021) suggests that the contribution of urine to SARS-CoV-2 excretion is negligible.

According to the results of the research of Crank et al. (2022), the contribution of different shedding routes on the total amount of SARS-CoV-2 in wastewater is presented in table 2.1. Although the load of the SARS-CoV-2 virus is comparable for samples from stool and respiratory fluids, table 2.1 shows that the contribution of stool to the total load of SARS-CoV-2 in wastewater is much higher compared to saliva, sputum and urine. Therefore the results from this research support the assumption that stool dominates the load of SARS-CoV-2 RNA (Crank et al., 2022).

Table 2.1: The contribution of different shedding routes on the amount of SARS-CoV-2 in the wastewater (Crank et al., 2022). The gene copies (GC) are the daily viral load produced by a population of 1000 SARS-CoV-2 infected individuals.

Shedding Route	Contribution (log_{10} SARS-CoV-2 GC*)	25th-75th interval
Saliva	8.05	6.86-11.52
Sputum	7.92	6.58-9.03
Urine	8.15	7.07-9.18
Stool	10.55	9.27-11.79

The assumption that stool dominates the load of SARS-CoV-2 RNA to the wastewater applies to community-level surveillance (Crank et al., 2022). However, to identify a single infected individual, any shedding route could contribute to a detectable signal (Crank et al., 2022). It is uncertain what the number of inhabitants in an area should be, or how many infected persons should be in that area to assume that the dominating shedding route is via stool.

Shedding load of SARS-CoV-2 by infected persons
Another factor that can influence the load SARS-COV-2 in the samples analyzed by the RIVM is the load virus gene copies shed by infected people. The load shed varies per person and the day of infection. Also, not all patients excrete the virus.

The research of X. Li et al. (2021) detected a mean shedding magnitude of $10^{4.52}$ copies/gram stool from pooled stool samples with a 95% confidence interval from $10^{4.26}$ to $10^{4.78}$ copies/gram. The research of X. Li et al. (2021) also states that the research of Bivins et al. (2020) found that RNA was decreased with 90% in 3-33 days.

There is a great uncertainty in the virus load shed by patients. Data availability is limited for the load and the variation over time. This variance has a longer influence on the estimation of the number of infected persons in an area, compared to monitoring the trend of virus gene copies in the wastewater.

Number of patients shedding SARS-CoV-2
The load SARS-CoV-2 in the sewer system is also influenced by the number of infected people shedding the virus. Like persons infected with COVID-19 being asymptomatic, meaning the persons are not having any symptoms, not all infected people shed the virus. Only a proportion of infected persons are shedding the SARS-CoV-2 virus via their stool.

The study of Roshandel et al. (2020) found that about 39.5% of the COVID-19 infected persons excrete the virus in their stool based on 30 earlier studies. The research of Xiao et al. (2020) found among 73 infected hospitalized patients 53.4% shedding the virus.

Although shedding of SARS-CoV-2 RNA varies between infected persons, the study of X. Li et al. (2021) states that the excretion uncertainty is limited for catchments with ten or more infected persons. Therefore, the influence on the accuracy of monitoring the trend of virus gene copies in wastewater by this factor is considered not to be significant for NRS purposes.

Influence of variants and vaccination on shedding SARS-CoV-2

As discussed earlier, it is unknown what load of virus RNA is excreted by infected people to the wastewater. The load shed is potentially influenced by different variants of the virus or vaccinations against COVID-19.

The SARS-CoV-2 virus mutates, resulting in different variants. The spike proteins differ for the various variants. With qRT-PCR the load of gene copies in wastewater are detected as well as the variants of the virus. The research of Crits-Christoph et al. (2021) states that in the municipal utility districts in the San Francisco Bay Area, the SARS-CoV-2 genotypes detected by the analysis from the wastewater were identical to clinical genomes. Therefore the study of Agrawal et al. (2021) suggests that the sequencing of SARS-CoV-2 RNA in wastewater can help to determine the virus variants. Nevertheless, there is no data availability about the variance of load of the virus in the wastewater between different virus variants. Consequently, the influence of the virus variants on the virus load in wastewater is uncertain.

Since January 2021 inhabitants of The Netherlands are vaccinated against COVID-19 infections. There are different types of vaccines produced by distinctive pharmacists, with varying protection. The vaccines protect people against an infection by the virus and causes less extreme symptoms. The excreted load of virus could also be influenced by whether or not infected persons are vaccinated. There is no data available on the influence of vaccines on the load virus excreted to wastewater, consequently the impact by this factor is unknown.

2.1.2. Location of people shedding SARS-CoV-2

The load SARS-CoV-2 measured from wastewater in The Netherlands is normalized to 100,000 inhabitants. In this normalization (equation 1.1) the 100,000 inhabitants are divided by the number of inhabitants in the area recorded by the Centraal Bureau Statistiek (CBS). This means that in the normalization it is assumed that people shed faeces to the sewer system in the area where they are living.

During the first lockdown most people stayed at home, which made this assumption applicable. Since there is no lockdown anymore, or less strict lockdowns, people travel around by reason of work, visits and holidays. This makes it difficult to know exactly where people go to the toilet. Consequently, the location where people possibly shed the virus to the sewer system is hard to determine.

Data availability on where people excrete their faeces to sewer system is limited, which makes it difficult to include this factor in the normalization. The number of shedders to a WWTP could be determined by using parameters analyzed from wastewater. Combining flow-based normalization with CrAssphage-based normalization could possibly increase the accuracy of monitoring the virus regarding this factor (Langeveld et al., 2021). Using total nitrogen and total phosphorous for determining the population size is an other possible method (Choi et al., 2018). Furthermore, cell phone usage and GPS data could be valuable to determine the population size.

2.1.3. Sampling location

The sampling location could influence the load of the virus measured (X. Li et al., 2021). This means that the sanitation concept of the sewer system could affect the virus load measured. In this sub-section two types of factors influencing load due to the sampling location are discussed. These are pandemic hotspots and the development of sewer systems.

Pandemic hotspots

Pandemic hotspots could influence the magnitude of load measured from wastewater. Nursing homes, hospitals and isolation facilities are examples of pandemic hotspots (X. Li et al., 2021).

Generally, there are many infected individuals at these hotspots and this could cause the measured load to increase for an area in which such a hotspot is located. It is necessary to consider the influence of this factor when the monitored data between different catchments is compared and decisions are made based on the results. The quantitative influence of pandemic hotspots on the viral load in wastewater is unknown.

Development of the sewer system
The possibilities of monitoring of the virus through WBE is influenced by the sanitation concept. The type of sewer systems, the structure and the ageing differ per country, as well as the regulations. Not in all countries in the world the wastewater produced is connected to a proper sewer system. For example, in India 80% of the wastewater is not connected to a proper sewage system (CPCB, 2020). This may cause WBE to be more difficult for monitoring the virus, since the information in wastewater is not representative for the entire population. Furthermore, the sanitation concept may influence the methodology of WBE. In The Netherlands, almost every household is connected to the sewage system. Since this research focuses on The Netherlands, influence of the development of the sewer system is considered to be negligible for this study.

2.2. In-sewer transportation

In sewer systems many processes take place. Processes like decay, adsorption and discharges can affect the load of SARS-CoV-2 virus measured in the samples collected at the WWTPs. For monitoring the virus it is useful to understand which processes affect the load of the virus in wastewater, and to what extent. The factors related to these processes discussed in this sub-section are processes that slow down the transportation of the virus to the WWTP, the decay of the virus, the loss of information by water discharged from the system or the failure of pumping stations, and lastly the sewage volume.

2.2.1. Delay of SARS-CoV-2 to treatment plant due to the sewer system

The transportation of the SARS-CoV-2 virus through the sewer system is possibly delayed by processes which take place in the sewer system. Sedimentation of substances takes place by gravity and substances in the sewer system interact with the biofilm. The degree of adsorption affects these processes, as adsorption affects the weight of the particles to which the virus is attached and how well these particles can potentially adhere to the biofilm. The cross-section of a sewer pipe is shown in figure 2.2 to highlight the different environments where the transport of the virus possibly is extended (Jensen et al., 2016). Also, the transportation of gross solids could possibly be delayed in the small diameter sewer pipes between a house and the main sewer system. In these pipes there is often intermittent flow of wastewater, resulting in storage of gross solids, like faeces, in the pipes until a flush of wastewater takes place.

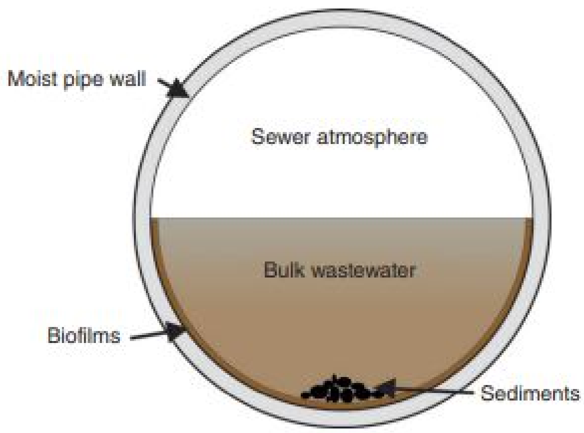

Figure 2.2: The cross-section of a sewer pipe highlighting the environments for sedimentation and biofilms. (Jensen et al., 2016).

The extended travelling time of substances to the WWTP, here the SARS-CoV-2 virus, make that these substances are not present in samples from the influent of the WWTP as expected. It can result in a lower load of virus in the sample if the virus copies are arriving after the sampling interval. Stormwater can cause the release of substances from the sediment or biofilm, due to resedimentation of substances by increasing flow rates in the sewer pipes. In this section the adsorption of the virus is discussed, as well as the possible effect of sedimentation and interaction with biofilm to the load of the SARS-CoV-2 in the samples measured. In addition, the effect on delay of information on the viral load by small diameter sewer pipes between houses and the main sewer pipes is explained. Due to a possible delay in information, it is necessary to understand the in-sewer delay of the virus for accurately monitoring of the virus.

Adsorption
Faeces mainly contributes to the load SARS-CoV-2 measured from wastewater. The virus is adsorbed to other substances present in faeces. Adsorbed virus particles will not cause the virus to inactivate or degrade. Adsorption is related to the partitioning of the virus in the wastewater. Adsorption is influenced by transport and physicochemical parameters like the flow intensity of wastewater, the concentration of solids, the pH, the ionic strength, and the organic load (Kostoglou et al., 2022).

When the SARS-CoV-2 virus is adsorbed to substances in the water, the detection of the virus is hindered, because this makes the virus less accessible for detection. The research of Petala et al. (2021) argues that the quantitative measurement of viral copies in wastewater can be mislead by adsorption. Knowledge about the effect of adsorption on the viral load in the wastewater and the detection is desired to know the effect of this phenomenon on the representativeness of the samples. It must be noted that samples in this research are filtered, which is different from the method used to analyze samples for the NRS.

To analyze the effect of adsorption for SARS-CoV-2, properties are investigated. A separator separates different sizes of suspended solids. This gives information on what number of the SARS-CoV-2 virus is attached to suspended solids, and if this is influenced by the size of the suspended solids. This is performed by the Canadian researcher Peter Vanrolleghem. He ran four samples through the separator at different sedimentation rates (80-100 m/h, 60-80 m/h, 40-60 m/h etc.). He found that at all fractions the same mass of SARS-CoV-2 RNA was adsorbed (in # SARS-CoV-2 RNA/gram suspended solids).

In the study of Westhaus et al. (2021) the adsorption of the virus in aqueous and solid phase were compared. This research found that the number of virus copies in the solid phase is 25 copies/mL, compared to 1.8 copies/mL for the aqueous phase of the inflow sample (Westhaus et al., 2021).

In The Netherlands samples are not filtered, meaning that the virus in analyzed wastewater is present in solid- and aqueous phase. To determine the virus load in a sample, 1mL of the sampled wastewater is pipetted and analyzed. Although it could matter whether or not there are large particles in the analyzed wastewater, it was found by experts that this does not significantly affect the measured load. Furthermore, samples are analyzed in duplicates. This also makes that the effect on the load by suspended solids present in the analyzed wastewater is negligible. However, the exact effect of adsorption on the representativeness is complex and therefore unknown.

Sedimentation and interaction with biofilm
The main mechanism behind sedimentation of substances in wastewater is gravity. Therefore, the degree of adsorption affects the in-sewer sedimentation of the virus. When the flow through the sewer system is not high enough to carry a substance in the current, the substance is settling down. When the flow velocity in the system is increasing, due to, for example, an increasing amount of water by rainfall, this possibly leads to resedimentation of substances. Biofilms are mainly microorganisms attaching to surfaces, caused by adhesion and growth of the microorganisms (Flemming, 1993). Viruses may also interact with the biofilm. Similar to the reaction of sediment to greater flows through the system, the substances attached to the biofilm can release when flow increases. During wet weather flow (WWF) the inflow of total suspended solids (TSS), Phosphorus, Total Kjeld Nitrogen (TKN), BOD and COD is at a higher load compared to dry weather flow (DWF) (Langeveld et al.,

2021). Resedimentation of these substances is taking place from the sediment and biofilm when there is an increased flow in the sewer system.

Since crAssphage is abundantly present in human faecal samples, it is seen as a microbial source tracking (MST) marker for human faecal pollution in water (Ballesté et al., 2019). The research of Langeveld et al. (2021) suggests that the load of crAssphage is not increasing in the influent of the WWTP during WWF, compared to DWF. This implies no correlation between an increase in flow and an increase in the load of crAssphage in the influent. Therefore, it can be assumed that the influence of sedimentation and interaction with the biofilm is limited for crAssphage looking at the flow, although crAssphage is found in high concentrations in the sediment of sewer pipes (Ballesté et al., 2019). If the SARS-CoV-2 virus sediment and attaches to the biofilm comparable to crAssphage, the assumption can be made that the influence of sedimentation and biofilm is limited for monitoring the SARS-CoV-2 virus.

If the load of SARS-CoV-2 virus is also affected by larger flows through the sewer systems, it is necessary to determine and quantify the effect of temporary in-sewer storage of the virus. As sedimentation and interaction with biofilm are complex processes, it is hard to exactly determine what is happening to the virus in the sewer system by these processes.

House connections
In the small diameter pipes between houses and the main sewer pipes, the transport of gross solids, like faeces, are possibly delayed. The flow regime in small diameter pipes is often intermittent (Littlewood and Butler, 2003). Solid movement is dependent on WC flush, bath and washing machine volumes, with WC's typically producing the most intense flush wave (Littlewood and Butler, 2003). Since faeces is not always immediately discharged to the main sewer system, the information on the virus (present in faeces) might be delayed by small diameter pipes. The effect of this factor on the representativeness of monitoring the virus is unknown. If decay of the virus in these pipes is significant, this factor can affect the representativeness of the monitored data. Knowing the average residence time in these pipes would be valuable.

2.2.2. Decay of SARS-CoV-2 in the sewer system

In the sewer system there is first order decay of the SARS-CoV-2 virus (Ahmed et al., 2020). For monitoring the SARS-CoV-2 virus it is important to know which factors influence the decay and to what extent. This is necessary for monitoring the virus, since the decay influences the load of the virus in the wastewater. Knowledge on the decay is also needed for the back-estimation of the measured load to the initial load shed at the loo. Many factors are possibly affecting the decay, for example the temperature, the residence time in the sewer system and the interaction with other substances in the water. The influence of these factors on the decay of the virus in the wastewater, as known from the literature, are elaborated in this section.

Temperature
The viral inactivation is increased by higher temperatures (Carducci et al., 2020). The decay rate for the RNA of the virus estimated by the research of Ahmed et al. (2020) is given in table 2.2 for four different temperatures in untreated wastewater. T_{90} is defined by the time needed to decrease the initial load of the virus by 90 %. The values for T_{90} confirm the presumption that the SARS-CoV-2 RNA is persisting long enough in untreated wastewater for reliable detection of the virus for WBE application (Ahmed et al., 2020).

Table 2.2: The decay rate and T90 for SARS-CoV-2 RNA in untreated wastewater for different temperatures of the water (Ahmed et al., 2020).

Temperature (°C)	**Decay rate (/day)**	r^2	T_{90} (days)
4	0.084 ± 0.013	0.79	27.8 ± 4.45
15	0.114 ± 0.012	0.71	20.4 ± 2.13
25	0.183 ± 0.008	0.87	12.6 ± 0.59
37	0.286 ± 0.008	0.74	8.04 ± 0.23

The temperature of sewage water varies over time, due to the type of system and the season. In The Netherlands the temperature of the wastewater varies between 8°C and 22°C. Table 2.2 shows that the decay of the SARS-CoV-2 RNA is affected by the temperature of the sewage. Consequently, for monitoring the virus from the sewage samples, it is necessary to understand the exact effect of the temperature on the decay. The fluctuating temperature of wastewater and the decay rate for different temperatures, makes it hard to exactly quantify the decay in the sewer system related to the temperature. Since table 2.2 shows a wide range in the days for T_{90} for the virus, it is expected that the temperature has a great influence on the virus load arriving at the WWTP. Therefore, the effect on representativeness by this factor will be examined quantitatively.

Residence time

The distance from a catchment to a WWTP varies for each sewer system. The flow velocity of the wastewater depends on the type of system (pressurized, or under gravity) and the type of sewer pipe (connection, or main sewer pipe). These factors make that the residence time of wastewater varies between catchments. Since the decay of viruses is time dependent, longer residence times of wastewater in the system can affect the decay of the SARS-CoV-2 virus. This influences the viral load measured in the samples arriving at the lab of the RIVM.

The residence time of the wastewater depends on the delay in the pipes between the house connections and the main sewer system (section 2.2.1), the travelling time through the main sewer system, the structure of the system (branched or looped structure) and the residence time in the (pressurised) pipe to the WWTP. The velocity of the wastewater through the system depends on the shape of the cross-section of the sewer pipes, the flow, the diameter, the pipes being pressurised or under gravity and the capacity of the pumping stations.

The distance from a catchment to the WWTP depends on the area. In The Netherlands the distance can vary from several kilometers for smaller catchments (e.g. on Schiermonnikoog the maximum length from a house to a WWTP is $\pm$ 5 kilometers) up to tens of kilometres for larger catchments. For example WWTP Harnaschpolder in Den Hoorn treats the wastewater of around a million inhabitants and 40.000 industries in the area of The Hague. Due to these large variations in distances and thus residence time, the decay of the virus can vary greatly from one sewer system to another. This means that the residence time can influence the load of the virus measured.

Interaction with substances in the wastewater

Besides adsorption, discussed in section 2.2.1, reactions with other substances present in the wastewater is also possible. The research of X. Li et al. (2021) states that the water matrix plays an important role in the inactivation and breakdown of viruses (Wetz et al., 2004). The concentration of substances that cause inactivation or decay could possibly increase by infiltrating water containing these substances to the sewer system.

Dissolved Oxygen (DO) affects the adsorption of the virus (Petala et al., 2021). However, no literature is found on the effect of DO on the inactivation or decay of the virus. Also, for other substances in the water this is not found. Therefore, it is hard to estimate the impact on the virus load in the samples. The study of Ahmed et al. (2020) concluded first-order decay of the SARS-CoV-2 RNA is mostly influenced by the temperature. This could mean that the influence on representative monitoring the virus is possibly limited, but the exact effect is not known.

2.2.3. Loss or delay of information

The virus in wastewater is possibly discharged from the sewer system by overflows and exfiltration. Besides, the virus can be temporary stored in the sewer system due to longer failures of pumping stations. Since these mechanisms potentially influence the load of the SARS-CoV-2 virus in the sewage, the representativeness of monitoring the virus can be affected. Therefore, this section explains the effect of overflows, exfiltration and failure of pumping stations.

Overflows
There are different types of sewer systems and overflows. In The Netherlands the most common overflows are storm sewer outfalls (SSO) and combined sewer overflows (CSO). The type of sewer varies per neighborhood. The wastewater arriving at WWTPs often is a combination of wastewater from different types of sewer systems.

SSO's generally discharge stormwater to surface water. There are many illicit connections in separated sewer sewers (Hoes et al., 2009). Illicit connections cause wastewater entering the pipes intended for stormwater. In The Netherlands 25% of the households are connected to separated sewer systems (Schilperoort et al., 2013). Approximately 2% of the connections are illicit connections. Therefore, a limited part of information on the SARS-CoV-2 virus is lost due to SSO's. To what extent the SSO cause information loss is difficult to estimate, since detection of illicit connections is hard and expensive.

In combined sewer systems the wastewater is mixed with stormwater. When large storm events occur, the capacity of the combined sewer system may be exceeded, resulting in discharge of sewage from the system through CSO's. This discharge of wastewater could also result in the loss of information on the virus, if the wastewater discharged is containing the virus. As the overflowing of the CSO's often happens only a few times a year, based on expert consultation approximately four times per year in The Netherlands nowadays, it is not expected that the representativeness of many samples is influenced by overflow event. Nevertheless, overflowing of the CSO's could affect the available information and therefore the representativeness of the samples analyzed if an overflow event takes place.

Since the great variation in structure per catchment the effect of the overflows can vary a lot per catchment. Effect on the representativeness of samples monitored by WBE also depends on the magnitude of influence per type of sewer overflow.

Exfiltration
In areas where the groundwater level is below the sewer pipes, exfiltration of wastewater from the sewer pipes could occur. Since the groundwater level is often lower in summer, in this season the appearance of exfiltration is expected to be greatest. Exfiltration of sewage from sewer systems can cause the loss of information, since the exfiltrating water can contain the virus. This water will not arrive at the WWTP and consequently will not end up in the sample. In The Netherlands it is expected that exfiltration will only take place in higher areas, like Limburg. In general, the other, low-lying areas of The Netherlands have relatively high groundwater levels. The proportion of exfiltration in The Netherlands is unknown and therefore the influence of this factor is not known. As a consequence of the generally high groundwater levels in The Netherlands, the influence of this factor is expected to be limited.

Pumping station failure and maintenance
The sewer system performance is directly affected by the pumping stations in the system (Korving and Ottenhoff, 2008). There are different causes for the failure of pumping stations. When a pumping station is failing for a longer time this can affect the representativeness of the sample, since the sample is possibly not representative for the quality of the wastewater in the sewer anymore. The virus can get blocked at the pumping station or discharged from the sewer system at an overflow due to overloading of the capacity of the sewer system. The reliability of pumping stations varies per type of pumping station, the network design, the number of pumping stations and on whether the pumping stations are connected in series or parallel. Therefore, the probability of failure can vary between catchments.

Also, maintenance of the sewer system can cause less representative samples, since not all the wastewater from the catchment flows to the WWTP and is therefore not sampled. This is especially a problem when the maintenance is not communicated correctly to the operators of the WWTP. The samplers then will not be informed. The samplers will then not have been informed of the anomalous area sampled.

2.2.4. Sewage volume

The wastewater produced by humans is diluted in sewer systems which influences the volume of sewage in sewer systems. The magnitude of dilution is depending on the type of sewer system (separated - or combined sewer system). The wastewater produced per person per day is $\pm$ 120 L in The Netherlands. The three factors that mainly influence the sewage volume are discussed in this section. The factors are infiltration or inflowing water, stormwater and industrial water. Flow is an important parameter in the normalization. Flow is measured at every WWTP and automated samplers are connected to these flow meters. Flow measurements have an inaccuracy due to the accuracy of flow meters. Also, sometimes the flow measured is over- or underestimated. By itself, dilution is not a problem. However, if the measured flow rate is not correct, this results in less representative samples and monitoring of the virus.

Infiltration
The research of Weiß et al. (2002) studied 34 earlier studies in Germany on combined sewer systems. This study found that the relation between sewage, stormwater and infiltration inflow on a WWTP from a combined sewer system is about 30%, 35% and 35% respectively. Infiltration flow therefore has a big contribution to the discharge of the influent of a WWTP. According to the research of Weiß et al. (2002) there is also a strong annual variability in the amount of infiltration. The research states that it is possible to have an infiltration inflow ten times larger in winter and spring compared to autumn and summer.

Industrial water
Industrial water is an other large contributor to the volume of sewage in sewer systems. Since this industrial water is expected to not contain the virus, this type of water is also considered as a dilution of wastewater. Industrial water is part of the DWF. Together with the infiltration water from obsolete pipes and higher groundwater levels, the infiltration water thus is approximately 35% of the inflowing water at the WWTP. The amount of water from industries depends on the catchment. The production of industrial water is fluctuating often over time. It is assumed that discharges are greatest during a working day and less during the evenings and weekends.

Stormwater
Separate sewer systems discharge stormwater directly into surface waters connected to the sewer systems. The sewage for a separate sewer system is connected to the WWTP. Since the sewage is not mixed with the stormwater, for systems without illicit connections, the dilution of the sewage by stormwater can be neglected.

In combined sewer systems the stormwater and sewage are mixed in the same system. Therefore, the sewage is diluted by the stormwater. The relation between sewage, stormwater and infiltration inflow given is 30%, 35% and 35% respectively (Weiß et al., 2002). This shows that stormwater contributes largely to the flow into the WWTP and consequently the sewage volume.

2.3. Sampling and storage

Sampling for the NRS is done at WWTPs and samples are stored afterwards. The following information on samples taken applies to samples taken for the NRS. At the WWTPs an automated composite samplers take small samples from wastewater at a frequency depending on volume. In 24 hours at least 100 samples of 50 mL should be taken from the influent, meaning after 24 hours at least 5 L from the wastewater is collected. For some locations this is adjusted to a minimum number of samples of 70 in 24 hours, due to a big difference in the DWF and WWF. After 24 hours a 50 mL sample is sampled from the collected wastewater. After transportation and preparation the samples are analyzed in the lab of the RIVM. Many factors can influence the accuracy of the sampling, which possibly influences the representativeness of monitoring the virus. The factors discussed in this section are the frequency of sampling, automated composite samplers and the location of sampling. The storage and transportation of the samples are also important for correct monitoring of the virus and therefore briefly discussed in this section.

2.3.1. Frequency of sampling

The NRS aims to monitor the SARS-CoV-2 virus to understand the spreading and its pace. A right balance has to be created between the number of samples needed, to give accurate information, and feasibility. There is just a certain capacity for analyzing samples. Too many samples are unnecessary, due to the incubation period and course of the disease. Moreover, many samples cost a lot of money. The RIVM currently aims to analyze four samples per week for each WWTP as part of the NRS. For this to succeed on average five samples a week are taken, since not all the samples will meet the requirements of a good sample. For some water labs, new sampling moments are planned in a week if a sample is rejected.

Some weeks not four samples are meeting the requirements and consequently, fewer samples are analyzed. The optimal frequency of sampling is not known. The mean incubation period for the virus is 4.3 days for Delta infections and 5.0 days for non-Delta infections (Grant et al., 2021). Assuming that patients shed the virus in faeces prior to having symptoms, four samples a week is assumed to be sufficient for accurately monitoring the virus. It is not known what the influence is on the quality of the analytical results by the spread of the samples over the week and the number of samples per week.

2.3.2. Automated composite samplers

Automated composite samplers take the samples that are later analyzed by the RIVM. For valuable analytical results a representative sample is required and this cannot be compensated by the number of samples, accurate chemical analysis, or statistics (Ort, Lawrence, Reungoat, et al., 2010). In this section it is discussed what the influence of several aspects is on whether or not the samples are representative. These aspects are the accuracy of automated composite samplers, the difference between volume- and time dependent samples, the type of sample, the duration of sampling, mechanical errors and human errors.

Accuracy automated composite samplers

The national regulation for sampling in The Netherlands (NEN6600) makes some demands on the samplers. The following requirements are stated in this standard (NEN6600). The suction pipe should be as short as possible, placed at an angle and must not contain any kinks or unnecessary bends. All parts from the suction point to the sample container should have an inner diameter of 12 mm. The minimum velocity in the suction pipe should be 0.3 m/s. Modern pumps can achieve minimum velocities in suction pipes of 0.5 m/s, which is advised to avoid settling of heavy sediment. The wastewater should be mixed well at the location where the sample is taken from. The suction point should not be in a stagnant zone. Also, air inclusion must be prevented. The sampling cannot be done in the bend or constriction of a sewer pipe. Filters must not be present at the suction point. Lastly, the sewer pipe should be completely filled with water.

Not all automated samplers are meeting the requirements. It is unknown to what extent non-compliance with the requirements affect whether a sample is representative. Since the start of monitoring the SARS-CoV-2 using WBE, the number of automated samplers meeting the demands is improved. The influence of this improvement on the representativeness of the monitored data of the virus is also not known.

Volume- and time-dependent samples

According to NEN6600 at least 100 aliquots within 24 hours should be taken from the wastewater for a representative sample. For some locations the minimum number of samples per 24 hours is set to 70, by reason of great fluctuations in volumes between DWF and WWF. These 70 or 100 samples can be sampled from the wastewater depending on time or volume. The sampling standard (NEN6600) indicates that volume-dependent samples are preferred in comparison to time-dependent samples. The study of X. Li et al. (2021) highlights that high-frequency flow-proportional sampling reduce the uncertainty in the prevalence estimation significantly. Also, the research of Ort, Lawrence, Reungoat, et al. (2010) states that time proportional sampling is never unbiased for the diurnal flow and the corresponding pattern in concentration, since time proportional sampling does not weight aliquots by flow.

Volume- or flow-dependent samples ensure that the samples are more representative. The samples taken for the NRS are volume-dependent. This makes that the influence of this factor on the accuracy of prevalence estimation is limited. For each automated composite sampler the volume after which a sample should be taken should be programmed, such that after 24 hours at least 70 or 100 aliquots of 50 mL are taken. This volume is estimated for each automated sampler by the average daily flow.

Grab- and composite samples
Two types of samples could possibly be taken from the wastewater for the detection of the SARS-CoV-2 virus, namely grab- and composite samples. Grab samples are taken directly from the wastewater on a certain moment. Composite samples are samples from collected water depending on time or flow for a certain time interval. 24-hour composite samples include the daily pattern in the production of waste-water and toilet use (Ort, Lawrence, Reungoat, et al., 2010). Composite samples are also required according to NEN6600 for representative samples. Therefore, WBE for the SARS-CoV-2 virus in The Netherlands is depending on composite samples collected at WWTP (Medema, Heijnen, et al., 2020). This makes that the influence on the representativeness of the samples by this factor is negligible.

Sampling duration
According to the research of Ahmed et al. (2021) 24-hour composite samples are more accurate compared to 1-hour composite samples. The NEN6600 standard requires samples for the analysis of the SARS-CoV-2 RNA to be 24-hour composite samples. The diurnal flow pattern is included in 24-hour composite samples that are flow-dependent. Therefore, this factor is assumed to be negligible for having an effect on the accuracy of monitoring the virus.

Mechanical errors
Several mechanical errors can affect the operation of the automated composite samplers. Some errors will result in samples that will be rejected, since the samples do not fulfill the requirements stated by NEN6600. When samples are rejected, they will not be analyzed by the RIVM. It is required by the standard NEN6600 that the temperature of the samples is in between 1 °C and 6 °C. The difference between the theoretical and the actual collected volume in the collector vessel must not be more than 7.5%. Lastly, the deviation between the aliquot volume in practice and the set aliquot volume is 5% at maximum. Some mechanical errors will not result in the rejection of a sample, since theoretically the samples meet the requirements. These very samples are analyzed by the RIVM and could affect the representativeness of the monitored data.

There are many types of mechanical errors that could occur. For example, the suction pipe can get blocked by dirt or objects in the wastewater. This dirt can form a floating layer, that relocates the suction point. This can cause failure in suction or incorrect samples. The suction pipe can also freeze, although the system should be protected against freezing. Technical problems or power failure could occur in the system. When these types of errors happen, the system has to be reset. This can give a variation in settings, which gives inaccurate results if not considered. Sometimes there is an inaccuracy in the flow measurements, resulting in under- or overestimated flows. Lastly, when the suction pipe is too long, the pressure may be insufficient. This will result in an inaccurate volume of the aliquots. Possibly, other mechanical errors also affect the operation of the automated composite samplers.

However, the exact effect by mechanical errors on the representativeness of the samples for monitoring the virus is unknown. To what extent these types of errors occur can be researched for many of the stated mechanical errors. When errors in the system occur and get noticed, they are registered by the samplers. Some errors are unnoticed and may affect representativeness without being noted. The mechanical errors should be considered and if possible adjustments should be made to reduce the frequency of errors.

Human errors
In addition to mechanical errors, there are also mistakes made by humans. Like mechanical errors, due to the requirements stated by NEN6600, some samples are rejected due to human errors. Also,

not all human errors cause rejection of the samples and therefore potentially affect the accuracy of the analytical results.

An important human error is making a mistake in the procedure for taking samples. Although most of the samplers are well educated for taking samples, a person cannot work without making errors. For some WWTPs the sampling is not done by an external party and the workers from the WWTP doing the sampling are not educated. Possible mistakes are imprecise reading of volumes or temperatures. The temperature is possibly incorrect sometimes, since the samplers do not read the temperature immediately when opening the sample cabinet. Also, there is a great variation in the volume of aliquots.

The volume of aliquots are determined by averaging the volumes of three aliquots. Reading errors in volume result in less accurate aliquot volumes measured. Previously, the sample volumes were noted with one significant number. Rounding of numbers can also cause this variation. Nowadays, the sample volumes are noted with two significant numbers. Also, sometimes the samplers miss part of the collection tanks, or only empty part of them.

Another important factor for correct sampling is communication. Many stakeholders are involved, which makes good communication difficult. This can result in incorrect sampling or sampling at the wrong location or time. Lastly, sometimes the structure of the sewer system is changed, by reconnecting the system. Additionally, sewer systems get cleaned once in a while. Often this information is not communicated and the information in the sample changes without being noticed.

As well as the mechanical errors, these human mistakes can affect the analytical results when the information is not considered in assessing the sample results for monitoring purposes. To what extent these factors influence the representativeness of the monitored data is not known, but it is expected that this factor indeed influences the results.

2.3.3. Location of sampling at WWTPs

Samples for monitoring the trend of the SARS-CoV-2 virus for the NRS are taken from the influent of WWTPs. The location of sampling could possibly affect the quality of the samples. Three factors according to the location of sampling which may affect the monitoring of the virus are discussed in this section. These factors are the influence of sampling in zones that are not well-mixed, the impact by screens and the influence of return flows in WWTP.

Well-mixed

According to NEN6600 samples should be taken from well-mixed water. If samples are taken from stagnant zones the samples will not be representative for the virus load in the wastewater. In stagnant zones the water is not mixed well with the incoming water containing the excreted SARS-CoV-2 virus. When samples are not taken from well-mixed wastewater and this remains unknown during the processing of data, this can give inaccurate information. It is not known to what extent this factor influences the representativeness of the monitored data of the virus. Since samples from wastewater that are not well-mixed do not meet the requirements for a representative sample, it must be questioned whether these samples can be included in the monitoring. The effect of this factor on the monitoring of the virus also depends on the number of WWTPs that do not take samples from the correct place. There has been an improvement in sampling at WWTP since the start of the NRS.

Screens

The first step in each WWTP is a physical barrier, a screen, to retain the greater, unwanted solids and objects in the wastewater. Most of the sampling locations for the NRS are downstream from screens. Since the wastewater is pre-treated before sampling at most of the sampling locations, it could be possible that the samples are less representative for the virus.

The diameter of the SARS-CoV-2 virus is approximately 100 nm (Bar-On et al., 2020). Due to the small diameter, the SARS-CoV-2 virus will not be blocked by the screens. The virus can adsorb to other solids in the wastewater, which are retained by the screens. The gravitational sedimentation of large suspended solids viruses attach to is considered as the main and first step in the removal of viruses (Teymoorian et al., 2021; Vickers, 2017). But, the physical barrier relying on gravitational sedimentation is not sufficient for removal of viruses entirely (Teymoorian et al., 2021).

It is generally determined by process technologists at WWTPs that when samples are taken from wastewater for water quality parameters at WWTPs downstream from a screen, that 5% load should be added to the measured quality parameter, due to the blocked suspended solids. According to an expert, for some locations with greater mesh sizes, this should be ±1% (for example 11 mm at Kralingse Veer). The amount of suspended solids retained by screens depends on the mesh size. This mesh size is less for newer screens, i.e. 3 - 6 mm, resulting in more blocked suspended solids. The amount of suspended solids retained by the screens also depends on the season, for example because of more leaves in the sewer system. Therefore, an addition of 1 - 5 % to the quality parameter should be correct if the quality parameter is measured downstream of screens.

In short, if the SARS-CoV-2 samples are taken downstream from screens, the influence on the load will be limited. Therefore, the effect of this factor on the representativeness of samples will be little. Nevertheless, there will be a small effect on the load.

Return flows in WWTP
Each WWTP has its own structure to treat the wastewater. Different types of treatment can be applied to obtain an effluent quality that is meeting the requirements. In part of the treatment plants there are return flows for efficiency of the system or to ensure good quality of the effluent. In some WWTPs the sampling is done from wastewater that is mixed with water from the return flows. If these return flows contain SARS-CoV-2 virus copies and the sampling is done from wastewater mixed with the return flows, the virus load in the samples is possibly affected by the return flows.

Return flows are often returned from the final clarifier into the system. The number of treatment steps prior to the final clarifier are varying per WWTP. The study of Abu Ali et al. (2021) concludes that 1 log removal of SARS-CoV-2 on average was attained by each primary and secondary treatment step. The research of Kumar et al. (2021) found that during Upflow Anaerobic Sludge Blanket (UASB) treatment a viral genetic loading reduction of the SARS-CoV-2 virus was more than 1.3 log removal of the virus. Tertiary treatment or chlorination could result in complete removal of the virus (Abu Ali et al., 2021). Assuming WWTPs have multiple treatment steps, the virus load in the return flows is negligible compared to the virus load in the influent. However, it is good to be aware of the return flows, as these flows may contain virus particles and thus influence the load.

2.3.4. Storage

After sampling, the samples are stored until lab-analysis. The NEN6600 standard also includes requirements for the storage of samples. If the storage is not done precisely, the quality of the samples can be affected. Two factors related to the storage of samples that may influence their quality are the temperature and duration of storage. These two factors are discussed in this sub-section.

Temperature of storage
The NEN6600 requires samples to be stored between 1°C and 5°C. For the NRS currently storage temperatures of samples up to 6°C are accepted. Based on recent studies, the research of X. Li et al. (2021) states that the SARS-CoV-2 virus was relatively stable for at least 14 days when the samples were stored at 4°C. The study of Baldovin et al. (2021) found no difference in the persistence of the virus after 24 hours of storage. However, the research of Hokajärvi et al. (2021) found a linear decay for the SARS-CoV-2 RNA over 28 days when storing the samples at 4°C. Freezing could ensure that there is no degradation of the virus. The study of Medema, Been, et al. (2020) indicates that the SARS-CoV-2 RNA is stable in samples at 5°C up to 15 days.

Due to small decay rates for the virus for lower temperatures, it is expected that storage temperatures will not affect the virus load significantly for immediate analysis of the samples. Therefore, the effect on the viral load in the samples is also expected to be negligible for analysis within days. For longer storage times, it is advised to include the decay rate in the analysis. An other possibility is to freeze the samples before storage. However, limited data is available on the effect of freezing on the quality of samples from wastewater (Medema, Been, et al., 2020). Pasteurization prior to the storage of the samples might be a solution, but due to conflicting information on its effect on the detection of the SARS-CoV-2 RNA should be avoided according to the research of (Ahmed et al., 2022).

Duration of storage
As discussed, the temperature of storage can affect the virus load in samples, due to decay. The duration of storage can have impact on the magnitude of decay. In general, samples should be analyzed within 48 and 72 hours when stored at 4°C, to minimize RNA degradation (Tan et al., 2017). The samples are not always analyzed within this period, because the samples have to be transported, the analysis is sometimes carried out over the weekend and the samples are sometimes analyzed later for other purposes.

The uncertainty in the decay of the SARS-CoV-2 RNA during storage at low temperatures also gives uncertainty to the effect of the duration of storage. It would be best to analyze the samples as soon as possible, or to determine the degradation and include this in the back-calculation. This limits the influence of this factor on the representativeness of the virus monitoring.

2.3.5. Transport and preparation

Next to sampling and storage, the transportation and preparation of the samples for the analysis is important. During transportation temperatures of 7°C are accepted for the storage of the samples. This is 1°C more compared to the requirement for storage. Although decay is expected to be limited at lower temperatures, this higher temperature could effect the quality of the sample. It is aimed to transport the samples as fast as possible. Also, part of the samples do not arrive at the lab, not because they have been rejected, but for unknown reasons. However, that is not the intention and in fact a pity, since valuable information might have been lost. An important factor that affects the preparation of the samples is labelling. More frequently than desired, labelling of the samples is done wrongly, resulting in incorrect data. The preparation should be done precisely, to avoid contamination, warming of samples and processing the incorrect data.

2.4. Analysis of SARS-CoV-2 RNA load

The analysis is the last step in determination of SARS-CoV-2 load in the samples. The lab staff of the RIVM carries out this analysis in the lab of the RIVM. In this stage of monitoring the virus, some factors may also influence the accuracy of the results. In this section the effect of false-positive and false-negative errors, virus variants, detection limit, adsorption and human errors are discussed.

2.4.1. False-positive and false-negative errors

As with PCR-tests, the results of WBE for detecting the virus can be influenced by false-positive and false-negative errors. False-positive errors can be monitored, minimized, and eliminated by quality assurance and quality control (QA/QC) procedures (Ahmed et al., 2022). This research discusses that it is more difficult to identify and mitigate false-negative errors. Many factors can result in false-positive and false-negative errors. The study of Ahmed et al. (2022) discusses these factors. Some factors causing false-negative errors are factors already discussed in other sections of this literature review. In addition to the possible effect on the accuracy of monitoring the virus discussed, these factors can affect the accuracy to a greater extent by false-negative results.

False-positive and false-negative errors might affect the representativeness of WBE for monitoring the SARS-CoV-2 virus, due to incorrect interpretation of the data. This will lead to distorted data and wrong decision making. To what extent this factor influences the representativeness is not known. Standardized protocols for the analysis of viruses and QA/QC procedures could optimize the quality and reliability of the SARS-CoV-2 analysis (Ahmed et al., 2022).

2.4.2. Virus variants

Since the outbreak, the SARS-CoV-2 virus has mutated, resulting in different variants. The study of Crits-Christoph et al. (2021) appoints that the strength of WBE is the ability of identifying different genotypes in the samples. WBE is helpful for detection of different variants in the community. Information on whether the detection of the virus in a sample is hampered by virus variants is unknown, and it is also unknown whether the number of gene copies shed by infected persons varies between infections of different variants. If these factors apply to the SARS-CoV-2 virus, this can give imprecision or difficulties to the data analysis, and this could result in less representative monitored data.

2.4.3. Detection limit

For lab-analysis a minimum load of the SARS-CoV-2 virus should be present in the samples. When the virus load is less than the limit of quantification (LOQ) or limit of detection (LOD) the virus will not be detected. This can give incorrect interpretation of the data, since the virus can still be present in the sample. Therefore, it can give false-negative results. For smaller catchments this can give difficulties in the analysis, especially when there are limited infected persons in a catchment. The research of Kumar et al. (2021) defines the LOQ for RT-qPCR as $1.7 * 10^2$ copies/L. According to the study of Ahmed et al. (2022) the assay limit of detection (ALOD) is the concentration of a target with a probability of 95% of detection. The detection limit should be known for the analysis of the virus, to ensure that the data obtained is not misinterpreted.

2.4.4. Adsorption

In the analysis of the wastewater sampled for monitoring the SARS-CoV-2 virus extraction is performed. This extraction ensures the detection of adsorbed RNA. The samples analyzed for the NRS are not filtered, meaning RNA is also present in solid phase in the wastewater analyzed. During the extraction of 1 mL of sample, care is taken to destroy the cell walls and proteins of the enveloped virus. Then the RNA is separated from other substances in the wastewater and the RNA is stabilized. Magnetic beads are used to attract the RNA and then the load SARS-CoV-2 RNA is measured. This method is performed twice, to ensure duplicate analysis on the samples. The extraction and duplicate analysis ensure limited affect of adsorption on the representativeness of the load measured.

2.4.5. Human errors

In section 2.3.2 the human errors in sampling have been already discussed. Human errors might also affect the quality of the analysis of the virus. Working with the samples in the lab must be done precisely and according to the established protocols. If this is not done carefully, the results may be affected. Since lab staff is educated for working in the lab and there are strict protocols the influence of this factor is expected to be limited. But making mistakes is human and therefore not inevitable. To minimise the number of human errors, it is necessary that lab staff are well aware of the protocols and the possible effect if they are not properly followed. During the COVID-19 pandemic, a great deal of pressure has been placed on these lab staff, so great care must be taken not to make any unnecessary mistakes due to fatigue.

2.5. Back-estimation

The last step in the prevalence estimation of the SARS-CoV-2 virus is the back-estimation. In this step, the collected data is converted to the goal of the WBE. In this study, the purpose is to monitor the virus to follow the trend over time. In this last step, the factors discussed in the sub-sections about step 1 to 4 can be accounted for in the monitoring. In the back-estimation no new factors influence the representativeness of monitoring the virus. The challenge is to include the influencing factors in the back-estimation, to obtain representative results for the trend of the virus. The back-estimation changes when the purpose of the WBE for the virus is different.

2.6. Conclusion

This chapter gives an overview on which factors are relevant for this research based on the literature review. Figure 2.3 shows the overview by using the structure of the dendrogram discussed in the introduction of this chapter. The green boxes are the relevant factors that are interesting to research for this thesis, the orange boxes are interesting factors but beyond the scope of this thesis and the red boxes are showing negligible factors. Based on the results of this chapter, seven factors were studied in more depth. The factors are: the location of shedding, temporary in-sewer storage, in-sewer RNA decay, deviant flow values, loss of information by overflow events, automated samplers and flow rate measurements.

Figure 2.3: Conclusion on the literature review. This dendrogram presents the factors that are investigated quantitatively in this thesis. Green is showing relevant factors that are interesting for this research, orange are interesting factors but beyond the scope of this thesis and red is showing negligible factors.

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3

Criteria for synthesis on factors

In this research factors, which influence the representativeness of monitoring the SARS-CoV-2 virus, are investigated. Seven factors are quantified by different methodologies. This quantification is based on the conclusion of the literature review. These factors are: the location of shedding, temporary in-sewer storage, in-sewer RNA decay, deviant flow values, loss of information by overflow events, automated samplers and flow rate measurements. These factors and their effect on representativeness are elaborated on and discussed in the next chapters.

A synthesis on the outcomes of the before-mentioned factors, discussed in the next chapters, is performed and is based on different criteria. This synthesis enables to create an overview on the effect of the various factors and thereby ensures comparison. Considering the results of this synthesis, advice is given on the future inclusion of factors in the methodology of monitoring the SARS-CoV-2 virus through WBE. Also, based on the results advice on potential exclusion of samples is given. This chapter explains which criteria are included in the synthesis and and how the results of this study are assessed against these criteria. The synthesis is based on four criteria and the results are presented in chapter 11.

The first criterion is whether the factor influencing the representativeness of monitoring the virus is caused by random or systematic errors. Information on the type of error helps with understanding the principle of the factor affecting the representativeness and how the effect could possibly be reduced. A random error is defined as unpredictably, resulting in values around the true value. Random errors give results within a certain range. A random error, for example, could be limited by performing multiple experiments. A systematic error on the other hand is defined as a predictable error with the same proportion for each measurement. If these errors are not considered, systematic errors can lead to results far from the truth. Calibration of measuring instruments reduces systematic errors.

The second criterion is the quantitative effect on the load of the virus. This criterion is included to give information on the degree of influence on the representativeness of monitoring by the different before mentioned factors. This facilitates an overview of the quantitative effect of the various factors. Based on the degree of effect, an advice could be given on how important it is to include a specific factor in the methodology of WBE for monitoring the virus or to exclude samples from the trend. The following ranking for the effect on the load monitored is used for this criterion:

- **0 - 10 %** Negligible effect
- **10 - 30 %** Significant effect, but other criteria has to be considered and prior to inclusion in WBE more research should (possibly) be done
- **30+ %** Significant effect, has to be included in methodology of WBE or samples should be excluded from monitoring the virus

The third criterion is the occurrence of less- or non-representative samples by the different factors. This criterion provides information on the relevance of inclusion of a factor in the methodology of WBE for the SARS-CoV-2 virus. It is important that the results of enough samples are included in the monitoring of the virus. Therefore, if the representativeness is regularly affected by a factor, it could be advised, for example, that it is important to include the factor in the methodology of the WBE. When the representativeness of a sample is highly influenced by a specific factor, but this does not occur frequently, it could be advised that those samples should not be included. However, it must be certain that the sample is not representative prior to exclusion. This will possibly require further research on the requirements for rejection.

The fourth criterion is whether the influence on the representativeness by a certain factor is recognizable. This means that the influence of the factor can be recognized by certain parameters that are measured. If the effect on representativeness can be recognized, the sample taken on that day, for example, could be excluded or labeled ’possibly not representative’.

Based on the results from the synthesis on the factors by these four criteria, advice on the different factors is given. This recommendation includes whether the factor should be included in the methodology of WBE or that sample results should not be included in monitoring the virus. Advice is also given on how the factors could be included in the methodology of monitoring the virus and on what conditions samples could be rejected. Information on data that are needed, as well as available data and additional measurements, are discussed in more detail.

4

Number of persons shedding to a WWTP using N and P data

4.1. Introduction

The RIVM uses equation 4.1 for the normalization of the SARS-CoV-2 measurements. In this equation, the number of inhabitants is based on the data of CBS. People travel around, due to work, day trips and holidays. Since individuals perhaps shed their wastewater to a WWTP other than the WWTP of their residence, the representativeness of the load monitored might be affected. Potentially, the spread of people should be accounted for, to correctly base the monitoring of the virus. The aim of this chapter is to get insight into the number of people shedding wastewater to a certain WWTP using total nitrogen (N) and total phosphorous (P) data from the influent. This provides knowledge of the distribution of people, using sewage data. It is aimed to elaborate on the effect of the spread of people on the representativeness of the virus load monitored.

$$Load\ (per\ 100,000\ inhabitants) = conc.\ (virus\ gene\ copies) * volume\ (24h) * \frac{100,000}{number\ of\ inhabitants} \quad (4.1)$$

4.2. Methodology

For determining the number of shedders to a WWTP, data on total nitrogen (N) and total phosphorous (P) is used (Choi et al., 2018). This is done for two locations, i.e. WWTP Houtrust and Westerschouwen. These locations are different in size, location and differ in the degree of tourism.

WWTP Houtrust is located in the west part of The Hague, close to the beach. According to the CBS, on the 1st of January 2021 there were 243.228 inhabitants connected to this WWTP. Next to citizens of The Hague, multiple industries are connected to this WWTP. The neighborhoods Scheveningen, Centre, Kijkduin and Ockenburgh, Moerwijk, Strijp, Leidschenveen and parts of Wassenaar are located in this catchment. The Hague is the third largest city in The Netherlands in terms of population, and the city has many businesses where residents of the city and others from other areas work.

WWTP Westerschouwen is located close to the village of Burgh-Haamstede in the province of Zeeland. As reported by the CBS in the beginning of 2021 there were 7978 residents of nearby areas connected to the WWTP. The catchment of WWTP Westerschouwen is a tourist coastal area with a combination of combined - and separated sewer systems (Liefting et al., 2011).

In figure 4.1 the daily flow of the influent for both WWTPs for 2021 is eleborated (in m^3/day). With the vertical colored lines the holidays for both locations are highlighted. WWTP Houtrust is located in the district 'Midden-Nederland' and WWTP Westerschouwen in 'Zuid-Nederland', which causes some of the holidays are on a different moment. For both plots the flow is much higher on rainy days compared to the dry weather days. Looking closely to the flow for WWTP Houtrust, the DWF seems to be relatively constant over the year. It is remarkable during the summer holiday that the DWF seems to be less compared to the rest of the year. This can be supported by inhabitants of The Hague travelling to other locations for their holidays. For WWTP Westerschouwen the DWF seems to be increasing when the weather is getting better in spring and summer, this can be explained by the high number of tourists coming to this area.

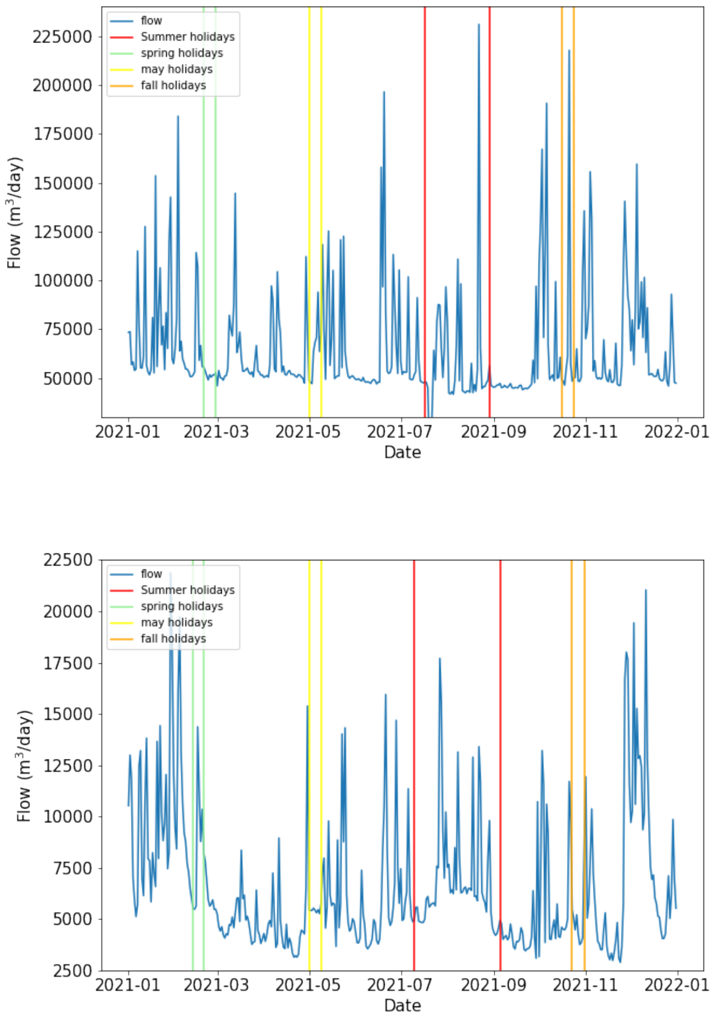

Figure 4.1: The flow (in m^3/day) for WWTP Houtrust (upper graph) and WWTP Westerschouwen (lower graph) for 2021. With the vertical colored lines the boundaries of the holidays in the areas are highlighted.

To determine the number of people shedding to the sewer systems connected to WWTPs Houtrust and Westerschouwen by total N and P the data on quality parameters of the wastewater influent from Z-info is used for both locations. Firstly, from this dataset the data on total N and P mass (per day) were selected. For WWTP Houtrust the data were available for each day of the year, and for WWTP Westerschouwen the parameters were measured approximately once a week.

The wet weather days are excluded from the data, to ensure no effect of temporary in-sewer sto- rage of N and P. Possible increased load of N and P at the WWTP due to rainfall is therefore negligible. This is done by ordering the influent flow per day of the WWTPs on volume and determining the 10th and 50th percentile. It is assumed that the DWF is in between these percentiles, based on expert consultation. The data on the days where the daily influent flow was between below the 10th and above the 50th percentile was excluded from the dataset, resulting in a dataset with the data on dry days.

The measured mass total N and P was divided by the load N and P that is approximately shed to the sewer system per person per day (pppd). The range in load of total N and P produced mainly by households coming from different studies is 11 - 13 gNpppd and 1.6 - 2.0 gPpppd (Zessner and Lindtner, 2005). N and P are consumed by inhabitants mainly by food and discharged into wastewater via urine, faeces and dish wash (Zessner and Lindtner, 2005).

This study assumes a load for total N of 13.2 gNpppd and for total P of 1.64 gPpppd. These loads are close to the values used in earlier studies. The per capita load of N and P was determined based on the mean number of inhabitants of WWTP Houtrust. This wastewater treatment plant was assumed to have a relatively constant number of shedders due to its size and location in an urban area. The load N and P pppd is determined by dividing the average load N and P during DWF by the number of inhabitants documented by the CBS.

In equation 4.2 the formula is shown for the determination of the number of shedders to the system according to the total N and P data.

$$Number\ of\ shedders\ =\ \frac{load\ N\ or\ P\ (in\ mg)}{load\ N\ or\ P\ /1000\ (mgpppd)} \tag{4.2}$$

The load N and P in wastewater is influenced by industries, which may cause a different load per capita to be used. By assuming a population equivalent (pe) of 60 g BOD_5/d, the research of Zessner and Lindtner (2005) states that industries have a specific contribution to municipal wastewater between 0 and 13 gN/(pe per day) and 0.3 to 2.0 gP/(pe per day). This results in average loads of 8.8 gN/(pe per day) and 1.5 gP/(pe per day) for municipal wastewater.

Examples of industries that contribute to the load N and P in wastewater are meat industry (Johns et al., 1995) and milk/dairy industry (Rauch et al., 2021). The research of Onet (2010) investigated the contribution to untreated municipal wastewater by meat and milk and dairy industries. This study determined a contribution of 2743.6 mgN/L and 328.4 mgP/L for meat product factories. For milk and dairy industries the investigated contribution was 663 mgN/L and 153.6 mgP/L.

With the number of inhabitants recorded by the CBS on the 1st of January 2021 for the locations, the relative number of shedders is determined. This is done by dividing the number of shedders according to the data on total N and P by the number of inhabitants by the CBS.

Lastly, the relative number of shedders by N and P was plotted against time. In the plots the holidays over the year for both locations are plotted as well. The y-axes have been adjusted to have the same values, to ensure the comparison of the results.

4.3. Results

The results on the number of shedders to the sewer systems for WWTP Houtrust and Westerschouwen according to total N and P data for dry days are given in this section. In figure 4.2 and 4.3 the relative number of shedders are plotted over time for Houtrust and Westerschouwen respectively, with the results on N in red dots and P in green dots. In both plots the horizontal blue line is representing the number of inhabitants recorded by the CBS. This line is plotted at 1.00 since the calculation to the relative number of shedders is done by dividing the number of shedders by N and P data with the number of inhabitants according to the CBS. This line enables to determine whether the number of shedders by N and P data is above or below the number of inhabitants stored by the CBS. Also, the holidays are highlighted with the vertical colored lines for both locations. The plots with the actual number of shedders for both locations are elaborated in Appendix A.

In figure 4.2 the results for WWTP Houtrust are given. In this figure it is visible that the number of shedders by N and P data are comparable to each other. For some days the number of shedders by

N is slightly higher compared to the data on P, and vice versa. Also, the number of shedders is fluctuating over time, but most of the time shifting between a relative number of shedders of 0.9 and 1.1. Although there are fewer datapoints during the summer period, the results suggest that there are considerably fewer shedders in this catchment during this period. The relative number of shedders in this period is ±0.8, meaning there are ±20 % fewer persons shedding to the system in this season. Figure 4.1 in the methodology of this section confirms this result, since the DWF seems to be as well less during this period.

Figure 4.2: The relative number of shedders by data on total N (red dots) and P (green dots) for WWTP Houtrust. The horizontal blue line represents the relative number of inhabitants by the CBS and the colored vertical lines are highlighting the holidays.

The results for WWTP Westerschouwen are shown in figure 4.3. The dispersion in the relative number of shedders for this location is striking. The number of shedders to this WWTP is underestimated by the data from the CBS for the entire year, compared to the number of shedders using N and P data. The difference in the results for number of shedders by N and P is less comparable, but most of the days the number of shedders by N is greater compared to the shedders by P data.

From the results it is visible that with better weather in spring and summer, the number of shedders to this WWTP is increasing, with a peak in the holidays in May. In the summer holidays and September the number of shedders is structurally higher. After the summer the number of shedders is decreasing again. Most of the year the number of shedders by N and P data is 1.5 - 3.0 times the number of inhabitants by the CBS. During the holidays in May, the summer and in the month September the shedders are 3.0 - 3.5 times the number of recorded inhabitants. Again, these findings are strengthened by the increasing DWF during the summer, visible in figure 4.1.

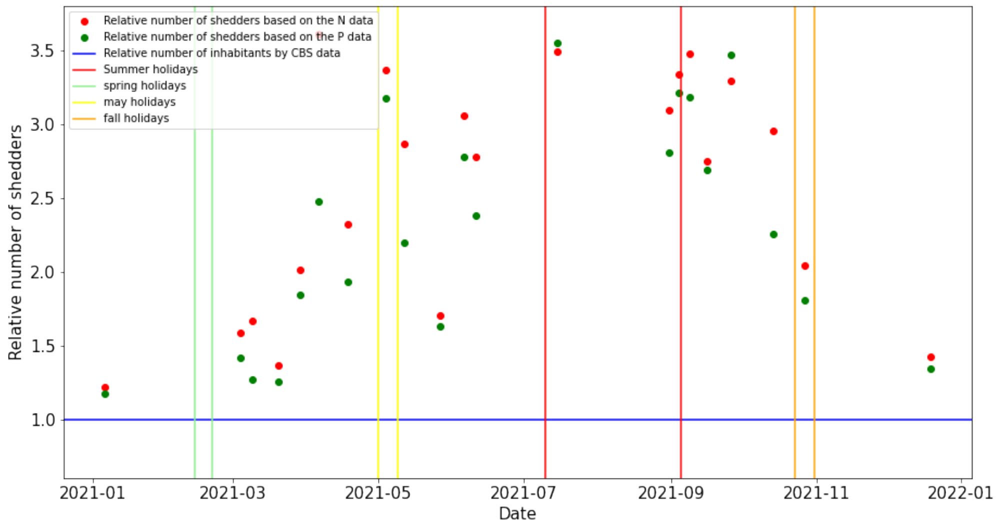

Figure 4.3: The relative number of shedders by data on total N (red dots) and P (green dots) for WWTP Westerschouwen. The horizontal blue line represents the relative number of inhabitants by the CBS and the colored vertical lines are highlighting the holidays.

4.4. Discussion

The results of the number of persons shedding to a WWTP are determined for dry weather days, to ensure no influence of increasing load of N and P due to rainfall by temporary in-sewer storage of the substances. To determine the days with DWF, the data on daily flow between the 10th and 50th percentile is used. The results show that the DWF was lower in summer for WWTP Houtrust and higher for WWTP Westerschouwen, probably because of the movement of people. As a result of the criterion used for days with dry weather, fewer data points were available in the summer. Especially for WWTP Houtrust, many data points were filtered out by this criterion, even though they were days with dry weather. Fortunately, the remaining data points do show the right trend. It is debatable whether this method of determining dry weather days is the right one for this issue.

In this research the results on this factor are based on the relation between the number of people shedding to a WWTP and the load total N and P. The load N and P pppd used are determined for WWTP Houtrust, and validated by literature. As mentioned, the load N and P in wastewater is influenced by industries. The specific contribution to municipal wastewater varies per catchment and degree of industries in an area. Some industries contribute high loads of N and P to wastewater. Since the same load of N and P per capita was assumed for the different sites, this variation in N and P load pppd was not included in the results.

The results on this factor given in this research are based on two locations. To validate the results, data analysis on more locations should be done. Furthermore, it would be of added value to look at data from several years. This would ensure that the randomness of the results is eliminated.

An advantage of the determination of the number of people shedding to a WWTP by data on N and P is the availability of the data, since these parameters are already measured from the influent. Therefore, no additional equipment or measurements are needed when this factor is included in normalization. However, for some locations these parameters are not measured every day. Furthermore, also other methods that can properly determine the location of individuals could be used. The accuracy and extra costs of each method could be compared to decide on which method to use.

4.5. Conclusion and input for synthesis

In conclusion, the results on the number of shedders to a sewer system by data on total N and P, suggest that there is a variability in the number of shedders in a catchment over time. This spread depends on the location, the degree of tourism and the time of the year. Moreover, for the city of The Hague in the summer holidays there are ±20% less persons shedding to the sewer system. The rest of the year the number of shedders is quite constant for this sewer system. For WWTP Westerschouwen these results suggest that the number of shedders is underestimated by the CBS data on inhabitants (up to 350%). Also, the number of shedders is higher in spring and summer, with peaks in the holidays in May and summer and the month September. A major advantage of this methodology used is that the data is already being measured and therefore would not require additional equipment or measurements. However, for some locations the frequency of measurements on N and P should be increased.

5

Temporary in-sewer storage of the SARS-CoV-2 virus

5.1. Introduction

The transportation of the SARS-CoV-2 virus through the sewer system is possibly delayed by processes which take place in the sewer system. This is defined as temporary storage of the virus. The main processes causing temporary storage considered in this chapter are sedimentation and interaction with biofilm. Sedimentation of substances takes place by gravity, which makes that larger particles sediment more easily. Therefore, adsorbed SARS-CoV-2 virus particles possibly settle. Since substances settle down when the flow in the sewer pipe is not high enough to carry the substances in the current, sedimentation is also depending on the flow velocity. The cross-section of a sewer pipe is shown in figure 5.1 to highlight the different environments where the transport of the virus possibly is extended (Jensen et al., 2016).

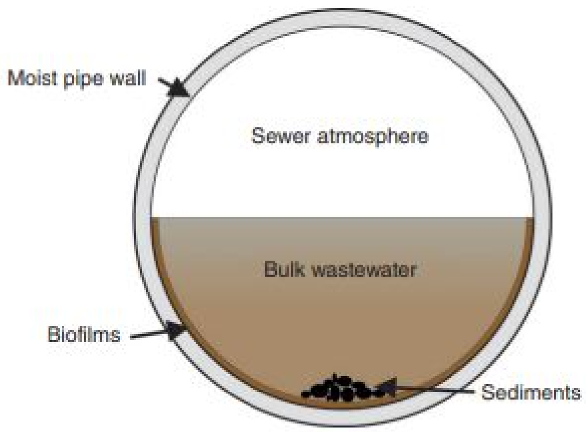

Figure 5.1: The cross-section of a sewer pipe highlighting the environments for sedimentation and biofilms. (Jensen et al., 2016).

Resedimentation of the virus particles in a combined sewer system will potentially take place when the flow velocity in the sewer system is increasing, due to a rainfall. If resedimentation of the SARS-CoV-2 particles by stormwater is significant, it could be stated that the load of the virus monitored at the WWTPs is increasing during a rainfall event. This would affect the representativeness of the samples taken during rainfall, since the load of the virus could be overestimated. Therefore, the aim of this chapter is to get more insight in the temporary storage of the SARS-CoV-2 virus by processes in the sewer pipes, to give more insight on the effect of these activities on the representativeness of samples.

5.2. Methodology

The effect of temporary storage of the SARS-CoV-2 virus by sedimentation and interaction with the biofilm has been examined in this chapter. This is done by plotting the the daily load of the virus per 100.000 inhabitants (in # virus copies) against the daily flow (in m^3) for three WWTPs. It is hypothesized that if there is a correlation between the flow and the load of the virus, resedimentation of the virus by stormwater is affecting the representativeness of samples. Since stored virus particles would arrive during rainfall due to higher flow velocities, the hypothesis is based on the statement that the load is increasing for higher flow. This assumes that flow measured at the WWTP is higher during rainfall.

The results are plotted for three WWTPs. These WWTPs differ in size and are Amsterdam West, Houtrust (The Hague), and Camperlandpolder. According to the CBS data for 2021 669,917 inhabitants are connected to Amsterdam West, 243,228 to Houtrust and 49,220 to Alphen Noord, large, middle and small WWTPs respectively.

Part of the people infected with the SARS-CoV-2 virus shed the virus to the sewer system. Therefore, it is stated that the load of the virus is increasing with more infected persons. In 2021 the number of infected people was varying over time in The Netherlands. Thus, the effect of varying number of people infected is excluded from the plots for the daily viral load against the daily flow. This is necessary to determine the effect of temporary storage of the virus on the representativeness of samples by these plots. Otherwise the load of the virus included in the plots would be influenced by the number of people infected. Based on the registered number of persons infected, a division on the number of people infected was made into five classes. For 2021 the number of registered persons was summed per day for the entire country. The classes used are the 0th - 20th, 20th - 40th, 40th - 60th, 60th - 80th, and 80th - 100th percentile. Percentiles are used to ensure the same number of datapoints in each plot.

In 2021 different measures were taken, people got vaccinated, and there were different variants of the virus that were more or less contagious. To exclude the effect of the omicron variant, data of 2021 is used until the 15th of December. For the three WWTPs the plots are made with subplots for each class. The coefficients of determination ($R^2 - values$) are determined to estimate the correlation between the flow and the viral load.

To examine the relation between the viral load and the number of positive tested persons, the viral load is plotted against the number of positive tested persons for the three WWTPs. This is done for 2021, until the 15th of December. The trendline for the datapoints is plotted in the same plot and the $R^2 - values$ are determined.

5.3. Results

In figures 5.2, 5.3 and 5.4 on the next pages the plots for the daily viral load against the daily flow are presented. In figure 5.2 the plot for WWTP Amsterdam West are given, in figure 5.3 for WWTP Houtrust, and in figure 5.4 for WWTP Alphen Noord. The results for the three WWTPs correspond well, although the sizes of the WWTPs differ. The magnitude of the flow depends on the size of the WWTP. It is hard to find a relation between the daily flow and the daily viral load for all subplots for the three WWTPs. This suggests a negligible effect of temporary storage of the SARS-CoV-2 virus on the representativeness of samples. Based on the results the viral load is not increasing with higher daily flow. The subplot for 7973 - 23699 infected persons show higher loads compared to the load in the other four plots for the three WWTPs. This indicated higher loads with more infected persons. Difference in load between the other four subplots is not visible.

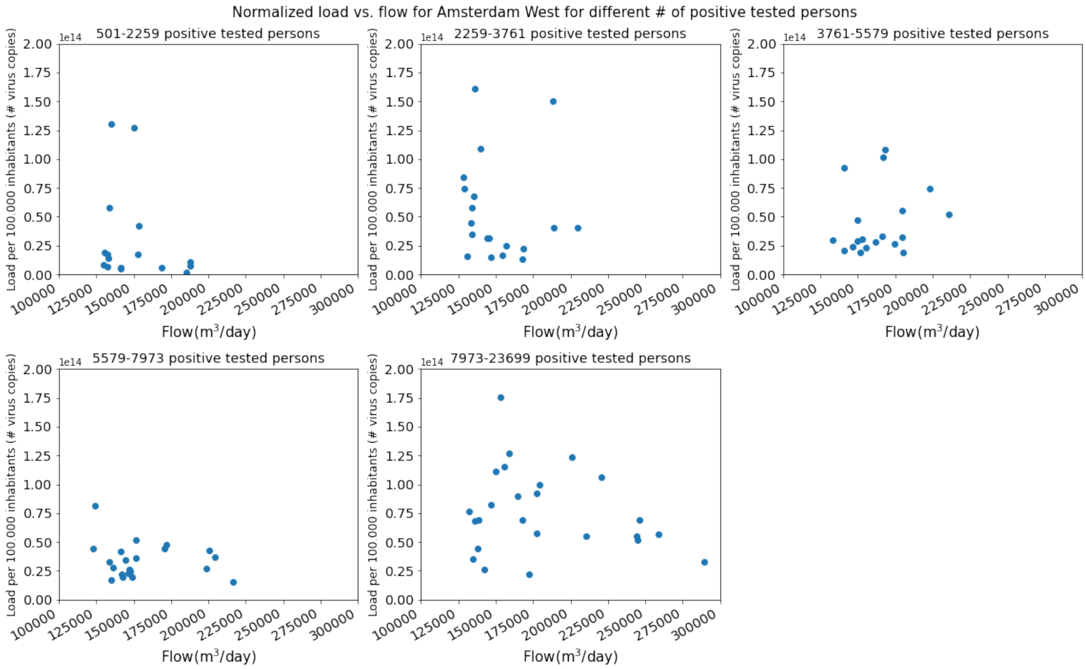

Figure 5.2: The normalized load of the SARS-CoV-2 virus plotted against the flow for the WWTP Amsterdam West.

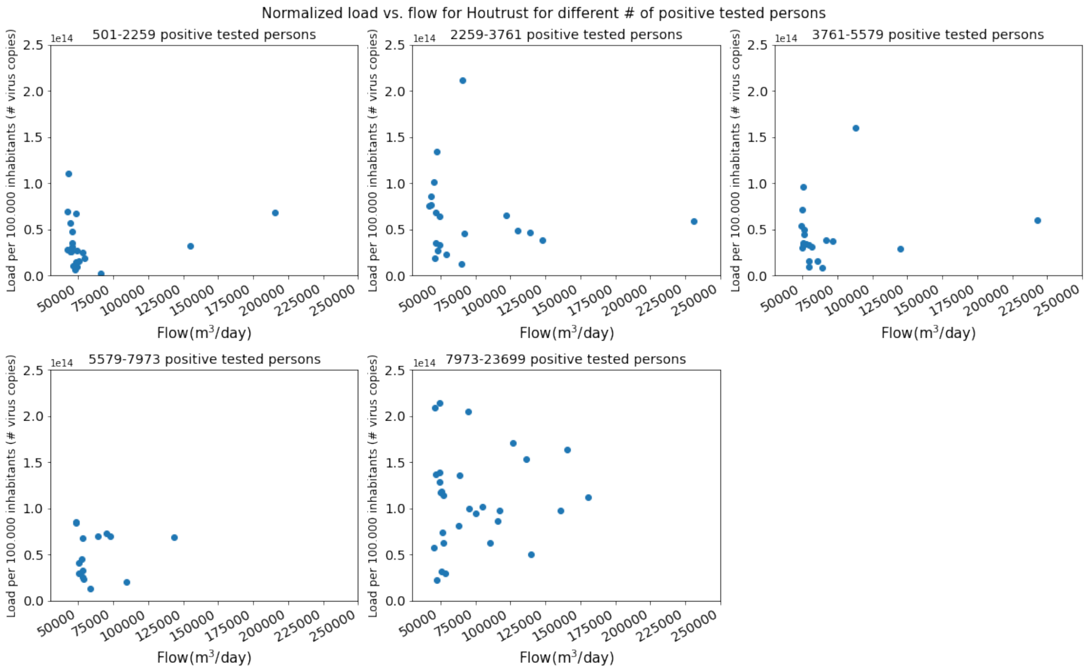

Figure 5.3: The normalized load of the SARS-CoV-2 virus plotted against the flow for the WWTP Houtrust.

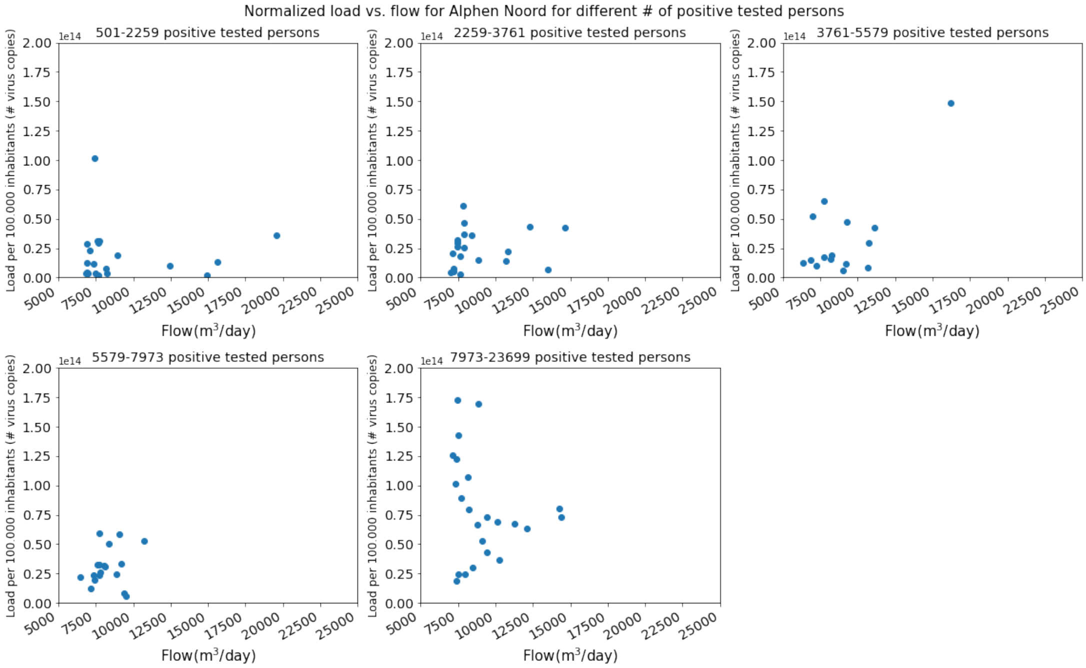

Figure 5.4: The normalized load of the SARS-CoV-2 virus plotted against the flow for the WWTP Alphen Noord.

In table 5.1 a summary on the $R^2 - values$ is given for the three locations per subplot based on the number of positive tested persons. The $R^2 - values$ confirm that the flow and load of the virus show limited correlation. This validates the suggestion that there is a negligible effect of temporary storage of SARS-CoV-2 virus on the representativeness of samples.

Table 5.1: $R^2 - values$ for plots given in figure 5.2, 5.3 and 5.4.

# positive tested persons	Amsterdam West	Houtrust	Alphen Noord
501-2259	0.049	0.028	$4.85*10^{-5}$
2259-3761	0.10	0.0091	0.023
3761-5579	0.096	0.030	0.50
5579-7973	0.027	0.017	0.046
7973-23699	0.042	0.007	0.066

Figure 5.5 shows the relation between the load of the virus for WWTP Alphen Noord and the registered number of infected persons. This figure shows that for a higher number of positive tested persons the daily viral load is increasing. This supports the higher viral load for the plots for 7973 - 23699 infected persons given in figure 5.2, 5.3 and 5.4. The $R^2 - value$ for this trendline is 0.47, which validates an average relation between the two parameters. However, the relationship is not strong. Therefore, the load of the virus and the number of registered positive tested persons are possibly influenced by other factors. In Appendix B the plots for Amsterdam West and Houtrust are shown.

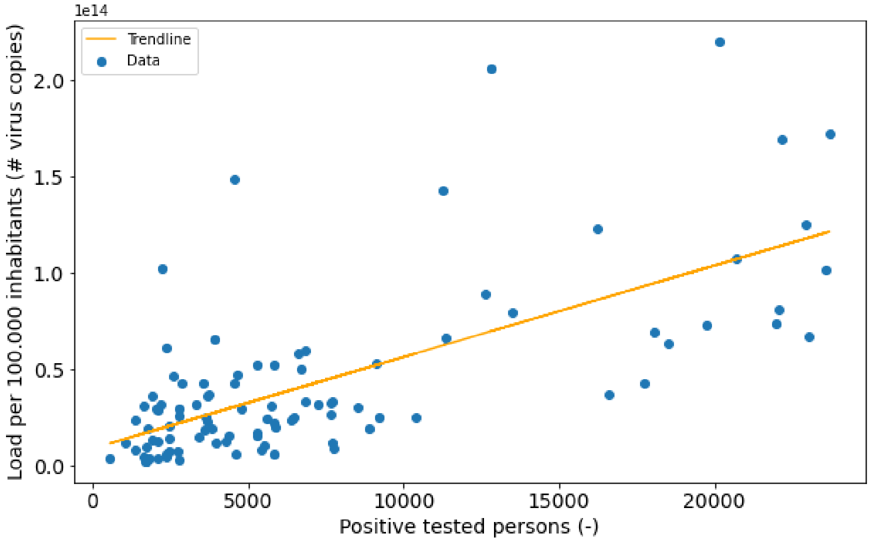

Figure 5.5: The normalized load of the SARS-CoV-2 virus plotted against the registered number of infected persons for WWTP Alphen Noord.

5.4. Discussion

This chapter provides insight in the effect of temporary storage of the SARS-CoV-2 virus on delayed arrival of viral load at the WWTPs with higher flow. The mechanisms underlying this delayed transport of virus are sedimentation and interaction with biofilm. Although the results show that temporary storage does not cause higher loads of the virus during rainfall, this does not mean that the in-sewer processes related to the virus are not influenced by sedimentation and interaction of biofilm at all. The representativeness of samples is possibly influenced by other in-sewer processes. For example, storage of the virus and factors related to adsorption could influence the representativeness.

The results are sorted by the registered number of persons tested positive. During this period there was a changing willingness to get tested, and the availability of self-testing eliminated the need for individuals to be officially tested. These factors possibly affected the registered number of persons tested positive, and therefore influenced the results on the relation between the load and the flow. Also, there is a delay between the first day of symptoms of a person infected and a positive test. The load shed by an infected person is greatest on the first day of symptoms. Therefore, this delay could results in days with many persons registered positive, but a relatively low load, since the persons were tested some days after the first symptoms. This delay could also result in days with a relative high load compared to the registered number of positive tests, for example, in the beginning of a peak in infections. The load of the virus in the sewer system is already increasing due to more infections, but the registered positive tests are available later.

The three factors discussed in the above paragraph, the willingness to get tested, the availability of self-tests and the delay between shedding and testing, potentially also affected the relation between the load of the virus and the registered number of persons tested positive. Figure 5.5 shows an average relation between the two parameters. If the discussed factors would be included in the relationship, the relationship between the two parameters will possibly be stronger.

Another factor that possibly influences the load of the virus in the sewer system is the virus variant. In 2021 multiple virus variants were dominant in The Netherlands. To exclude the effect of the omicron variant, the results are determined for 2021 until the 15th of December. From this day it is known that the omicron variant was dominant. However, the load of the virus could possibly be influenced by this variant before this date, since this variant was already detected in The Netherlands. Based on expert consultation, the other variants that were dominant in The Netherlands in 2021 seem to shed the same load. Therefore, it is expected that other variants did not influence the results.

5.5. Conclusion and input for synthesis

Based on the results of this chapter it is concluded that there is no relation between the daily flow and the daily load of the virus per 100.000 inhabitants. Therefore, there is a negligible effect of temporary storage effect on delayed arrival of viral load of the SARS-CoV-2 virus with higher flow at the WWTPs. The influence on the representativeness of the samples by temporary storage of the virus is thus expected to be limited. The results are not affected by the sizes of the WWTPs. Furthermore, there is a relation between the registered number of persons tested positive and the load of the virus.

6

In-sewer decay of SARS-CoV-2 RNA

6.1. Introduction

The SARS-CoV-2 virus and RNA are degrading in the sewer system. For monitoring the virus, the load of RNA is analyzed. Therefore, this chapter focuses on the decay of SARS-CoV-2 RNA. The load of the virus in samples taken from wastewater is influenced by decay. This chapter provides information on the decay of the virus, to give insight on the effect of decay on the representativeness of samples.

This research focuses on two parameters related to decay: temperature and residence time. According to the study of Ahmed et al. (2020) the RNA of the virus is degrading first-order and most greatly influenced by temperature. Decay is time-dependent and therefore the residence time of the wastewater in the sewer system is an other important parameter.

Decay of viruses is affected by the temperature, with increasing inactivation at higher temperatures (Carducci et al., 2020). In The Netherlands the temperature of the wastewater varies between 8°C and 22°C. In figure 6.1 the temperature of the activated sludge is shown for WWTP Amstelveen to see the spread in temperature over time. This plot shows that the variation in temperature of wastewater is less compared to the air temperature. According to the Koninklijk Nederlands Meteorologisch Instituut (KNMI) the air temperature fluctuated in between -16.2°C and 34.0°C for 2021. The temperature of wastewater in a sewage system is mainly determined by the soil temperature, which results in limited variation in temperature of wastewater.

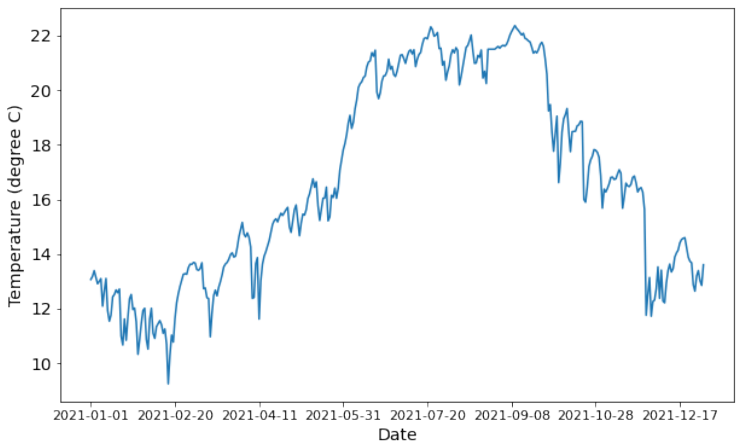

Figure 6.1: The temperature of the activated sludge tank of WWTP Amstelveen for 2021.

Data on the decay rate related to temperature for the virus varies in literature. Therefore, this chapter will give better understanding of the relationship between the decay rate and temperature for the SARS-CoV-2 RNA.

The residence time of wastewater varies per sewer system, due to the distance to the WWTP and the flow velocity in the sewer pipes. Knowledge on residence time is necessary for determination of the decay of the virus. If there is a significant spread in residence time, the viral load could be affected by decay. If the load of the virus is significantly influenced by decay, the samples might not be representative. Variation in decay between locations in the same sewer system or between different sewer systems, could complicate the interpretation of the monitored data. This chapter provides information on the residence times of wastewater in the sewer systems of The Hague and Rotterdam.

6.2. Methodology

This chapter contributes on information about the effect of decay on the representativeness of samples. This section describes the methodology. Firstly, the procedure of determining the relation between decay rate and temperature is defined. Secondly, the methodology on calculating the residence times for the sewer systems in The Hague and Rotterdam is described. Lastly, it is discussed how the parameters are combined to define the decay.

6.2.1. Temperature

The relationship between temperature and the decay rate is determined by combining the information of two studies. The research of Hokajärvi et al. (2021) found the k-value 0.06 /day with no standard deviation. In addition, the study of Ahmed et al. (2020) concluded the reaction rates for different temperatures presented in table 6.1.

Table 6.1: The decay rate for SARS-CoV-2 RNA in untreated wastewater for different temperatures of the water Ahmed et al., 2020.

Temperature (°C)	Decay rate (/day)
4	0.084 $\pm$ 0.013
15	0.114 $\pm$ 0.012
25	0.183 $\pm$ 0.008
37	0.286 $\pm$ 0.008

The thermal inactivation of a virus can be predicted by the Arrhenius equation (Yap et al., 2020). This equation gives the relation between ln(k) and 1/T for first-order reactions. The Arrhenius equation is the following equation (equation 6.1):

$$ln(k) = -\frac{E_a}{RT} + ln(A) \tag{6.1}$$

In this equation R is the gas constant, E_a the activation energy and A the frequency factor (Yap et al., 2020). By plotting ln(k) (/min) and 1/T (10^4/°K) the against each other, the linear relationship between the reaction and the temperature is determined. For respiratory pathogens the change of the relative survival rate (C/C_0) is an exponential equation, given in equation 6.2 (Luyao et al., 2021).

$$\frac{C}{C_0} = e^{-kt} \tag{6.2}$$

Next, the temperatures of the wastewater for 20 WWTPs is analyzed for 2021. This analysis is based on the measured temperatures of activated sludge at WWTPs, since the temperature of wastewater is not measured at WWTPs. According to experts the temperature of wastewater corresponds well to the temperature of activated sludge. It is assumed that the temperature of the wastewater is equal to the temperature of the activated sludge. A histogram and boxplot on the variance in temperature for these 20 WWTPs is elaborated.

Furthermore, the relation between the reaction rate and the actual temperatures of the wastewater in

The Netherlands are combined. In a table the decay rate (per day) is given per 3°C, starting with the minimum value and ending with the maximum value according to the boxplot given in the results.

6.2.2. Residence time

The average and extreme residence time for wastewater, and the virus, are estimated for WWTP Houtrust and WWTP Dokhaven. The average residence time is determined by comparing the daily pattern for the drinking water supply to the daily pattern for the influent of the wastewater at the WWTP per hour. Assuming that most of the drinking water is immediately discharged to the sewer system, comparing the patterns gives insight in the average residence time. The extreme residence time is determined with different methods for Houtrust and Dokhaven. For Houtrust information on the sewer system from the online portal Viewer Duopp is used. Results by Johan Post (Partners4UrbanWater) on the distance of random points in the sewer system to the WWTP Dokhaven are used.

Based on expert consultation, assumptions on the flow velocities in sewer pipes are made in this research. For pressurised sewer systems and sewer systems under gravity a flow velocity of 0.25 m/s is assumed for the connections to the main sewer pipes. In the main sewer pipes a flow velocity of 0.5 m/s is assumed. The flow velocity in pressurized pipes to the WWTP is assumed to be 0.25 m/s, to limit the energy needed, but avoid sedimentation. These are the minimum velocities, since this research focuses on extreme residence times. The average and extreme residence times are compared for both locations.

WWTP Houtrust is located in The Hague and treats water of industries and 243,228 inhabitants according to the CBS. The sewer system is mainly a combined sewer system, except the neighborhoods Wateringse Veld, Ypenburg and Leidschenveen with a separated sewer system. The flow of wastewater through the sewer is under gravity, with pressurized pipes to the WWTP. The data on the hourly supply of drinking water used is provided by Dunea, the drinkwater company for The Hague and surroundings. It supplies drinking water to all areas in The Hague with zipcodes starting with 25XX, except Wateringse Veld (2548). Drinking water for this area is produced at Pompstationsweg in Scheveningen and Haagweg in Monster. This data includes the drinking water usage of inhabitants, as well as resource and process water for industries. The influent data of WWTP Houtrust is provided by Water Board Delfland. This data includes the wastewater produced by the inhabitants and industries.

The average residence time for The Hague is estimated by plotting the pattern for the supply of drinking water against the pattern for the flow of the influent per hour. Since the wastewater data was stored differently from the 5th of January, the pattern is determined from the 6th of January 2021 until the 31th of December 2021. First the days with DWF are selected according to the 10th and 50th percentile of the total daily flow. The days with DWF are used, to ensure that the pattern is not influenced by stormwater. Due to leakage loss and unbilled consumption, experts from Dunea assume that 8% of the produced water is not arriving at the users. This percentage was taken from the data on the volume consumed.

For the dry days the mean flow values per hour are determined for the drinking water and wastewater. It is ensured that the mean flow values are determined for the same hours, since the data is given for different timesteps, i.e. 03:59 is assumed to be 4:00 for the data on the influent of the wastewater. The mean values for drinking water and wastewater are plotted against each other for each hour. The first derivative for both patterns was determined for each hour and plotted over 24 hours. These figures substantiate when there is an increase or decrease in the patterns. This allows the minimum and maximum values of the two patterns to be easily compared.

The extreme values for the residence time are determined for two locations in the catchment of WWTP Houtrust and one location in the middle of the catchment. This is done with information from Viewer Duopp on the sewer system of this catchment. From the points highlighted with stars in figure 6.2 the residence time is determined. The star close to the beach is WWTP Houtrust.

Figure 6.2: The locations in The Hague for which the residence time has been determined. The stars show the three locations, as well as WWTP Houtrust. The blue lines represent the flow direction to WWTP Houtrust.

For each location the pipes are followed from the starting point to the sewer pumping station or WWTP. Then the pressurized pipes are followed until the WWTP. The distance of the pressurized pipes to the WWTP Houtrust are determined by the distance measuring function in Google Maps, following the pressurized pipes. In Appendix C an overview of the pressurized pipes in The Hague is given. The lengths of the pipes are noted down from Viewer Duopp, as well as information if the pipe is a connection, main sewer or pressurized pipe to the WWTP. The connection pipes to the main sewer pipe are assumed to have a diameter of at maximum 750 mm. The diameter of the main sewer pipes is assumed to be above 750 mm. Lastly, the residence time is determined by dividing the length of each pipe by the assumed flow velocity (0.25 m/s for connection pipes, 0.50 m/s for main sewer pipes and 0.25 m/s for pressurized pipes to the WWTP). The total residence time is a summation of all pipes.

Next, the average residence time of the wastewater in the sewer system connected to WWTP Dokhaven is determined. This sewer system is also under gravity. The influent of WWTP Dokhaven is coming from five influent pipes and one pipe for internal flow from the sludge processing plant (pipe 1). The influent pipes 2, 3 and 4 come from Rotterdam South. The wastewater coming from the Northern side of Rotterdam is coming to the WWTP by one pipe. This pipe is split into two pipes, influent pipes 5 and 6, since the total flow is exceeding the capacity of one influent pipe. A schematic scheme is given in figure 6.3. The data on the hourly supply of drinking water used is provided by Evides, the drinkwater company that supplies drinking water to the catchment of WWTP Dokhaven. The hourly pattern is based on four zipcodes in the area. These zipcodes are 3015, 3044, 3068 and 3071. This data includes the drinking water usage of inhabitants, as well as resource and process water for industries. The influent data for WWTP Dokhaven is provided by Water Board Hollandse Delta. This data includes the wastewater produced by the inhabitants and industries.

The methodology on determining the average residence time for the sewer system connected to WWTP Dokhaven is similar to WWTP Houtrust. First, a summation of influent pipes 2 to 6 is done per hour. Influent pipe 1 is not included, since this pipe for internal flow is not relevant for this research. Then, the days with DWF are selected and the mean values for the hourly flow for these days are determined. These mean values for the drinking water and wastewater are plotted against each other per hour. Furthermore, the first derivative for both patterns was determined for each hour and plotted over 24 hours.

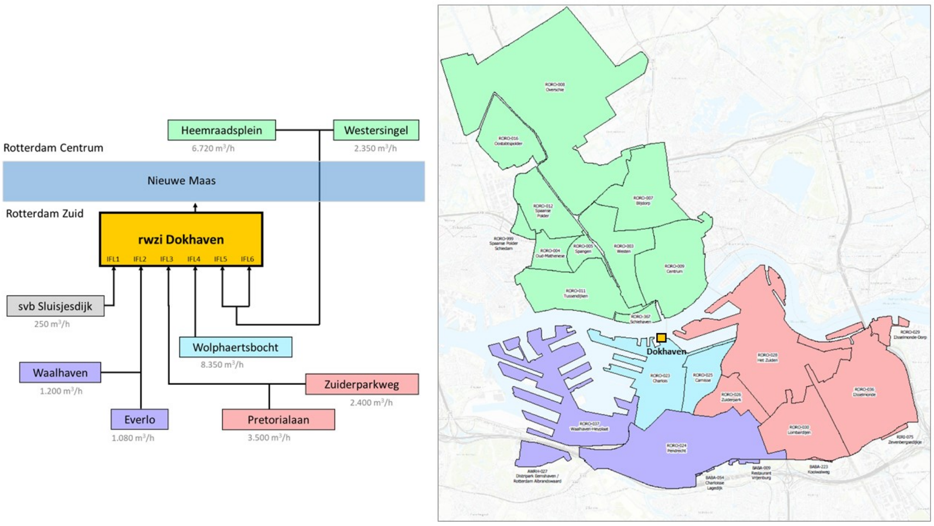

Figure 6.3: Overview of the sewer systems connected to WWTP Dokhaven and the different strains of influent.

For the sewer system connected to Dokhaven the distance from a random point in the system to the sewer pumping station is determined. This distance is the shortest distance, non-euclidean, meaning it follows the pipes of the system. This is created for 500 random points in more than 100 systems. The median distance is determined, as well as the 95% interval. This is given in a plot. The wastewater arriving at a sewer pumping station is pumped to the WWTP via a pressurized pipe. For the sewer system there is not an overview available on the pressurized pipes to WWTP Dokhaven. Consequently, the distance from the pumping stations to the WWTP is determined by an Euclidian line and adding 10% to this distance, based on expert consultation. The Euclidian lines from the pumping stations to WWTP Dokhaven are given in the overview shown in Appendix C. This is done for 14 pumping stations, which drain a large number of kilometers of sewer. From this data the minimum, mean and maximum distance to the WWTP is determined. The residence time is determined, based on an assumption of 0.25 m/s flow velocity in the entire system.

6.2.3. Decay based on temperature and residence time

The results of the relation between the decay rate and the temperature and the residence times are combined to determine the effect of decay on the representativeness of samples. The hourly decay for the minimum and maximum wastewater temperature are used. These are 0.32 % and 0.76% respectively. By multiplying this decay with the residence time, the total decay is calculated.

6.3. Results

This section provides the results of the study on decay of the SARS-CoV-2 RNA. Firstly, the results on the relation between decay rate and temperature are given. Secondly, the results on the residence times are presented. Lastly, the decay based on a combination of temperature and residence time is discussed.

6.3.1. Temperature

The relation between the reaction rate (k) and temperature (T) is given in figure 6.4 according to the decay rates determined by the research of Hokajärvi et al. (2021) and Ahmed et al. (2020).
From this figure the relation given in equation 6.3 is determined with R^2 = 0.9623. In this equation the unit for k is (/min) and for T (/°K).

$$ln(k) = -0.366 * (1/T * 10^4) + 3.268 \tag{6.3}$$

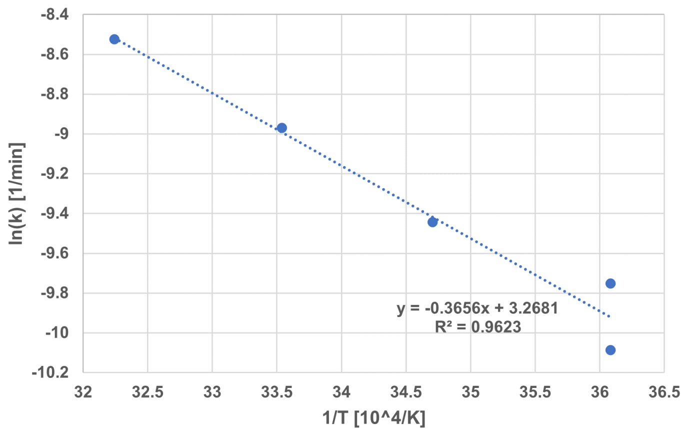

Figure 6.4: The relation between the reaction rate and temperature for SARS-CoV-2 according to the results of two researches (Ahmed et al., 2020; Hokajärvi et al., 2021).

In figure 6.5 the histogram related to the measured temperatures of the activated sludge of 20 WWTPs is given. Here the 25th percentile is given in yellow, the mean value in red and the 75th percentile in purple. The median value is not given, since this value is equal to the mean value. In Appendix C the results are presented in a boxplot. The minimum temperature measured is 6.22°C and the maximum temperature measured is 25.87°C. The mean temperature for all 20 WWTPs is 16.48°C. The left bin shows that there are some outliers in the data. These outliers are caused by an error in the measuring device at WWTP Eindhoven.

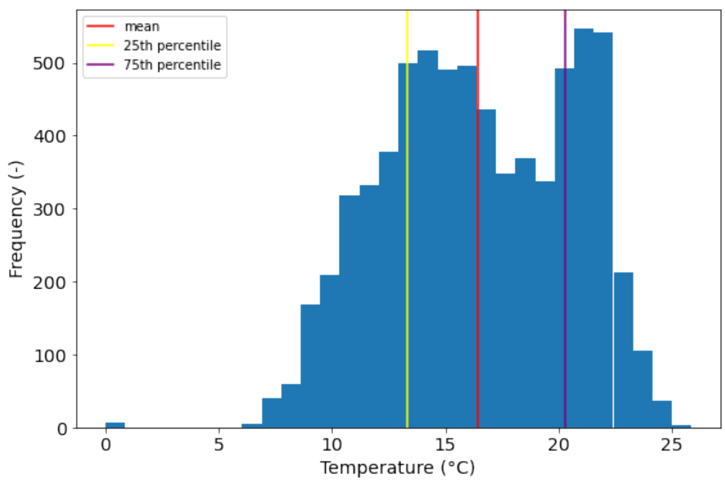

Figure 6.5: Histogram on the data of the temperature of the activated sludge of 20 WWTPs (with 30 bins).

In table 6.2 the decay rates per day and per hour are given per 3°C and the minimum and maximum temperature measured. In the left column the temperature is given in °K, and in the second left column the same temperature in °C. This table shows that the decay of the virus is increasing with higher temperatures.

Table 6.2: The decay rate determined by equation 6.3 per 3°C and the minimum and maximum temperature of the wastewater.

Temperature (°K)	Temperature (°C)	Decay rate (/day)	Decay rate (/hour)
279.37	6.22	0.077	0.0032 (0.32%)
282.15	9.0	0.088	0.0037 (0.37%)
285.15	12.0	0.101	0.0042 (0.42%)
288.15	15.0	0.115	0.0048 (0.48%)
291.15	18.0	0.131	0.0055 (0.55%)
294.15	21.0	0.149	0.0062 (0.62%)
297.15	24.0	0.169	0.0071 (0.71%)
299.02	25.87	0.183	0.0076 (0.76%)

6.3.2. Residence time

In figure 6.6 the relative drinking water and wastewater patterns are shown for The Hague. In Appendix C the plot of the patterns against each other for the actual values is presented. The graph with the first derivative for both patterns is also given in Appendix C. Figure 6.6 illustrates that the patterns for drinking water and wastewater show the same course over the day. The pattern for wastewater is flattened during the night. This means that the wastewater flow is relatively higher during the night compared to the drinking water supply. Comparing the drinking water and wastewater pattern no delay is visible. Therefore, the average residence time of wastewater in the system of Houtrust cannot be determined based on the patterns. The graph on the first derivatives, presented in Appendix C, also show that the peaks are at the same time. This substantiates that the average residence time for Houtrust's sewer system cannot be determined from the drinking water and wastewater pattern.

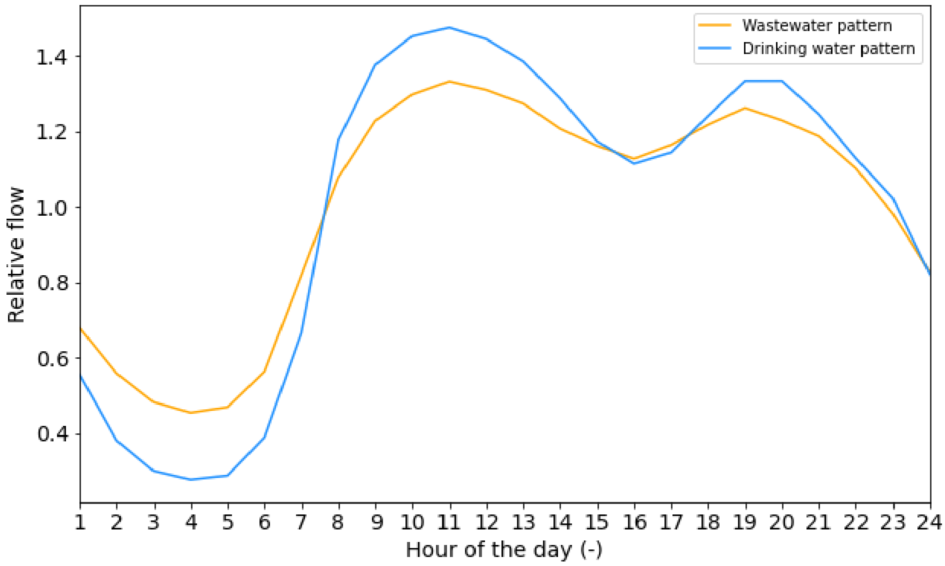

Figure 6.6: Drinking water and wastewater patterns for The Hague to estimate the average residence time of wastewater in the sewer system with relative values.

From the data in Viewer Duopp and the distances of the pressurized pipes from Google Maps the residence times are determined for three points in The Hague. The results are given in table 6.3. In Watering the starting location was Laan van Wateringse Veld and the sewer pumping station was Lageveld. The starting location for Mariahoeve was Robertaland, and the sewer pumping station Schiestraat. The point in the centre started at Helmerstraat. There was no sewer pumping station between this point and WWTP Houtrust. The results show much longer residence times for the points at the boarder of the catchment, compared to the centre.

Table 6.3: Distance and residence time for three locations in The Hague to WWTP Houtrust.

	Wateringen	Mariahoeve	Centre
Distance to sewer pumping station (m)	2331.2	3997.91	-
Distance to WWTP Houtrust (m)	9065	6590	2856.82
Residence time (s)	38591.2	41055.8	6248.48
Residence time (h)	10.72	11.40	1.74

Figure 6.7 elaborates on the drinking water pattern and wastewater pattern for Rotterdam. In Appendix C the plots of the patterns are presented with the actual values, as well as the first derivatives for both patterns. The graph in figure 6.7 shows that the drinking water supply is increasing from 4:00 am and a first peak at 11:00 am. The wastewater flow is increasing at 7:00 am and peaks at 1:00 pm. These results give an average residence time of wastewater in the system of Dokhaven of 2 - 3 hours. This average residence time is validated by the peaks presented graph on the first derivatives for both patterns (presented in Appendix C).

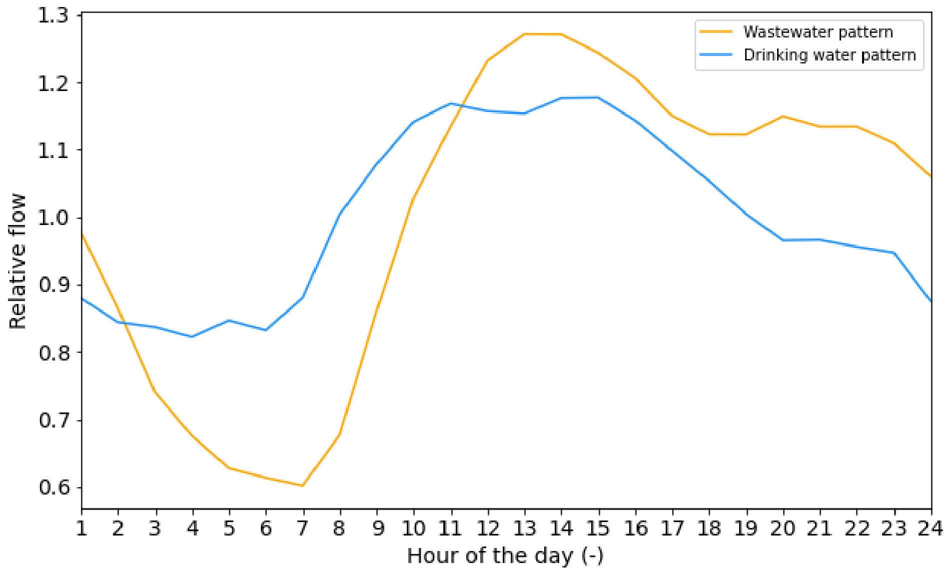

Figure 6.7: Drinking water and wastewater patterns for Rotterdam to estimate the average residence time of wastewater in the sewer system with relative values.

Figure 6.8 shows distribution on the distance from a certain point in the sewer system of Rotterdam to the closest sewer pumping station. This graph is based on 500 points for over 100 smaller sewer systems connected to WWTP Dokhaven. The graph shows that for 100% of the data the distance to a sewer pumping station is in between ±0 and ±2700 m (95% interval). The median distance is ±1450 m.

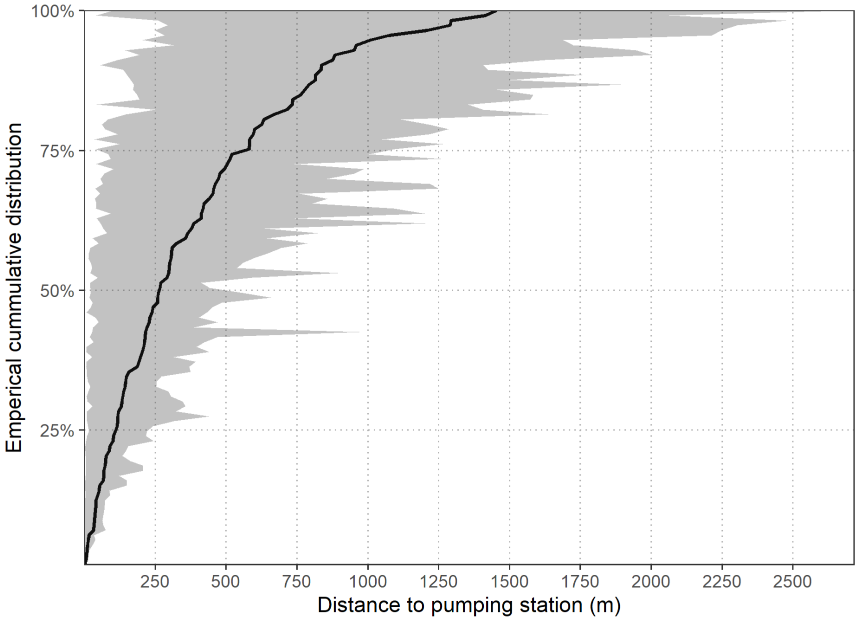

Figure 6.8: Overview of the distribution on the distance from a certain point in the sewer system of Rotterdam to the closest sewer pumping station. The 95% interval is given in grey and the median value with a black line.

Based on the Euclidean distance between 14 sewer pumping stations and WWTP Dokhaven the minimum, mean and maximum distance are determined. In table 6.4 distances to the sewer pumping stations and distances from the sewer pumping stations to WWTP Dokhaven are given. The median distance from a certain point in the system to the pumping station is combined with the mean distance from a sewer pumping station to WWTP Dokhaven. In table 6.4 this is named as 'median + mean' distance. The minimum, 'median + mean' and maximum distance are given, as well as the residence time in seconds and hours. Notably, the average residence time and the residence time using the median and mean distances are significantly different.

Table 6.4: Minimum, median/mean and maximum distance from points in the sewer system of Rotterdam to WWTP Dokhaven.

	Min. distance (m)	**Median + mean distance (m)**	**Max. distance (m)**
To pumping station	0	1540 (median)	2700
To WWTP Dokhaven	533.5	3640.1 (mean)	7632.9
Residence time (s)	2134	20720.4	41331.6
Residence time (h)	0.6	5.8	11.5

6.3.3. Decay based on temperature and residence time

The residence times of wastewater in the sewer system is combined with decay of the virus by temperature. The results are given in table 6.5. The minimum (6.22°C) and maximum temperature (25.87°C) for wastewater are used, with an hourly decay of 0.32 % and 0.76% respectively. The results show a decay between 0.19 % an 3.68 % for the minimum wastewater temperature for the determined residence times. For the maximum wastewater temperature the decay is in between 0.46% and 8.74%. It was not possible to estimate the average residence time of wastewater in the sewer system of The Hague, and therefore this is not included in the overview in table 6.5.

Table 6.5: Residence times for different locations in The Hague and Rotterdam combined with the minimum and maximum decay.

Location	Residence time (h)	Min. decay (%) (6.22°C)	Max. decay (%) (25.87°C)
The Hague Centre	1.7	0.55	1.32
The Hague Mariahoeve	11.4	3.65	8.66
The Hague Wateringen	10.7	3.43	8.15
Rotterdam average	2 - 3	0.64 - 0.96	1.52 - 2.28
Rotterdam minimum	0.6	0.19	0.46
Rotterdam median + mean	5.8	1.86	4.41
Rotterdam maximum	11.5	3.68	8.74

6.4. Discussion

This chapter gives information on the decay of the virus by temperature and residence time. There are some limitations to the research performed on this topic. These points of discussion are discussed in this section. The limitations on temperature, residence time and the combination of these parameters are explained respectively.

6.4.1. Temperature

The determined relation between the decay of the virus and temperature is based on the findings of two studies. To argue that the relation accurately reflects reality, more data points should be included. If this cannot be obtained from previous studies, more lab experiments need to be done. This would give insight in the standard deviation of the results and ensure that the relation could be validated.

In this research it is assumed that the temperature of the wastewater is equal to the temperature of the activated sludge. If the sensor is placed after aeration of the active sludge, there could be a bias in the temperature. In winter the temperature of the active sludge is possibly slightly lower due to the cold temperature of the air, and in summer possibly slightly higher.

6.4.2. Residence time

For the determination of the residence time it is assumed that the flow velocity in main sewer pipes is 0.5 m/s. For the sewer pipes that are the connection pipes to the main sewer pipes a flow velocity of 0.25 m/s is assumed. For the pressurized pipes between the sewer pumping stations and WWTPs the assumption is made that the flow velocity is 0.25 m/s. These flow velocities are assumptions based on literature and knowledge of experts, and indicate the minimum flow velocities. The actual flow velocities could be different in the sewer system, resulting in other residence times. Also, a constant flow velocity in each type of pipe is assumed, which is not feasible in the actual situation.

In Appendix C the flow pattern with the actual flow for drinking water and wastewater through the day is shown for The Hague. This figure shows a much higher volume used for drinking water compared to the production of wastewater. Leakage loss and unbilled consumption is considered (assumed to be 8%, based on information by an expert). The large difference can be explained by sprinkler consumption and the use of water as raw material. Also, it could be possible that the areas do not fully match for drinking water and wastewater data.

The average residence time of wastewater in the sewer system is determined by comparing the drinking water and wastewater patterns. For The Hague this methodology did not work, since both patterns had peaks on the same moment. This could be explained by the dynamics of the wastewater system. In The Hague the wastewater flows rapidly through the pipes under gravity. Then, the wastewater is pumped from sewer pumping stations to WWTP Houtrust via pressurized pipes. These pressurized pipes are always filled with water. This means that if wastewater is entering the pressurized pipes connected to the WWTP, this already measured by the flow meter in WWTP Houtrust. Since the measured flow at the WWTP are the data used, the methodology on determining the average residence time does not include the residence in the pressurized pipes. This makes that the method is unusable.

For Rotterdam the result of the average residence time does not match the outcome of the residence time by the median distance to the sewer pumping station and mean distance from sewer pumping stations to WWTP Dokhaven. The average residence time is 2 - 3 hours, compared to 5.8 hours based on the median + mean residence time. The wastewater in the system under gravity of Dokhaven is flowing less rapidly to the sewer pumping stations compared to the system of Houtrust. There are storage tanks in the system that slow down transportation of the wastewater. Probably the 2-3 hours average residence time is based on this delay. The difference between both residence times determined could again be explained by the pressurized pipes from the sewer pumping stations to the WWTP. The methodology on the determination of the average residence time does not include the residence time in the pressurized pipes, and therefore is not applicable.

Furthermore, the drinking water pattern of Rotterdam is based on four zip codes from the catchment, due to data availability. In total over 35 zip codes are located in this catchment. Therefore, the pattern given in the results is not the actual drinking water pattern. If data from the entire area had been used, the pattern might be different. However, the pattern does correspond well to typical points in a general drinking water pattern. Examples of iconic points are less supply during the night, an increase in supply in the morning and a peaks around noon.

Due to no data availability on the structure of the pressurized pipes from the sewer pumping stations to the WWTP Dokhaven, an estimation of the distances is made by using the Euclidean distances and adding 10%. These measured distances do not represent the actual distance to the WWTP.

This study investigated the residence time for two cities in The Netherlands, both with a sewer system under gravity. The residence time of wastewater might vary between urban and rural areas, as well between different catchments with pressurized systems and systems under gravity. The WWTPs for the systems studied in this chapter are both centrally-located in the catchment. For other catchments the location of the WWTP perhaps is less central. Therefore, the results elaborated in this chapter potentially do not represent the residence times of all catchments in The Netherlands.

6.4.3. Decay based on temperature and residence time

As discussed, there are limitations to the investigation on the relation between the rate of decay and temperature and the residence time of the wastewater in sewer systems. These drawbacks potentially affected the results of decay based on temperature and residence time. Inaccuracy in the outcomes of both parameters are combined, by bringing together the results on both parameters.

In the literature review it is discussed that there are multiple factors that possibly influence the decay of the virus. This research focused on the influences of temperature and residence time. It could be as well that different parameters influence the decay and each other, so combining the influence of different factors could lead to new insights regarding the degradation of the virus.

This research focuses solely on The Netherlands. In other countries, the temperature of the wastewater in sewer systems is potentially different. Also, if the present substances in the water differ, the degradation is possibly different, especially when interaction between substances have a certain effect on decay of the virus. In addition, the sewer systems vary between different countries. For example, there are closed and open, pressurized and under gravity, combined and separated, branched and unbranched sewer systems. All these factors could cause a variance in decay of viruses between countries and regions within countries.

6.5. Conclusion and input for synthesis

From the results, a relation between the decay of the virus and temperature is concluded. Equation 6.3 gives this relation, with k in (/day) and temperature in (/°K). Furthermore, from this study on the decay of the virus it is deducted that there is a fluctuation in the temperature of the wastewater. Seasonal variation in temperature is shown in figure 6.1. The temperature of the wastewater for 20 WWTPs is in between 6.22 °C and 25.87 °C for 2021, with a mean value of 16.48 °C. From table 6.2 and equation 6.2 it is clear that the number of virus copies at the maximum temperature is degrading 2.38 times faster compared to the minimum temperature of the wastewater.

Based on the results elaborated on in this chapter, it is expected that decay of the virus in the sewer system differs between neighborhoods, due to a fluctuation in residence times. The results on the residence time show a variation between 0.6 and 11.5 hours. For most wastewater of a catchment mean residence times of several hours apply. Considering the minimum and maximum temperature of wastewater, these residence times mean a variation in decay between 0.19 and 8.74 %.

7

Deviant flow values

7.1. Introduction

The daily flow is an important parameter with respect to normalization, since samplers are volume proportionally controlled. DWF is fairly constant, with a certain pattern over the day. Industrial - and infiltration water are also part of DWF. Due to stormwater the daily flow will increase on rainy days. The flow is measured at pumping stations in sewer systems and in (pressurized) influent pipes at WWTPs. Flow values that vary enormously from the expected value are defined as deviant flow values in this study. Deviant flow values could be caused by (unexpected) errors in the system, and could affect representativeness of samples for the monitoring of the SARS-CoV-2 virus, since transport of the virus through the sewer system is not as expected.

There are different factors that can result in deviant flow values measured at WWTPs. The focus in this research are deviant flow values at WWTPs, due to the focus of the NRS. The most common causes of deviant flow values are maintenance in the sewer system, pump failure in sewer pumping stations or pump failure in influent pipes at WWTPs. Maintenance is not always communicated or registered correctly, but affects the transportation through the sewer network. In figure 7.1 a schematic overview of the different causes is elaborated.

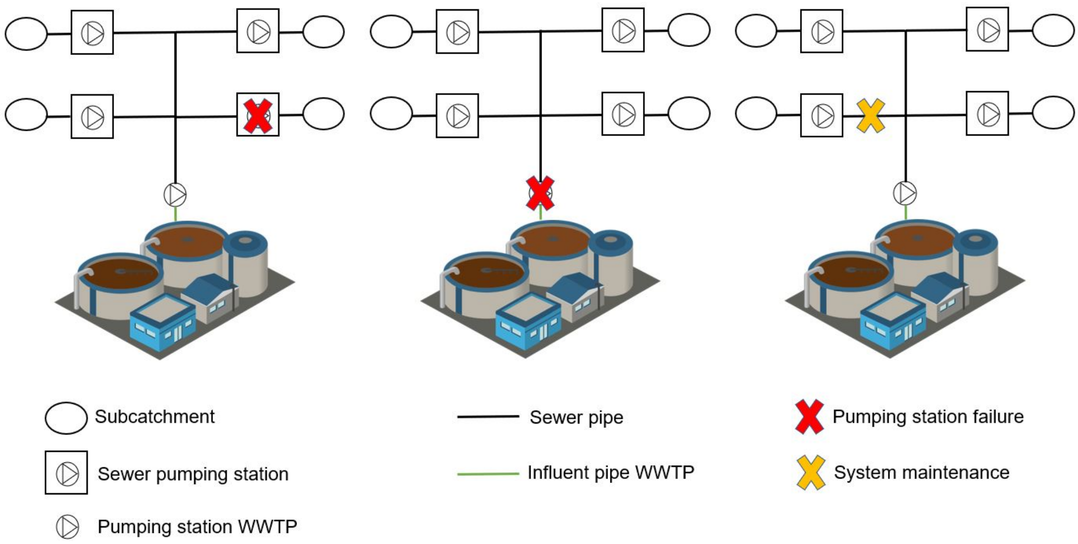

Figure 7.1: Schematic overview on three examples of causes for deviant flow values measured at WWTPs. On the left sewer pumping station failure of one subcatchment is elaborated. The middle picture shows pumping station failure of the influent pipe of the WWTP. The right picture represents system maintenance of a subcatchment.

More insight into the occurrence of deviant flow values is needed to assess whether samples are representative for the viral load in the sewer. This chapter gives insight in two types of deviant flow values: 1) Flow rates with a significantly lower flow rate than the DWF on dry- or wet days, and 2) Flow rates with a significantly low value compared to the rainfall. The different types of deviant flow values are explained, as well as the occurrence and how the deviant flow values can be recognized.

7.2. Methodology

Although the objective of NRS is the scope of the research, the results in on the deviant flow values were obtained for the pumping stations Kanaalweg and Europaplein in Utrecht. Pumping station Kanaalweg is located in the western part of Utrecht, close to the neighborhoods Transwijk-Noord, Oog in Al, Leidseweg and Dichterswijk. Europlein is a pumping station in the south of Utrecht, close to the neighborhoods Kanaleneiland, Transwijk-Zuid, Zuidwest and Rivierenwijk.

The used data on Kanaalweg was from Januari 1st 2021 until April 14th 2022, and data on Europaplein from Januari 1st 2021 until March 30th 2022. From the online platform Sensight, owned by IMD, the data on the hourly flow for this pumping station is extracted. IMD uses this platform to see the functioning of the automated composite samplers, store data send by the samplers and adjust settings remotely. Since data on hourly flow is available for the samplers located at pumping stations via Sensight, this data is used. The methods are described per type of deviant flow values, flow rates with a significantly lower flow rate than the DWF on dry- or wet days, and flow rates with a significantly low value compared to the rainfall respectively.

7.2.1. Type 1: Days with flow rates with a significantly lower flow rate than the DWF on dry- or wet days

The first type of deviant flow values investigated are flow rates with a significantly lower flow rate than the DWF on dry- or wet days. DWF is relatively constant over the year. Infiltrating water is part of DWF. Since infiltrating water is less during dry periods, due to lower groundwater levels, the DWF could be less during dry periods. Also, periods with many persons leaving a catchment due to, for example, holidays could reduce the DWF. On some days the flow measured at the WWTP is far below the average DWF. If the flow on this day is more than the minimal volume samples (more than 70-100 times the aliquot volume), possibly something in the system caused this low amount of flow measured at the WWTP. If wastewater is not flowing to the WWTP as expected, the samples taken on the day with low flow values could be, as well as the next day could be not representative.

To give insight in this first type of deviant flow values the dataset of Kanaalweg is analyzed to see if this type occurs. With the following methodology an example is elaborated of a type 1 deviant flow value. This example gives understanding of type 1 deviant flow values. Since hourly flow is not measured at all WWTPs in The Netherlands, it is not possible to elaborate a warning system based on hourly flow. Therefore, criteria on daily flow and pulse information is used to extract dates with type 1 deviant flow values for pumping station Kanaalweg.

Firstly, the S-curve of the data is plotted. Ordered data of the cumulative daily flow is plotted against the number of days. Since the DWF is approximately between the 10th (q10) and the 50th (q50) percentile, these percentiles are determined and plotted with horizontal lines to show the range of DWF. Secondly, the minimum volume sampled is elaborated by multiplying the minimum amount of pulses (70) with the pulse volume for Kanaalweg (30 m^3). This pulse volume is determined from Sensight. Below this volume samples are rejected according to the NEN6600-1 standard. Thirdly, it is assumed that 30% below q10 is a significant lower flow rate than DWF. This assumption is based on fluctuations in DWF by dry periods and holidays and the S-curve. Therefore, the maximum threshold volume for deviant flow value of type 1 has been estimated to be 0.70 * q10.

To extract dates with deviant flow values of type 1 from the dataset of Kanaalweg, the dataset is analyzed for the minimum volume sampled and the maximum threshold volume. The extracted dates are added to a data frame. For these days the days before and after are added to the data frame. Subsequently, the DWF-pattern is estimated according to the assumption that the days with dry weather are between the 10th and 50th percentile. For these days the mean hourly flow is determined.

Then the data on the hourly flow measured at the pumping station, the DWF and the hourly rainfall (measured by and used from KNMI) are selected for 8:00 the two days before until 8:00 two days after the sampling interval with an error. This is done since sampling takes place from 8:00 until 8:00 the next day and it wanted to get information as well on the days before and afterwards. The data on the hourly rainfall by the KNMI is adjusted by converting the hourly rainfall from 'x 10 mm' to 'mm' and changing the values of -1 (given when the rainfall is < 0.05 mm) to 0, assuming this rainfall is negligible. Finally, plots are made on the hourly flow, DWF and rainfall for the days with a daily flow rate in between the minimum volume sampled and the maximum threshold volume.

7.2.2. Type 2: Days with flow rates with a significantly low value compared to the rainfall

The second type of deviant flow values studied are flow rates with a significantly low value compared to the rainfall. The volume of incoming wastewater at WWTPs is increased during a rainfall event, if stormwater is drained by sewer systems to WWTPs. If the flow rate of influent is not significantly higher than DWF during an intense rainfall event, an (unexpected) error might results in lower flow values than expected. In this research it is investigated whether this type of deviant flow values occurs.

The occurrence of this second type of deviant flow values is elaborated on by plotting the flow for days with a daily rainfall ≥10 mm against the precipitation for pumping station Europaplein. Days with rain ≥10 mm are considered as days with significant rainfall events. For this analysis the data of the KNMI on the daily rainfall at De Bilt is used between the 1st of January 2021 and the 30th of March 2022. The data of the KNMI is adjusted by dividing the rainfall per day by 10 to get the rainfall in mm/day. From Sensight the hourly flow for the pumping station Europaplein is used. The hourly flow is summed per day to get the cumulative flow per day in m^3/day.

Firstly, the days with a precipitation ≥10 mm are selected from the dataset of the KNMI for De Bilt on daily rainfall. Then corresponding cumulative flow is selected from the data set of Europaplein. Lastly, the daily flow and daily precipitation are plotted against each other, as well as the trend line. This plot is analyzed on the occurrence of deviant flow values on days with significant rainfall.

7.3. Results

In this section the results are shown for the two types of deviant flow values explained in the methodology of this chapter. Firstly, the results for days with flow rates with a significantly lower flow rate than the DWF on dry- or wet days are discussed. Secondly, the results for days with flow rates with a significantly low value compared to the rainfall are given.

7.3.1. Type 1: Days with flow rates with a significantly lower flow rate than the DWF on dry- or wet days

The S-curve for the daily flow at Kanaalweg is shown in figure 7.2. In this plot the minimum volume sampled, maximum threshold value, 10th and 50th percentile are given with colored horizontal lines. This figure elaborates on the flow values for dry weather days are assumed to be between 3334.6 (q10) and 4000 (q50) m^3/day. The minimum volume sampled is 2100 m^3, which is 37% of q10, and the maximum threshold volume for deviant flow values of type 1 for this pumping station 2334.2 m^3. This S-curve shows one day with a flow value in between the minimum volume sampled and the maximum threshold volume. This means that for pumping station Kanaalweg one day between the 1st of January 2021 and the 14th of April 2022 has a deviant flow value of type 1.

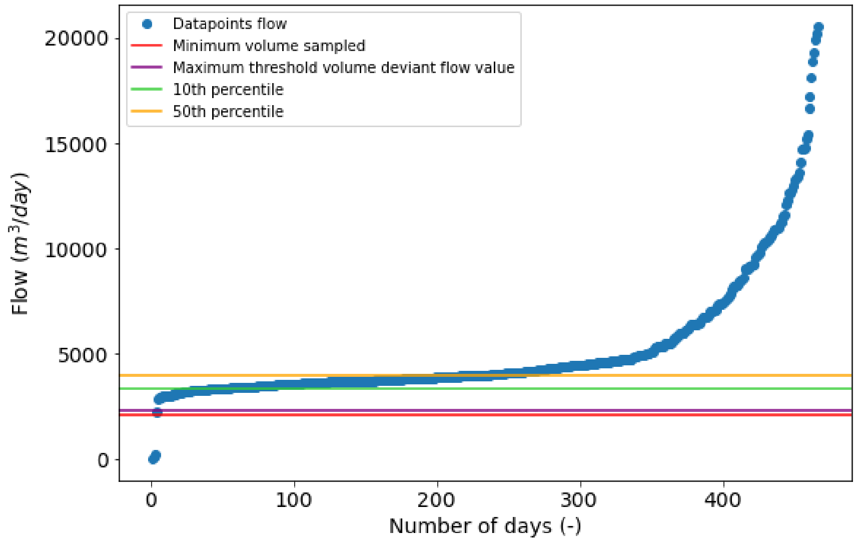

Figure 7.2: The S-curve for the flow at Kanaalweg, Utrecht, for 01-01-2021 until 14-04-2022. The minimum volume sampled, maximum threshold value, 10th and 50th percentile are shown with the colored horizontal lines.

In figure 7.3 the plot on the hourly flow, DWF and rainfall over time is elaborated for the day with a deviant flow value of type 1. This plot shows that the pump is failing from 21:00 until 8:00, since there is no flow during this time interval. The next sampling day, from 8:00, the pump is working again. The plot also shows that the hourly volume corresponds well to the DWF.

The frequency of taking an aliquot from the wastewater is depending on the flow. Since there is no flow during part of the sampling interval, no information of wastewater during that time interval will be present in the sample. This wastewater will stay in the sewer system until the pump starts working again. At the beginning of the day after the pump failure, the pumped volume is much higher than the average DWF. This is because the wastewater that remained in the sewer system due to the failure of the pump is then pumped.

This plot shows that information on the load in wastewater is missing on the day with pump failure, and therefore is not representative. Furthermore, the day after the pumping station failure, the sample will contain information of the day before and therefore also not be representative.

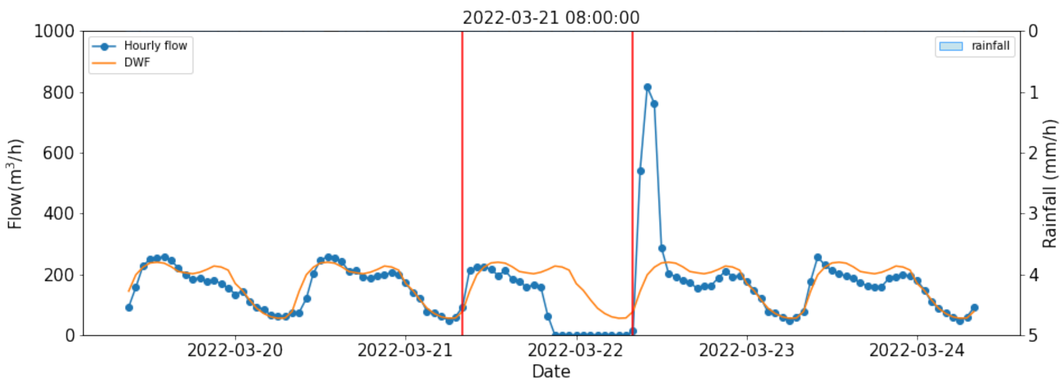

Figure 7.3: Plot on a deviant flow value of type 1 for Kanaalweg in Utrecht on 21-03-2022. The plot elaborates the hourly flow and DWF over time, as well as the rainfall. The day sampled is highlighted with vertical red lines.

If there is a longer pumping station failure, the sample on that day will be rejected according to the regulations stated in NEN6600-1. However, the samples on the day afterwards could contain information of the day(s) before. Due to wastewater stuck in the sewer system, like the above example. Also, when there is a shorter pumping station failure at the end of a sampling interval, the wastewater will possibly be sampled during the next sampling interval. Therefore, the quantitative effect on the load on the day after pumping station failure can be in between 0 and 100+% (+ if the pumping station was failing for multiple days).

7.3.2. Type 2: Days with flow rates with a significantly low value compared to the rainfall

In figure 7.4 the plot is given for the flow for days with minimal 10 mm of rainfall over the day against the precipitation for 2021 and part of 2022 for pumping station Europaplein. In this graph the 10th and 50th percentile are shown, as well as the trendline for the data. It is obvious in this graph that there is a relation between the rainfall and the flow, since the flow is increasing for more rainfall. This is as expected, since more rainfall will result in more water in the sewer system. In the purple box a potential deviant flow value is highlighted. 18 mm of rainfall during a day is significant. It is expected that the flow on this days is much higher than the DWF. This means that for this dataset on Europaplein one possible deviant flow value of type 2 occurs.

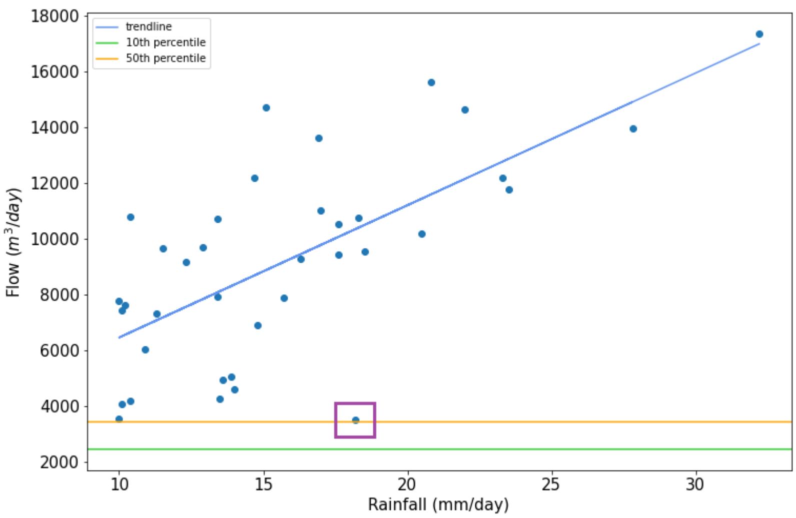

Figure 7.4: The measured flow plotted against the precipitation for pumping station Europaplein, for days with at least 10 mm of rainfall. The vertical lines represent the 10th and 50th percentile in red, green and orange respectively. The trendline is given in blue. The possible deviant flow value is highlighted with the purple box.

7.4. Discussion

Firstly, the results on the deviant flow values are based on pumping stations. The focus of this study actually is on the purpose of the NRS, and consequently on WWTPs. The results and occurrence are possibly different for WWTPs, since the amount of water passing through pump stations is often lower. It is also common in pumping stations that no water is pumped for a while, as there is a threshold for the pump to start pumping that has to be reached.

However, there has been contact with the operator of Dokhaven and it occurs up to about 5 times a year that there is maintenance of the system for about eight hours. Whether pump failure in the influent pipe of a WWTP can result in a less representative sample depends on the robustness of the system. When the system is redundant, failure of a pump will not affect the representativeness of samples. The wastewater will be pumped by another pump and will therefore still be sampled in the same time interval. Dokhaven, for example, has a redundant system.

This study elaborates on knowledge on two types of deviant flow values. However, there could be more types of deviant flow values. There are possibly days with a flow rate higher than DWF on dry days, for example, due to pumping station failure the day before. Also, there are possibly days with a much higher flow rate than the stormwater from a rainfall event. This study provides understanding of deviant flow values, but to know the exact (quantitative) effect, further research is recommended.

In addition, the results are provided for one pumping station for each type of deviant flow values. To ensure a reliable warning system multiple locations should be researched. The results are based on a dataset of just about 15 months, so there may also be coincidence in the outcomes. Furthermore, it would be good to compare the results with documented failures, maintenance and changes in the sewer structures. For Kanaalweg and Europaplein, the results were compared with data on the course of the flow in Sensight. Data on documented failures, maintenance and changes in structure were not available.

The results on type 2 deviant flow values suggest one day with a lower flow rate than expected, based on the precipitation on that day. The data on precipitation used is based on KNMI data of De Bilt. De Bilt is located approximately six kilometers from pumping station Europaplein. If there was a local, intense rainfall event at De Bilt, this could argue the low flow with high precipitation for the deviant flow value elaborated. The results on type 2 deviant flow values therefore should be validated with information on precipitation of multiple weather stations closer to the pumping station. If there was indeed a lot of rainfall for this day with a relatively low flow rate, the cause could be figured out. With that information, the quantitative effect could be determined.

7.5. Conclusion and input for synthesis

The results show that for Kanaalweg one deviant flow value of type 1 occurred. The plot (figure 7.3) on the hourly flow and the DWF over time illustrate that pumping station failure explains the deviant flow value. This plot explains that the sample taken on the day with the deviant flow value is not representative, since part of the wastewater is not included in the sample. Also, the plot shows that the water stuck in the sewer system due to pumping station failure, is sampled the next day. This means that the sample the day after pumping station failure is as well not representative.

The quantitative effect on the load is in between 30% and 37% for days with deviant flow values of type 1 (so days with pumping station failure), based on the minimum volume sampled and the maximum threshold volume. The quantitative effect on the samples taken on the day after pumping station failure can be from 0% to more than 100%.

The results on type 2 deviant values, based on pumping station Europaplein, show that for 15 months one day with a deviant flow value occurs. On this day the flow rate at the pumping station is much less compared to the significant rainfall event on that day. No quantitative effect on the load is determined for this type of deviant flow values.

8

Loss of information due to overflow events

8.1. Introduction

Sewer systems have a certain storage- and discharge capacity. This storage is constituted by the sewer pipes, streets and surface waters. When the storage of a sewer system by the sewer pipes and the streets is exceeded, overflows will spill the surplus water towards surface water. The sewer system capacity can be exceeded during an heavy rainfall event. Rainfall events with a minimum intensity of 10-20 mm/h can cause exceeding of the capacity of the sewer system. When it is a longer rainfall event the capacity will be exceeded from approximately 20 mm/h. For shorter, intense rainfall events an intensity of 10 mm/h can already cause overloading of the system. In figure 8.1 an overview is elaborated of the sewer capacity and typical values on storage and discharge capacities.

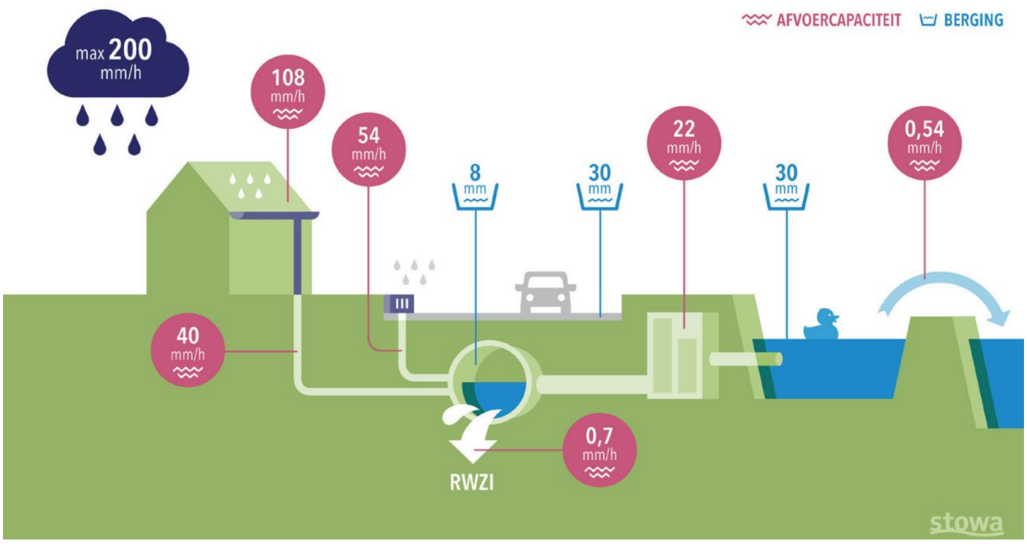

Figure 8.1: Schematic overview of the storage capacity and the discharge capacity for sewer systems in The Netherlands. (Langeveld et al., 2019)

The sewage flowing from the sewer system into surface water is mainly containing stormwater, however this sewage could include wastewater. If this wastewater contains the SARS-CoV-2 virus, information about the load of the virus is spilled from the system and not included in the samples taken during an overflowing event. This chapter aims to elaborate on the effect by overflows on the representativeness of the samples.

8.2. Methodology

In this section the effect by overflows on the representativeness of the samples is described. This is done for the city of Eindhoven, located in the southern part of The Netherlands. In figure 8.2 an overview is shown on the sewer system structure of the city, as well as the location of the 29 combined sewer overflows.

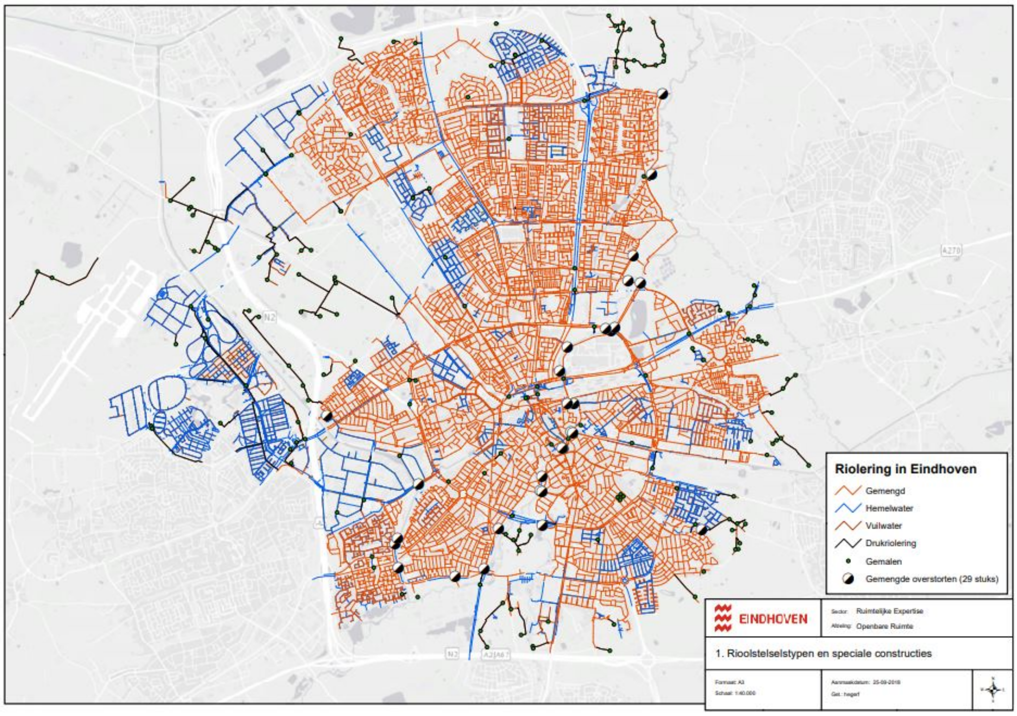

Figure 8.2: Overview of the sewer system of the city of Eindhoven with the location of the 29 combined sewer overflows. (Eindhoven, 2018)

In this chapter the data on the duration and volume of overflow events of 16 of the overflows is used for 2011, 2012 and 2013 obtained from Erik Liefting (Partners4UrbanWater). The duration of an event is given per 15 minutes and the volume in m^3/s. The data used is a combination of in-situ level measurements and modelled values for the discharge measurements, based on whether or not the level measurements were reliable. Eindhoven had 218.433 inhabitants in 2013 according to the CBS. Also, in 2018 Eindhoven had a total paved surface connected to the combined sewer system of 1825 ha Eindhoven, 2018.

According to KNMI the annual rainfall in 2011 was 781 mm, in 2012 876 mm, and in 2013 741 mm. The average annual rainfall in The Netherlands is 847 mm. The year 2011 had remarkably dry and very wet periods, 2012 was a relatively wet year and 2013 was a fairly dry year.

There are four questions that are answered in this chapter to give better understanding of the effect on the representativeness by overflows, which are:

1. How often do overflow events take place?
2. What is the duration of overflow events?
3. How much water is spilled during an overflow event?
4. What percentage of the total volume of wastewater in the sewer system is spilled during an overflow event?

These questions are answered by determining the duration and volume from the dataset for each available overflow in Eindhoven. Per overflow this dataset gives information on the occurrence of overflows, the volume spilled per event and the duration of an event. In total the information of 386 overflow events in 2011, 2012 and 2013 is used. The number of events in 2011 was 124, in 2012 154 and in 2013 108. Next, the data on the occurrence, duration and volume is plotted in boxplots with the pandas function in Python. The volumes per event are multiplied by 900 to convert the data from m^3/s to m^3, since the data on the volume was given in 15-minute intervals.

Also, information on the volume spilled is determined. The inflow capacity of WWTP Eindhoven is 15.000 m^3/h based on expert consultation. The duration of each overflow event is multiplied with this inflow capacity, which is the capacity of the WWTP. The total volume processed in the system is a summation of the volume discharged by an overflow during an overflow event and the capacity of the WWTP. Percentage sewage discharged during overflow event is determined by equation 8.1 for each overflow event. A boxplot on the percentage sewage discharged is again plotted with the pandas function in Python.

$$Sewage\ discharged\ (\%) = \frac{Volume\ discharged\ by\ overflow\ (m^3)}{Total\ volume\ processed\ in\ the\ system\ (m^3)} * 100\% \tag{8.1}$$

8.3. Results

The results according to this factor are given in this section. First, the results for the occurrence of overflow events are given. Secondly, results on the duration of an overflow are elaborated and then the results on the volume. Lastly, the results on the percentage sewage discharged during overflow event are shown. The results are presented in boxplots. In these boxplots the lower limit of the blue rectangle is the 25th percentile (Q1) and the upper limit the 75th percentile (Q3). The interquartile range (IQR) is the range between Q1 and Q3. The upper black line is the minimum (Q1 - 1.5 * IQR) and the upper black line is the maximum (Q1 + 1.5 * IQR). The green line is the median value and the green triangle the mean value. The circles are outliers.

8.3.1. Occurrence of overflows

In figure 8.3 the number of overflows per year for the 16 locations is presented in a boxplot. The number of occurrence per year is given for 2011, 2012, 2013 and the years together per year. The median values are 7.0 overflow events per overflow for 2011, 9.0 overflow events per overflow for 2012, 6.5 overflow events per overflow for 2013 and 7.0 overflow events per overflow per year from the data of the three years together.

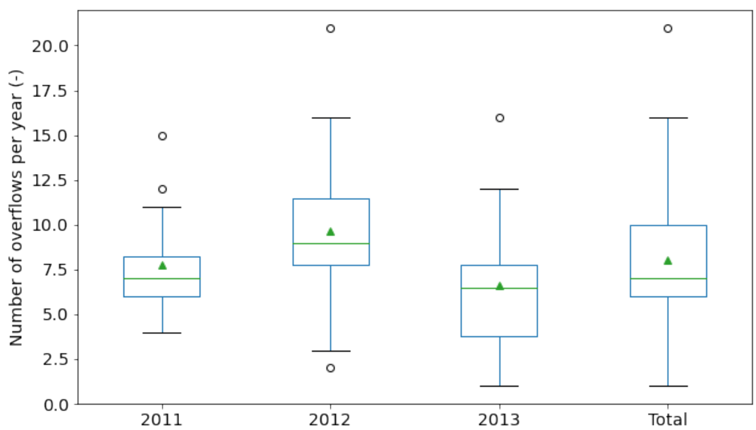

Figure 8.3: Boxplot of the occurrence of overflow events for 16 overflows in Eindhoven for 2011, 2012 and 2012. The green line is representing the median value, which is 7.0 overflows for 2011, 9.0 overflows for 2012, 6.5 overflows for 2013 and 7.0 overflows per year from the data of the three years together.

8.3.2. Duration of overflows

For the 16 overflows the boxplot in figure 8.4 shows the spread of the duration of an overflow event for 2011, 2012 and 2013. The median value is 127.5 minutes for 2011, 135.0 minutes for 2012 and 120.0 minutes for 2013.

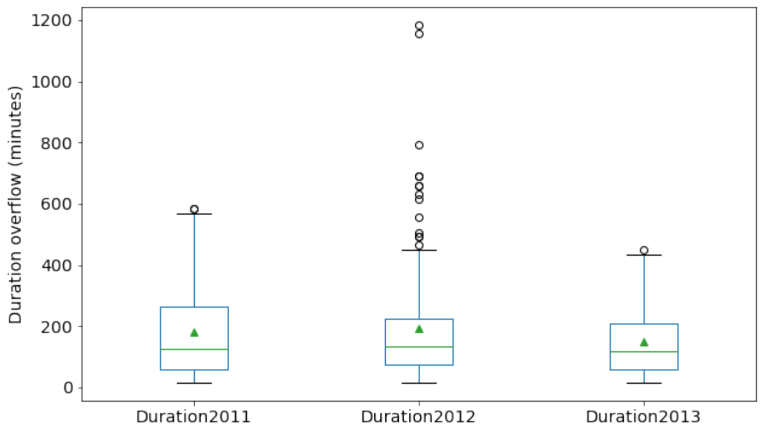

Figure 8.4: Boxplot of the duration of an overflow event for 16 overflows in Eindhoven for 2011, 2012 and 2012. The green line is representing the median value, which is 127.5 minutes for 2011, 135.0 minutes for 2012 and 120.0 minutes for 2013.

8.3.3. Volume spilled during overflow event

The boxplot for the total volume spilled during an overflow event is shown in figure 8.5. From this figure it can be seen that there are many outliers. The median value are 1712.25 m^3 for 2011, 1375.65 m^3 for 2012 and 1192.95 m^3 for 2013. The maximum values are 58859.1 m^3 for 2011, 25180.2 m^3 for 2012 and 55252.8 m^3 for 2013.

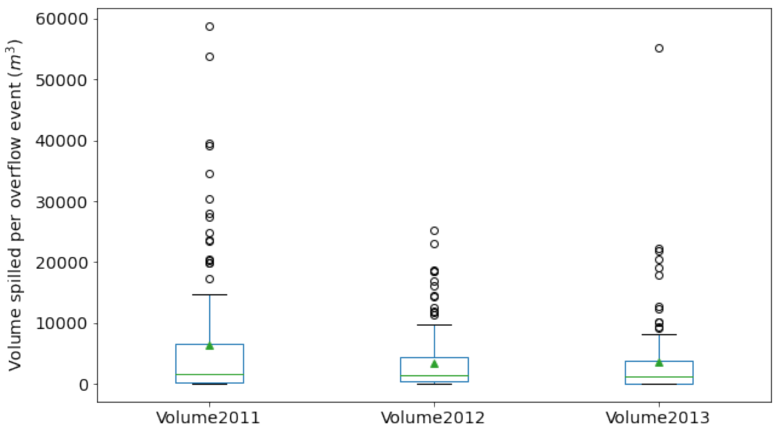

Figure 8.5: Boxplot of the volume spilled during an overflow event for 16 overflows in Eindhoven for 2011, 2012 and 2012. The green line is representing the median value, which is 1.90 m^3 for 2011, 1.53 m^3 for 2012 and 1.33 m^3 for 2013.

8.3.4. Percentage sewage discharged during overflow event

Figure 8.6 elaborates on the percentage of sewage spilled from the system from the total volume of wastewater by an overflow event. The boxplot gives information on the percentage spilled for each overflow event in 2011, 2012 and 2013, with median percentages of 5.0, 3.8 and 3.3 % respectively. The minimum percentage wastewater spilled is 0.012, 0.072 and 0.024 % for 2011, 2012 and 2013 respectively. The maximum percentage wastewater spilled is 82.2, 20.6 and 33.7 % 2011, 2012 and 2013 respectively.

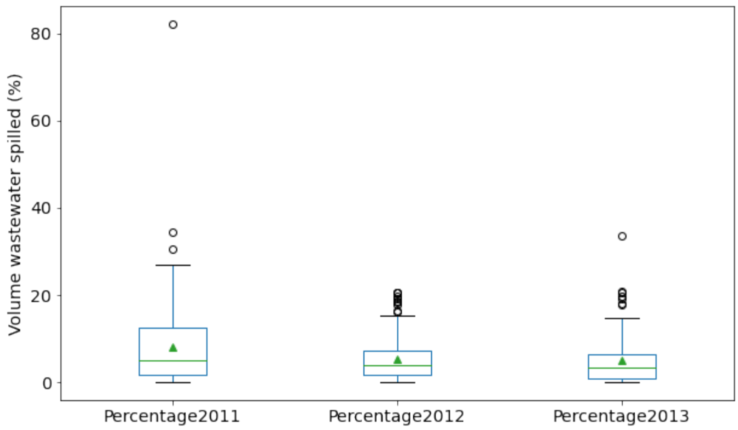

Figure 8.6: Boxplot of the percentage sewage discharged during overflow events of 16 overflows in Eindhoven for 2011, 2012 and 2012. The green line is representing the median value, which is 5.0 m^3 for 2011, 3.8 m^3 for 2012 and 3.3 m^3 for 2013.

8.4. Discussion

The results on the effect of overflows is only based on data of 16 overflows in Eindhoven. This is quite a large city in The Netherlands. Since there are many rural areas or cities of other sizes in The Netherlands, it would be of added value if overflows in different areas were investigated. It is expected that the number of overflow events correlates with the size of paved area. In rural areas where overflows are present, it is expected that overflow events are more scarce. Also, the results are just based on three years of data. More data will ensure exclusion of randomness.

Furthermore, the results do not provide information on what fraction of discharged water is wastewater possibly containing the SARS-CoV-2 virus. The results do not include the volume of precipitation. This makes it difficult to exactly know the effect on the representativeness of the viral load in samples by overflow events.

8.5. Conclusion and input for synthesis

On average seven overflow events in a year are elaborated for 16 overflows in Eindhoven. In 2012 the number of overflows was higher compared to 2011 and 2013, since 2012 was a wet year. For the dry year 2013, the number of overflows was varying greatly between the 16 overflows compared to 2011. In 2011 the annual rainfall was comparable to 2013, but in this year there were very dry and very wet periods.

The duration of an overflow event was 127.5 minutes for 2011, 135.0 minutes for 2012 and 120.0 minutes for 2013. In a year with a higher amount of annual rainfall, the duration of a spillage on average is taking longer. Also, in 2012 there are many outliers, up to 1200 minutes. This suggests that in a wet year there are more extremes in the duration of an overflow event.

The volume sewage discharged during an overflow event is on average 1712.25 m^3 for 2011, 1375.65

m^3 for 2012 and 1192.95 m^3 for 2013. The outliers on the volume show much higher volumes of water that is discharged from the system. The results suggest that in a year with very dry and very wet periods (2011) there are more outliers with large volumes spilled.

The results on the percentage wastewater spilled during overflow event show that there is a great range in the percentage wastewater spilled in respect to the volume of wastewater flowing to the WWTP. The median percentages of wastewater spilled are 5.0, 3.8 and 3.3 % for 2011, 2012 and 2013 respectively. If the discharged water contains wastewater with viral load, on the days with extreme volumes spilled via an overflow, it is possible that a significant amount of information on the virus is discharged from the sewer system, resulting in less representative samples.

9 Automated samplers

9.1. Introduction

Samples analyzed by the RIVM are taken by automated composite samplers. The sampling takes place at all WWTPs in The Netherlands. Sampling is also performed at some pumping stations for purposes other than the NRS. In figure 9.1 in the left picture the outside of the sampler is shown. At some locations it is not possible to place the sampler on guarded area, this does not apply for samplers placed at WWTPs. Then the sampler is placed in a protective cabinet that is vandalism proof. These pictures are taken at pumping station Delft and Leyweg (The Hague), which are not samplers used for the purpose of the NRS. On the right of both pictures it is visible that a tube is placed in between the sampler and the pumping station. When a sample is taken, wastewater flows through this pipe.

Figure 9.1: Automated composite samplers located in Delft (left) and at Leyweg, The Hague (right).

In figure 9.2 the process of taking an aliquot is shown. The samples taken for the NRS are volume-dependent, meaning that a pulse is given to the sampler to take an aliquot if a set amount of volume is measured by the flow meter connected to the sampler. If a pulse is given to the sampler, wastewater is suctioned through the suction tube. The flask in the sampler fills under vacuum, shown in the left picture of figure 9.2. With sensors the volume in the flask is measured. When the flask is filled, wastewater is pumped back again until the volume in the flask is the set volume of an aliquot (often 50 mL). This is shown in the middle picture of figure 9.2.

Lastly, the wastewater flows through the carousel, the black component in the lower part of the sample in the right picture of figure 9.2. The flow through the carousel is often under gravity, but can be pressurized. When there are many particles in the wastewater that can cause blockage of the tubes in the sampler at a certain location, pressurized flow is often instituted. The pictures in figure 9.2 are taken during the sampler's work to determine the aliquots volume. When the 24-hour composite samples are taken there are four containers in the lower part of the sampler, like the ones in the back of the picture.

Figure 9.2: The process of sampling: filling of the flask (left), measurement of an aliquot (middle), flow of the aliquot through the carousel (right). The pictures are taken during the sampler's work to determine the aliquots volume.

In the middle picture of figure 9.2 the volume of the aliquot is measured in the flask of the sampler. The flask is in a vacuum at that time. To ensure that the flask is in vacuum, the tube to the carousel is squeezed shut by an iron rod. This is shown in the left picture of figure 9.3. When the aliquot flows to the carousel, the iron rod slides backwards to open the tube. In the right picture of figure 9.3 this opening of the tube to the carousel is visible.

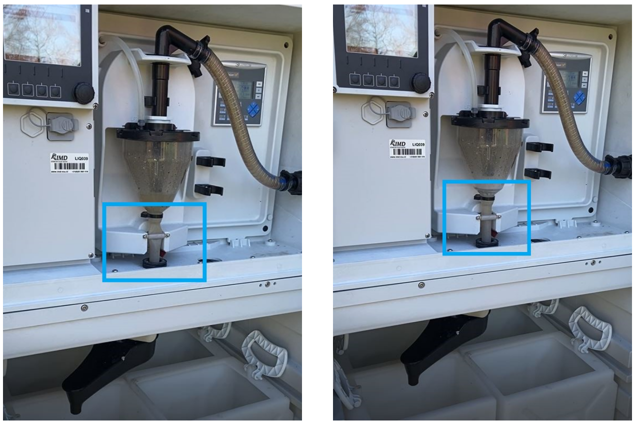

Figure 9.3: Measuring and flowing of an aliquot, with the tube to the carousel squeezed shut by an iron rod (left) and opened again (right).

The equipment used by a sampler to perform the sampling is elaborated in the left picture of figure 9.4. The first step a sampler must take for a good sample is measuring the temperature of the bottle with glycerol in the lower compartment of the sampler. It is assumed that glycerol remains at the same temperature for a longer period of time when the sampler is opened. The next step is to take three aliquots in a graduated cylinder, to check whether a sample may be taken from the sampled. The volume is read and documented separately for the three aliquots. The volume of each of the three aliquots may only vary 5% from the set aliquot volume. The mass of the filled collection tank is measured on the scale.

If the checks are good, then the sampling has gone well and a sample can be taken for analysis. Then, a jar is filled with the wastewater, this is shown in the right picture of figure 9.4. Prior to the filling of the flask, the spoon and funnel are rinsed with the wastewater to avoid contamination. The jar is labelled and stored in a compartment, which is cooled. Then, the collection tank is emptied, cleaned with a paper towel and weighted again to measure the mass of the empty collection tank. The volume collected during 24-hours is determined by subtracting the mass of the empty collection from the mass of the filled collection tank. A density of 1000 kg/m^3 is assumed for wastewater. Lastly, the collected volume is compared with the theoretical volume (the flow divided by the sampling frequency multiplied by the average volume of the three measured aliquots volumes).

Figure 9.4: The equipment needed for taking samples (right) and the filling of a jar with collected wastewater (left).

The standard NEN6600-1 specified requirements for samples, as well as the location of sampling. In section 2.3.2 the requirements are mentioned. If the sample does not meet the requirements or if the sampler does not work (properly), samples are rejected. These samples are not included in the monitoring of the virus. The reason of rejection is mentioned by the sampler. Sampling is done by employees of water labs or WWTPs. During the pandemic samplers are placed at the influent of many WWTPs. Previously, samplers were often not present in the influent of WWTPs. In addition, the location of the samplers, the protocol of sampling, the settings and the requirements are adjusted and improved for some samplers. Despite this, part of the samples is rejected for multiple reasons, with examples discussed in section 2.3.2.

During a visit on location (three pumping stations), three examples of mechanical failures emerged. Since the flows are less continuous at pumping stations than at a WWTP, sampling and failure at these locations could be slightly different compared to WWTPs. Figure 9.5 gives images of these mechanical errors. The notation of the reasons of rejection varies from one water lab or WWTP to another. Errors in sampling can affect the representativeness of samples. Also, (accurate) sampling is essential for the monitoring of the virus. Therefore, this chapter aims to give insight in the type of errors that lead to rejection, as well as the occurrence.

Figure 9.5: Images of mechanical errors during a visit to samplers at three pumping stations. In the left picture the contamination of the suction pipe by wipes is visible. In the middle picture, the angle of the suction hose is adjusted so it could be placed back in the proper position relative to the direction of flow in the pumping station. In the right picture it is shown that the suction pipe, connected to the sampler, was clamped.

9.2. Methodology

Insight in the type of errors according to sampling, as well as the occurrence, is elaborated by data analysis. Firstly, for the period from December 20th 2021 till February 28th 2022 the notated errors by five water labs are analyzed for rejected samples. For some WWTPs multiple influent pipes are sampled. The samples are mixed into one sample, that is analyzed by the RIVM. If an error is occurring for the sampling of one pipe, the samples of total influent pipes are also rejected. Therefore, the data on the rejected samples is corrected for mixed samples. This is done by selecting one of the rejections on that day at the WWTP with mixed samples. The other notated rejections are deleted.

The rejections are sorted per water lab, due to the system in error notations used per water lab. The errors are categorized according to 13 types of errors. This is done manually, since there is no unified notation of errors, also not within data of one water lab. For all water labs the number of occurrence per type of error is determined. This is summed to the total number of occurrence per type of error for the five water labs together. Also, the reasons given for rejection are noted down per type of error. The number of occurrence per type of error has been divided by the total number of errors, to estimate the relative number of occurrence. To give a direct impression of the overarching theme of the errors, the results on the occurrence per type of error are resorted.

One of the requirements in the standard NEN6600-1 is the maximum deviation of 7.5% between the collected volume of wastewater and the theoretical volume of wastewater. This type of error is occurring most often, and therefore more insight into this error is given. The information of one water lab is used. For the same period (from December 20th 2021 till February 28th 2022) the information from the samples that were definitely rejected for the reason of the 7.5% deviation or not meeting the NEN6600-1 criteria was used. The deviation is determined for all rejected samples, using equation 9.1.

$$Deviation\ (\%) = \frac{Measured\ volume\ (L) - Theoretical\ volume\ (L)}{Theoretical\ volume\ (L)} * 100\% \tag{9.1}$$

The days with 7.5% deviation (negative and positive) or more were selected. The dataset used, was again corrected for mixed samples. Lastly, the histogram is plotted with values on negative deviation (20 bins) and positive deviation (50 bins). The mean and median values are plotted with colored vertical lines.

For most water labs it is documented how many samples are planned in a week, accepted and rejected. To see if there are locations were many samples are rejected, this data has been analyzed. It is aimed by the RIVM to receive four samples per WWTP per week for at least 90% of the WWTPs. To fulfill this aim, for most locations sampling is planned for five 24-hours intervals per week. When a sample is rejected, sometimes a new sample is scheduled for that week.

For 177 WWTPs it is determined how many weeks in the period of December 20th 2021 till February 28th 2022 there were not enough samples (<4 accepted) or enough samples (≥4 accepted). For 139 locations only information on how many samples were rejected was available, this is also analyzed. Both datasets were corrected for mixed samples at some WWTPs as described above.

Information on the rejection of samples at locations with mixed samples is determined. It is calculated how much of the rejections approximately come from locations with mixed samples. It is estimated if the number of samples rejected is influenced by the number of influent strains sampled at WWTPs with mixed samples. Based on expert consultation a probability of success of sampling is assumed to be approximately 0.9.

9.3. Results

In table 9.1 the number of occurrence, as well as the percentage of the total number of rejections (1567 rejections for this period) is given for 13 types of errors. An overview on the explanation given by the samplers for the different types of errors is available in Appendix D. Some of the types of errors are related to each other, but the types of errors are separated in this table. 'Too many volume and/or pulses' also gives a greater deviation than 7.5%, but mainly has to do with the overflowing of collection tanks. For urban areas, this type of error is probably not common, due to a maximum difference between of DWF and WWF of 10-15 times. Based on expert consultation this difference can be up to 30-40 times for some sampling locations in rural areas. The DWF is relatively low for these locations compared to WWF on days with large rainfall events. 'Too much volume (others)' mainly have to do with mechanical or technical errors, and sometimes with human errors like communication. 'Maintenance' means maintenance of other things than the sampler, for example maintenance of pumps or the sewer system.

Table 9.1: Overview of 13 types of error messages during the period from December 20th, 2021 to February 28th, 2022. * For one water lab there was no distinction between mechanical and technical errors, therefore the errors are added to the technical errors.

Type of error	Number of occurrences (-)	Percentage (%)
Deviation > 7.5% (NEN6600-1)	633	40.4
Temperature (NEN6600-1)	18	1.1
Not enough volume or pulses (NEN6600-1)	21	1.3
Not meeting the standard NEN6600-1 (others)	51	3.3
Too much volume and/or many pulses	194	12.4
Too much volume (others)	147	9.4
Empty collection tank	199	12.7
Technical error sampler	120	7.7
Mechanical error sampler	35	2.2
Blockage	69	4.4
Maintenance (excluding sampler maintenance)	5	0.3
Pre-announced cancelled sampling	65	4.1
Others	10	0.6
Total	1567	100

It must be noticed that for one lab it was assumed that the notation 'rejection due to not meeting the standard NEN6600-1' meant '7.5% deviation', and therefore included in the number of occurrence for this type of error. The data on this notation was analyzed, and indeed most the rejections were due to the >7.5% deviation. ±6% was rejected due to temperature, no determination of the aliquot volume or no reason. This is adjusted in the results. For one lab it is assumed that the same notation meant the rejection because of 7.5% deviation, due to results of the analysis on the data of the other lab. For one lab there was no distinction in mechanical or technical errors. For that very lab, the rejections related to technical or mechanical errors were added to technical errors.

To have a direct impression of the overarching theme of the errors, the results on the occurrence per type of error are resorted. The assumed overarching themes of errors are: samples do not meet the requirements (NEN6600-1), sampler is not working properly, human errors, blockage and others. The number of rejections per theme are elaborated in figure 9.6. The distinction of the 13 types of errors is:

- Samples do not meet the requirements (NEN6600-1): deviation > 7.5%, temperature, not enough volume or pulses, not meeting the standard NEN6600-1 (others) and too much volume and/or many pulses;
- Sampler is not working properly: too much volume (others), technical - and mechanical error sampler;
- Human errors: empty collection tank and two rejections of too much volume (others);
- Blockage: blockage;
- Others: maintenance, pre-announced cancelled sampling and others.

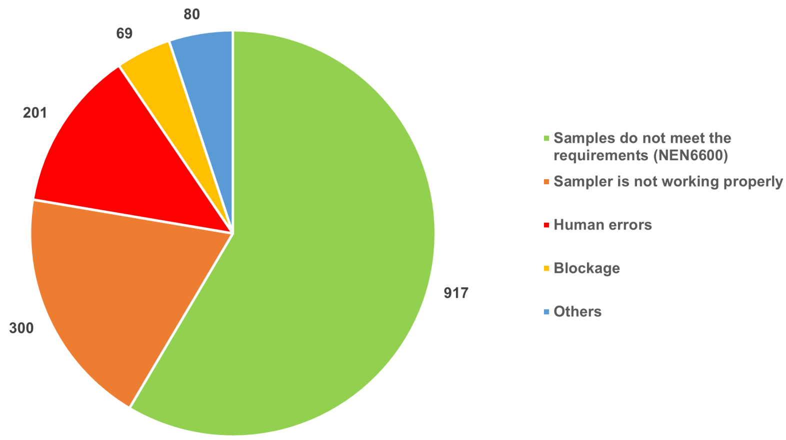

Figure 9.6: Pie chart on five themes, based on the results presented in table 9.1, with the number of rejections per theme outside the parts.

The histogram on 471 rejections of samples by deviation > 7.5% is elaborated in figure 9.7. As a reference, in the same period for this water lab there were 5670 samples accepted and 413 samples rejected due to other reasons. The negative deviation means that less water was collected during sampling than expected, based on the flow. Positive deviation means that there was more volume in the collection tank than expected. It is visible that there are more rejections for the negative deviations compared to the positive deviations. The range is greater for positive deviation, since the volume collected can be over 100% relative to the theoretical volume. When the collection tank is empty, this would give a deviation of -100%. The difference between the median and mean value for rejections is greater for rejected samples due to positive deviation. This supports the greater range in deviation for the positive deviations.

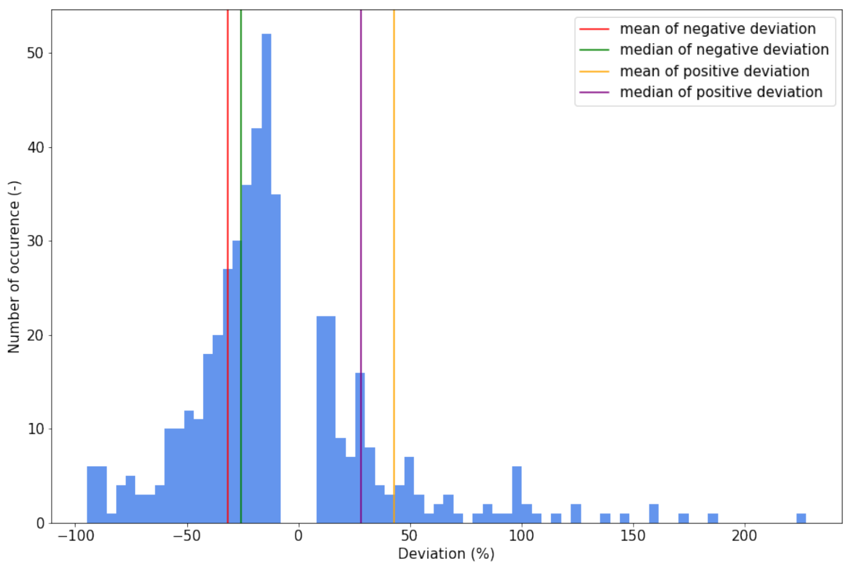

Figure 9.7: Histogram on the deviations >= 7.5% (positive and negative) for December 20th, 2021 to February 28th, 2022 for one water lab. The mean and median values are presented with colored vertical lines.

The histogram clearly shows that most of the rejected samples by the deviation have a deviation much larger than 7.5%. The mean deviation is -31.4% for negative deviations and 43.2% for positive deviations. The median deviation is -25.9% for negative deviations and 28.0% for positive deviations.

If the requirement of the deviation would be changed to 20%, this would result in 296 rejected samples by deviation. This means that if the requirement is changed, 37.2% less samples would be rejected by the requirement on deviation.

Insight in the reliability of receiving correct samples from certain location, based on 177 WWTPs, is given by the stacked diagram in figure 9.8. In ten weeks 9154 samples were taken and 1257 samples were rejected. This means in in ten weeks 13.7% of the samples taken were rejected. The results on the number of weeks a location cannot supply enough samples are subdivided in 0-1 weeks, 2-3 weeks and 4 weeks or more. The NRS aims to accept 90 % of the samples taken. Therefore, it is assumed that not enough samples for 0-1 weeks is good, 2-3 weeks is not sufficient, but could for example be an exception, and 4 or more weeks means that sampling is not sufficient at those WWTPs.

From the diagram it is visible that most of the locations investigated do supply enough correct samples (0-1 weeks). 49 WWTPs perform moderately with respect to sampling (2-3 weeks). 10 of the locations studied (5.6% of total) do not succeed in supplying enough samples more than 6 out of 10 weeks (4+ weeks). These sites have a low performance with respect to sampling and are far below the NRS's target based on the results.

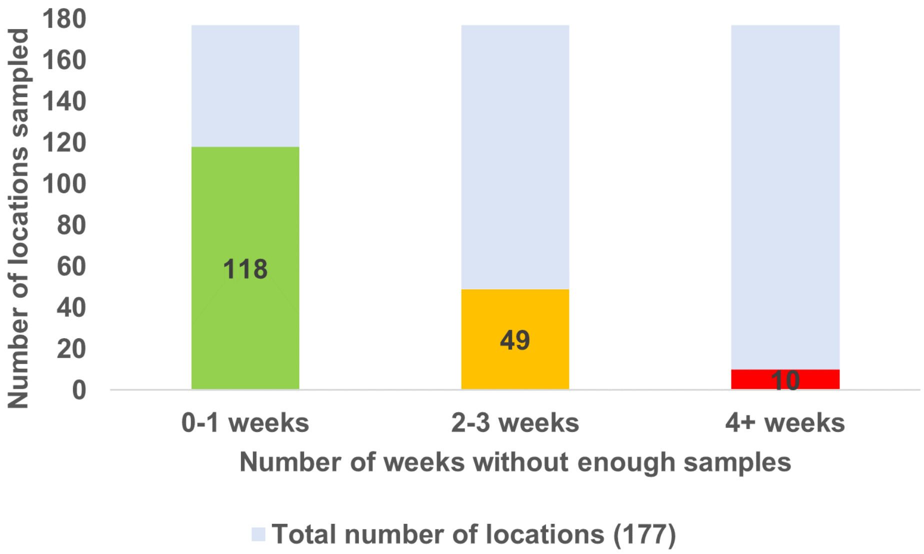

Figure 9.8: Stacked diagram on the reliability of receiving samples from 177 WWTPs for December 20th, 2021 to February 28th, 2022. In total 9154 samples were taken during this period, and 1257 samples rejected.

At last, the mixed samples are analyzed. For 139 locations there were 322 samples rejected in ten weeks, with 122 originated from WWTPs were the samples of different influents are mixed (37.9%). The location have two, three or four influent pipes. A probability of success of sampling is assumed to be 0.9, based on expert consultation. From the data used, there are 12 WWTPs with mixed samples with rejection of samples. Eight of the locations have two influents sampled, three of the locations three influents and one location four influents. The results on the number of samples rejected per number of influent pipe sampled for WWTPs with mixed samples are presented in table 9.2. With more influent pipes, the percentage total influent sample rejected is increasing.

Table 9.2: Number of samples rejected per number of influent pipes sampled for WWTPs with mixed samples, based on data for 10 weeks. For the locations with two or three influents it is assumed that 5 samples are taken per week.

# of influent pipes	# of locations	Mean percentage mixed samples rejected(%)
2	8	16.0
3	3	32.0
4	1	37.7

9.4. Discussion

The results on the occurrence of different rejections of samples is categorized to 13 types of errors. Since the notation of reasons for rejections is not unified, this is done manually. Not all descriptions gave a clear reason of rejection. So for some of the explanations, there has been a personal interpretation. This may have caused a bias in the results. For example, for one water lab the notation of 'deviation from criteria' is assumed to be a a minimum deviation of 7.5% between the theoretical and collected volume. For one lab there was no distinction in mechanical or technical errors. For that lab, the rejections related to technical or mechanical errors were added to technical errors. Furthermore, it is possible that types of errors are missing, and should be added.

Arranging of the types of errors was done manually, this was time consuming work. The results are just based on 10 weeks of data, all in winter. For example high temperatures of samples are not expected during this season. A different conclusion is not expected for more data, but there could be a bias in the results.

The histogram on the 7.5% deviation is based on data of just one water lab and based on the data with a deviation of 7.5% or more. The explanation of rejection that the collection tanks were flooded also means a greater deviation than 7.5%. These results are not included, mainly due to difficulties with determining the exact deviation. The results show that rejections for 7.5% deviation, more often are based on too little volume in the collection tanks, compared to more volume in the collection tank than expected. If the rejected samples for flooded collection tanks are also included, the results be shifting more to the right/positive side of the histogram.

Since the beginning of sampling for SARS-CoV-2 virus until now, there have been improvements in the protocol, education of the samplers and the sample locations. Information on this was not included in this study. No investigation is done on the location of samplers at WWTPs, due to lack of information and the scope of this research. The relation to the water authority, type of samplers and the location of samplers at WWTPs has not been examined. It is difficult to make recommendations on how to improve sampling, because not all required expertise is available and the variation in locations and wastewater origins. Furthermore, there are also external legislations that conflict with possible improvements. For example, in a number of locations, collection tanks often overflow as the WWF is a lot higher than the DWF. However, the placement of larger tanks is not possible, since the lifting of these is not allowed by the dutch working conditions law (Arbo-wet).

9.5. Conclusion and input for synthesis

In conclusion, not meeting the requirements of the standard NEN6600-1 is resulting in most of the rejected samples. The requirement of this standard that samples must have a maximum deviation between the theoretical and collected of 7.5% is the leading. Changing this requirement to, for example, 20% would result in more approved samples, but would affect the representativeness of the samples. The deviation is mostly reasoned by not enough water in the collection tank, compared to the expected volume by the flow. Besides difficulties with meeting the requirements or the performance of the sampler, human errors like communication errors lead to a significant number of rejections. There are many types of errors and the missing unified notation for the reason of rejection makes analysis difficult and time-consuming.

Furthermore, there are some locations that have difficulties with the regularly supply of enough samples. Most of the locations meet the aim of four accepted samples a week. However, some locations do not perform good in respect to the supply of enough correct samples. Some of these locations are improved in 2022.

Finally, the locations where samples are mixed, due to multiple influent pipes, are likely to reject more samples. This makes sense, since the likelihood of errors is increased due to multiple samples that need to be taken. The probability of failure of a mixed sample is increasing by the number of influent pipes. Between three and four strings there is not much difference. The analysis of the individual pipes instead of mixed samples will improve the amount of samples. This would improve the number of samples to monitor the virus load in these areas.

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10
Flow rate measurements

10.1. Introduction

Flow meters are installed in WWTPs to measure the flow. These flow meters are installed at different places in the treatment plant; at the influent, in between treatment steps, and/or at the effluent. Flow meters are electromagnetic, meaning a magnetic field is induced in the meter. A conductor generates an electrical voltage in this magnetic field equal to its velocity. The velocity of the water flowing through the flow meter is determined by the voltage measured. With a known diameter this velocity is converted to the flow. An electrode placed in the top of the flow meter measures if the pipe is completely or partly filled with water.

The automated composite samplers are electrically connected to the flow meters, meaning pulses to take an aliquot are determined by the measured flow rate. Flow meters at several WWTPs do not meet the requirements, for example because valves or pressure sensors are placed too close to the flow meters. Inaccuracies related to the flow meters might affect the representativeness of the samples. Furthermore, equation 10.1 shows that the volume of water in 24 hours (flow) is one of the parameters for normalization. Inaccuracy in the measurements of this parameter will directly affect the load monitored.

$$Load\ (per\ 100{,}000\ inhabitants) = conc.\ (virus\ gene\ copies) * volume\ (24h) * \frac{100{,}000}{number\ of\ inhabitants} \quad (10.1)$$

10.2. Methodology

In this chapter insight in factors that influence the accuracy of the measured flow by flow meters is given. This is explained theoretically. The results were obtained through information provided by the company IMD and conversations with experts. Furthermore, information on the quantitative effect of this factor is elaborated on by information from literature and expert consultation.

10.3. Results

Eight factors of influence on the accuracy of the measured flow by flow meters are discussed in the following sub-sections. These are the location, air in pipes, diameter of pipes valves in combination with DWF, calibration, automated samplers connected to the effluent flow meters, misinterpretation of data and low flow cut off respectively.

10.3.1. Location of flow meters

The structure of every WWTP differs, as well as the location of the flow meters. The influent flow meter for treatment plants is often located at the end of the pressurized pipe or influent pipe under gravity. Some WWTPs have multiple influent pipes. The lengths of the pressurized pipes could differ, and often the pipes contain bends in between the pump and the flow meter. This is not the optimal location, due the risk of incorrectly processing the signals received. Part of the water might flow in the opposite direction to the flow direction, which is defined as water recoil. This water recoil is, for example, caused by leaking valves, due to limited pressure by water to fully close the valves. To obtain the most reliable flow measurements, the location of the flow meters which are close to the pump is most appropriate.

10.3.2. Air in the pipes

The ideal situation for flow measurements is when the pressurized pipes are completely filled with water. Due to bends in the pipes, in the actual situation, the pipes can also be partly filled with air. This makes accurate measurements of the flow difficult. The electrode in the top of the flow meter does measure if the pipe is completely filled with water, but small amounts of air are hard to detect and do influence the flow. With air in the system, there is more resistance in the pipe for example. Also, the conversion from velocity to flow by the diameter of the pipe is affected.

10.3.3. Diameter of pipes

Flow meters measure the flow velocity of water in pipes. The flow velocity is converted to flow by the inner pipe diameter. This inner pipe diameter is not always measured or documented correctly, which could affect the amount of flow measured.

10.3.4. Valves in combination with DWF

Valves are placed at the pumps and ensure no water recoil. The valves depend on the weight that exerts pressure on them. Especially with DWF, on weekends with less industrial water, or during the night, water recoil of wastewater is occurring, due to less pressure on the valves.

10.3.5. Calibration of flow meters

Flow meters should be calibrated once in a while, optimally once every three years for discharge on surface water owned by the water authority and once every five years if the treated wastewater is discharged to National Water. Calibration ensures accurate flow measurements, with a maximum error of 5%. There are different possibilities for calibration. It is preferred to perform in-situ calibration, which means that the calibration is done for the 24-hour flow pattern. Even better are the in-situ calibrations where the pattern for DWF and WWF are included in the calibration, which takes some weeks. IMD performs this by placing the calibrated flow meter in the pipe near the fixed flow meter. The measurements of the calibrated flow meter are stored on a computer, a datalogger. After several weeks of measuring, the measurements of both flow meters are compared and, if necessary, the fixed flow meter is calibrated against the calibrated flow meter.

In-situ calibration is costly and therefore some flow meters are calibrated by a device that squeezes the pipe and measures the flow for 15 minutes. This is three times performed and is also considered as in-situ calibration. But this calibration is not accurate, since it is performed over a very short time, which does not include large variations in flow velocity (i.e. diurnal pattern, DWF and WWF). Other water authorities decide not to calibrate the flow meters, due to the costs and because it is not necessary according to their regulations. Also, it is often assumed that calibration is not needed, because it is believed that the flow measurements are accurate.

10.3.6. Automated samplers connected to the effluent flow meters

Automated samplers take 24-hour composite samplers to monitor the SARS-CoV-2 virus. As preferred, the automated samplers used for the NRS are volume-dependent. The volume that needs to flow into the system to give a pulse to the device is installed based on the DWF and maximum flow. Some WWTPs do not have a flow meter for the influent. The pulses for these devices are installed based on the effluent flow meter. These sampling locations do not provide representative samples, since the flow of the effluent is much more constant compared to the influent. Also, effluent pipes must have enough capacity, and therefore these pipes often have a big diameter. It is more difficult to calibrate these pipes, due to the installation of the calibrated flow meter. This makes the calibration more expensive, as well as that effluent flow meters often are not correctly calibrated, also resulting in less accurate results.

10.3.7. Misinterpretation data

The flow meters at WWTPs are bi-directional, meaning the forward flow and the reversed flow due to water recoil are measured. The bi-directional flow meters have two statuses, 1 for forwarding flow and 0 for reverse flow. The operators of the WWTPs sometimes misinterpret the statuses, the 1 is interpreted as no error and 0 as an error. This misinterpretation leads to incorrect settings for the pulses to the automated sampler. The volumes with status 0 are added up to the volumes with status 1, meaning the total volume is overestimated. Especially on dry weather - and weekend days, since there is less industrial water used on these days. This misinterpretation in the settings of the flow meter can influence the representativeness of the samples.

10.3.8. Low flow cut off

Another setting of the flow meter is the ignorance of noise (low flow cut off) in the volumes measured. It can be installed that the flow meters do not receive volumes under a certain amount (volumes below +/-1% of the total pumping capacity). Due to sloshing and siphoning of water in the influent pipe, less insight can be given into the operation of the flow meter. Low flow cut off is set based on pulses or mA signals. Often the volumes of reversed flows are low flow values and therefore these volumes are ignored due to the low flow cut off. These volumes thus are not registered, which could affect the amount of flow measured. Especially when this setting is based on pulses, an erroneous signal is sent out to the sampler.

10.3.9. Quantitative effect by flow meters on representativeness load monitored

Equation 10.1 shows that flow is one of the parameters in normalization of the virus. Therefore, inaccuracy in the flow measured will directly affect the load monitored. Based on four studies, the research of Ort, Lawrence, Rieckermann, et al. (2010) states that in measuring flow systematic and random errors around 20 % are reported, even in optimal conditions. Based on expert consultation random errors in measuring flow of 5 % at maximum are accepted, due to the accuracy of the flow meters. On DWF days flow measured could deviate from the actual flow around 30 %. This deviation is mainly caused by incorrect settings of the flow meters.

10.4. Discussion

Insight on the possible effect of flow rate measurements is described theoretically and by literature. This research provides knowledge on the possibly factors of influence on the flow measured. Quantitative insight by this study on this factor is limited. Data availability on the quantitative effect of this factor is difficult, since data on the performance of flow meters at WWTPs is not publicly available. Also, the number of studies on the accuracy of flow measurements by the different factors is limited.

This study describes the main factors influencing the measured flow rate by flow meters, based on interviews with experts on flow meters. Nevertheless, it is possible that not all factors affecting the uncertainty of the measured flow rate have been named in this study. Further research will possibly result in other factors affecting the accuracy of flow measured by flow meters.

10.5. Conclusion and input for synthesis

In conclusion, there are multiple factors that could influence the accuracy of the flow measured by flow meters. Since the load of the SARS-CoV-2 virus is normalized to the flow, this parameter is important in representative monitoring of the virus. The measured flow is affected by systematic and random errors. Literature reported errors around 20 % and based on expert consultation it is stated that errors occur up to 30 %.

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11

Synthesis on factors

This chapter performs a synthesis of quantified factors. This synthesis is based on four criteria, which are discussed in chapter 3. The criteria included in this synthesis are the type of error (random or systematic), the quantitative effect on the load of the virus, the occurrence of less- or non-representative samples by the different factors, and whether the influenced representativeness is recognizable.

Firstly, an overview of the different criteria are elaborated in table 11.1. Secondly, based on the results of the different criteria advice is given on which factors should be included in the methodology of monitoring the virus through WBE and which samples should be excluded. Thirdly, it is discussed how factors could be included and what data is needed.

In table 11.1 the results of the synthesis are presented per factor and criteria. The factors 'deviant flow values' and 'automated samplers' are divided into sub-factors. It is elaborated on whether the factor is based on unpredictable errors with results within a certain range, or predictable errors with results with the same proportion for each measurement, random and systematic errors respectively. Some factors are based on both types of errors.

The results on the quantitative effect on the load and the number of occurrences are derived from the results of the chapters on the different factors. The occurrence of less- or non-representative samples by the different factors provides information on the relevance of the inclusion of a factor in the methodology of WBE for the SARS-CoV-2 virus. Also, it is assessed whether the influence of a specific factor on the representativeness is recognizable. This gives information on whether it is possible to ensure that the factor does not affect representativeness. The quantitative effect on the load is assessed in the following manner:

- **0 - 10 %** Negligible effect
- **10 - 30 %** Significant effect, but other criteria has to be considered and prior to inclusion in WBE more research should (possibly) be done
- **30+ %** Significant effect, has to be included in the methodology of WBE or samples should be excluded from monitoring the virus

Table 11.1: Synthesis on factors based on four criteria. **Depending on settings automated sampler. ** It is not known which fraction of the discharged water is wastewater containing viral load. ***These samples are not included in monitoring the virus. **** This frequency is based on sewer pumping station data, not on WWTP data. Also, the occurrence of samples that are not representative due to pumping station failure the day before is not investigated in detail. ***** Random errors occur every day, systematic errors mainly on days with DWF (+/- 235 days a year).*

Factor	Sub-factor	Random or systematic error	Quantitative effect on load	Number of occurrence	Recognizing influence
Location of shedding		Systematic error	20 - 350%	Mainly during holidays	Yes
Temporary in-sewer storage		Systematic error	No relation (negligible)	Irrelevant	No
Decay		Systematic error	0.19 - 8.74%	Every day	No
Deviant flow values	Day with not enough flow	Random error	30 - 37%*	≥1 day a year****	Yes
	Day after failure	Random error	0 - 100+%	1 day a year****	No
Information loss by overflows		Random error	0.012 - 82.2%***	+/- 7 times a year	Yes
Automated samplers	Rejected samples	Random error	Unknown, great range**	13.7 % of samples taken	Yes
	Deviation > 7.5 %	Random error	> 7.5%**	7.7% of samples taken	Yes
	Locations not supplying	Random + systematic errors	Unknown, great range**	20-30% of weeks: 27.7%	Yes
	enough samples	Random + systematic errors	Unknown, great range**	30+% of weeks: 5.7%	Yes
	Mixed samples	Random error	Unknown, great range**	37.9% of rejected samples	Yes
Flow rate measurements		Random + systematic errors	0 - 30%	Every day*****	No

In the following sections, counsel is given on taking into account the factors with a significant effect on the load in the methodology regarding the monitoring of the SARS-CoV-2 virus. Equation 11.1 shows the current normalization as part of monitoring the virus through WBE performed by the RIVM. Based on table 11.1, it is concluded that the effect on the load of SARS-CoV-2 monitored by temporary in-sewer storage is negligible, as well as the effect by in-sewer decay of SARS-CoV-2 RNA by temperature and residence time. These factors are therefore not discussed in the next sections.

$$Load\ (per\ 100,000\ inhabitants) = conc.\ (virus\ gene\ copies) * volume\ (24h) * \frac{100,000}{number\ of\ inhabitants} \quad (11.1)$$

11.1. Number of persons shedding to WWTP

Table 11.1 clarifies that the factor on the number of persons shedding to a WWTP compared to the number of inhabitants by the CBS significantly affects the load, especially during holidays. Based on the magnitude of effect and occurrence, it is definitely recommended to include this factor in the normalization for monitoring of the SARS-CoV-2 virus. It is advised to include this factor for locations with lots of inbound and outbound tourism during holidays. The importance of inclusion is supported by the systematic error related to this factor and the direct impact on the load monitored (see equation 11.1).

The influence of this factor is recognizable by, for example, load N and P. This factor should be included by using a methodology for determining the number of shedders to a WWTP. This research determines the number of shedders to a WWTP by using the methodology based on N and P data. N and P load is measured at each WWTP in The Netherlands. This data is available on Z-info, which is an advantage for this methodology. For some WWTPs it is advised to measure the N and P load more frequently during holidays to increase the reliability of normalization by N and P data in the periods with a lot of variation in the number of persons shedding to a WWTP.

11.2. Deviant flow values

In this synthesis, two types of deviant flow values are included, 1) days with significantly low flow values compared to the expected flow rate, due to failure or maintenance in the system and 2) the day after failures or maintenance in the system. Type 1) might lead to non-representative samples, since information about the load of the virus from part of the produced wastewater is missing. The effect of this factor could be recognized by the daily flow rate. The quantitative effect of this factor depends on the minimum number of pulses to be taken at the WWTP and the pulse volume. The maximum threshold volume of a day with a deviant flow value could be determined based on the location, and also influences the potential quantitative effect. Type 2) could result in non-representative samples, due to wastewater of the day before, that was stored in the sewer system, possibly flowing to the WWTP the day after a failure or maintenance. This affects the representativeness of the samples since information on the viral load of the day before is included in these samples.

Both types of deviant flow values are caused by random errors and occur on a limited basis in a year. The results elaborated in this study show that, a deviant flow value of type 1) occurred once in approximately 15 months for a pumping station, with a quantitative effect between 30% and 37% (based on the criteria used). The effect on the representativeness of type 2) can be 0% to over 100%. This depends on the duration of the outage or maintenance and whether the water that was left in the system had already been pumped at an increased flow rate on the day itself. This type of deviant flow value also occurred once in 15 months for a pumping station. The occurrence of both types of deviant flow values is unknown for WWTPs.

It is advised not to include samples with these types of deviant flow values in virus monitoring when the representativeness is significantly affected. It is recommended to develop an alert system with critical requirements, to detect days with deviant flow values and to ensure that non-representative samples could be rejected. Critical requirements must be established prior to rejecting a sample. Type 1) could be detected by the daily flow, with data available from Z-info. For type 2) it is hard to detect if stuck water of the day before is sampled the next day based on measured flow on the day after maintenance or failure. Flow rates can increase for multiple reasons. Therefore, information on the flow of a day with failures or maintenance, which is again available from Z-info, could be used to determine whether the sample taken on the next day is representative.

11.3. Loss of information by overflows

The results in table 11.1 show that overflow events occur on a limited basis. If an overflow event discharges great amounts of sewage for a longer period, based on the results up to 82.2% of the sewage is possibly spilled. However, not all this sewage is wastewater containing information on the viral load. Knowledge of the fraction of wastewater containing viral load spilled during an overflow event is unknown. Therefore, it is recommended to perform further research on the fraction of wastewater, containing viral load, discharged during an overflow event. If an overflow causes, say, 30% of the wastewater is not sampled, this sample is not representative. Using information about which fraction of the discharged sewage is wastewater containing viral load, it can be determined when the representativeness of a sample is significantly affected. It is advised not to include these samples in the monitoring.

11.4. Automated samplers

The results investigated on automated samplers in this research provide knowledge on the reason for rejected samples. Since this factor is based on rejected samples, it has already been recognized that these samples are less- or non-representative. In addition, insight on the performance of locations in meeting the sampling goal and locations with mixed samples is given. The errors that cause rejections of samples are random and/or systematic errors. The results elaborated in table 11.1 do not give information on the quantitative effect on the load, since the quantitative effect is not determined for the different reasons for rejection. The quantitative effect on the representativeness of the samples varies per reason of rejection. Only the quantitative effect on the deviation between the theoretical volume and actual volume of wastewater collected by samplers is included in the table. Therefore, consultation on this factor is done based on the information and statistics on the rejection of samples.

Based on the results of sampling during ten weeks 13.7% of the taken samples are rejected. The goal of the NRS is to accept 90% of the samples taken. According to the results, this 90% is not achieved. As a consequence, the number of rejected samples should be reduced. Change the requirement on deviation should, however, be considered as a possible solution to reduce the number of rejected samples. The number of samples that have been rejected could be reduced by formulating the criterion differently. For example, accepting a 20% deviation instead of 7.5%, would reduce the number of samples rejected by deviation by approximately 37.2%. Fewer samples rejected would maybe create a more balanced picture. Nevertheless, the extra samples included in monitoring the virus would be less representative, which is not desirable.

Another possibility to reduce the number of rejected samples is to investigate the performance of WWTPs to supply enough correct samples. Based on information about 177 WWTPs (with one location having mixed samples, with a higher probability of failure in sampling), 33.4% of these locations do not succeed in the supply of enough correct samples in most (i.e. about 90%) of the weeks. However, 5.7% of the locations do not provide enough samples for at least 30% of the weeks. Therefore, it is recommended to analyze the cause of the high number of rejections for these locations and make adjustments to these sites in response to the findings. Research could be done into the relationship between certain error messages and the conditions and location of automated samplers. The results could be applied to the settings of automated samplers at WWTPs with the same situation, with the purpose of increasing the number of correct samples.

11.5. Flow rate measurements

Reliable flow measurements are necessary for representative samples and accurate normalization of the SARS-CoV-2 virus. Based on the results of this research, flow rate measurements can affect the representativeness of the load measured up to +/- 30%. This is caused by random, but more important, systematic errors.

Therefore, it is recommended that water authorities carry out proper in-situ calibrations every three years. This ensures detection of influence by this factor, which reduces systematic errors. The settings of the flow meters should also be checked to ensure that the flow is not overestimated and that water recoil is detected. Operators should be aware of the factors discussed in chapter 10, to ensure that they are aware of the system and the accuracy of the flow measurements.

12 Discussion

The results of this research give insight into factors affecting the representativeness of WBE for the monitoring of the SARS-CoV-2 virus. From literature, the possible effect is determined for many factors from loo to lab. Based on the results of the literature review, the seven most important factors with respect to representativeness are quantified. The results of the quantification of the various factors have been synthesized. Although the research adds knowledge to the understanding of factors influencing the accuracy of WBE, there are limitations to this research. The points of discussion per factor are already discussed in the chapters on the different factors. Therefore, this chapter discusses the general limitations to this research, as well as points of discussion on the literature review and the synthesis.

12.1. General limitations

The validation of the results elaborated in this research is limited. The quantification of the factors is based on one or multiple locations, due to the availability of data and time constraints. The methods used come from experts or literature and thus are applicable. As a result, some results might have been obtained by chance or randomness, and would result in a different effect in reality. However, this research aims to get insight into the factors that influence the representativeness. Despite the fact that the quantified influence of the factors may have variance, the results of this study do give a good overview of the possible influence.

Based on the results of the literature review it is concluded that there are many factors that possibly influence the representativeness of samples for the monitoring of the SARS-CoV-2 virus. The seven most important factors with respect to the representativeness are quantified. However, other factors mentioned in the literature review could also affect the representativeness significantly. Due to the background knowledge on in-sewer transportation, sampling and partly the shedding stage, quantification of factors in these stages was elaborated. Other factors are not quantified due to the scope and time constraints of this thesis work.

12.2. Literature review

The literature review on the identification of factors is performed from November 2021 to January 2022. Since the COVID-19 outbreak was quite recent, many researchers are working on obtaining new insights related to the SARS-CoV-2 virus. Therefore, constantly new scientific knowledge is elaborated on the virus and WBE regarding the virus. Potentially new studies have been published recently with insights on certain factors that were not included in the results of this study.

The literature review is based on a selection of papers. As a result, while conducting the literature review, interesting results might already have been excluded. Possibly, some influencing factors are missing in the dendrogram. Mainly about the analysis, there was limited background knowledge, so possibly not all factors regarding this stage have been included in the results.

The literature review states for some factors that the influence on the representativeness of the monitoring of the virus through WBE is limited or negligible. These findings were conducted based on the results of the literature review and the focus of this study (the NRS). For other countries or a different purpose of WBE, these factors could play a role. For other applications, the classification given in the conclusion of the literature review should be revised.

12.3. Synthesis

The results of the quantification of the various factors have been synthesized. This synthesis created an overview of the effect of the various factors and thereby ensured comparison between the different factors. Since the results are based on single locations, datasets, or situations, the results are partially biased. The description of the table on the results shows several comments. If the situation or location is different, the results in the table could be different. However, the recommendations based on the results provide general opportunities for improvement in WBE-based virus monitoring.

The synthesis elaborated the effect of different factors on the methodology of WBE from different perspectives as best as possible. Nevertheless, not all factors mentioned in the literature review that potentially affect the representativeness are included in this synthesis. If more research is done on the quantitative effect of the factors highlighted with orange in the dendrogram, the results on these factors should also be added to the synthesis to give a complete overview. In this thesis work, it was not possible to include these factors, for example, since there is not yet enough knowledge about these factors or the factors are too complex and could fill out a research project on their own. In addition, some of the factors are outside the field of water management or the focus of this thesis work.

13 Conclusion

This report answers the following research question:

"What is the effect of factors influencing the representativeness of the wastewater-based epidemiology for monitoring the SARS-CoV-2 virus in wastewater as part of the national sewage surveillance?"

The conclusion of the literature review (chapter 2) is given in the dendrogram, which points out the relation between the various factors. This dendrogram gives an overview of the factors and the degree of influence on the representativeness of WBE for monitoring the SARS-CoV-2 virus from loo to lab for five stages: 1) virus shedding, 2) in-sewer transportation, 3) sampling and storage 4) analysis of the SARS-CoV-2 virus and 5) back-estimation). Based on the degree of influence and the purpose of the NRS, the factors that were further examined in this study were determined. The effect on the representativeness of the seven most important factors based on the literature review was quantified. These factors are: the location of shedding, temporary in-sewer storage, in-sewer RNA decay, deviant flow values, loss of information due to overflow events, automated samplers and flow rate measurements.

A ynthesis was performed on the quantified factors by four criteria. The results of this synthesis are elaborated in table 13.1. This synthesis created an overview of the effect of the various factors and thereby ensured comparison between the different factors.

Table 13.1: Synthesis on factors based on four criteria. **Depending on settings automated sampler. ** It is not known which fraction of the discharged water is wastewater containing viral load. ***These samples are not included in monitoring the virus. **** This frequency is based on sewer pumping station data, not on WWTP data. Also, the occurrence of samples that are not representative due to pumping station failure the day before is not investigated in detail. ***** Random errors occur every day, systematic errors mainly on days with DWF (+/- 235 days a year).*

Factor	Sub-factor	Random or systematic error	Quantitative effect on load	Number of occurrence	Recognizing influence
Location of shedding		Systematic error	20 - 350%	Mainly during holidays	Yes
Temporary in-sewer storage		Systematic error	No relation (negligible)	Irrelevant	No
Decay		Systematic error	0.19 - 8.74%	Every day	No
Deviant flow values	Day with not enough flow	Random error	30 - 37%*	≥1 day a year****	Yes
	Day after failure	Random error	0 - 100+%	1 day a year****	No
Information loss by overflows		Random error	0.012 - 82.2%***	+/- 7 times a year	Yes
Automated samplers	Rejected samples	Random error	Unknown, great range**	13.7 % of samples taken	Yes
	Deviation > 7.5 %	Random error	> 7.5%**	7.7% of samples taken	Yes
	Locations not supplying	Random + systematic errors	Unknown, great range**	20-30% of weeks: 27.7%	Yes
	enough samples	Random + systematic errors	Unknown, great range**	30+% of weeks: 5.7%	Yes
	Mixed samples	Random error	Unknown, great range**	37.9% of rejected samples	Yes
Flow rate measurements		Random + systematic errors	0 - 30%	Every day*****	No

Using the results in this table, a recommendation was elaborated on for the five factors that had a higher quantitative effect than 10% on the load monitored. These factors are: the location of shedding, deviant flow values, loss of information due to overflow events, automated samplers and flow rate measurements. The synthesis concluded that temporary in-sewer storage and in-sewer RNA decay are negligible factors for the purpose of the NRS to monitor the SARS-CoV-2 virus. The main conclusions on the recommendations given in the synthesis per factor are:

- **Number of persons shedding to a WWTP using N and P data:** It is recommended to include this factor for locations with lots of inbound and outbound tourism during holidays, since the number of persons shedding to a WWTP compared to the number of inhabitants by the CBS significantly affects the load (20% - 350% based on the results of this research).
- **Deviant flow values:** The virus load in samples taken on days with deviant flow values, due to problems in sewer systems or maintenance, could significantly be influenced. As well as the samples taken on the day after (unexpected) problems. Therefore, it is recommended that the samples that are significantly less representative should not be included in the monitoring of the virus and these samples should be detected using an alert system.
- **Loss of information due to overflow events:** Since great amounts of wastewater are possibly discharged during an overflow event, it is recommended not to include samples whose representativeness are significantly affected in the monitoring. To know the effect on the representativeness, more knowledge about the ratio of stormwater and wastewater in discharged water during an overflow event must first be obtained.
- **Automated samplers:** Based on the results, the goal of the NRS to accept 90% of the taken samples, is not achieved. To reduce the number of rejected samples it is recommended to analyze the cause of the high number of rejections for locations that often do not supply enough samples. Possibly adjustments could be performed at these sites in response to the findings.
- **Flow rate measurements:** Flow rate measurements can affect the representativeness of the load measured up to +/- 30%. Therefore, creating awareness of operators for this factor is recommended, as well as in-situ calibration and checking the settings.

To summarize, there are many factors that possibly influence the representativeness of samples for the monitoring of the SARS-CoV-2 virus. This research gives a broad view of the different factors and the relation between them. In addition, the quantitative effect of seven important factors influencing the representativeness of the WBE for monitoring the SARS-CoV-2 virus in wastewater is determined. In conclusion, WBE is a valuable method to use for pandemic preparedness. The results elaborated by this study hope to contribute to the possible early detection of epidemic viruses, allowing government agencies to take appropriate measures early to reduce the number of infections of viruses like COVID-19. Furthermore, this thesis hopes to contribute to the application of WBE for other health indicators detectable from wastewater.

14
Recommendations

The COVID-19 virus poses a threat to human health, health systems and socio-economic systems. This study on the research question “What is the effect of factors influencing the representativeness of the water-based epidemiology for monitoring the SARS-CoV-2 virus in wastewater as part of the national sewage surveillance?” hopes to contribute knowledge on representative monitoring of the SARS-CoV-2 virus through WBE.

Based on the findings of this research and points of discussion, the recommendations are provided for further research. First, some general recommendations, and in addition, recommendations based on the literature review and the synthesis are described and elaborated, followed by recommendations on the various factors discussed in this research. In chapter 11 on the synthesis recommendations on which and how factors should be included in the methodology of monitoring the virus through WBE and which samples should be excluded. The recommendations mentioned in this chapter in combination with the recommendations based on the synthesis could, in the future, contribute to the scientific knowledge on WBE for pandemic preparedness of viruses like COVID-19 or other health indicators present in wastewater.

14.1. General recommendations

To ensure reliable quantification of results for the factors that potentially affect the representativeness of the samples for SARS-CoV-2 monitoring, validation of the results is imperative. This would remove uncertainty and randomness from the results. Examples of validation include obtaining and comparing results for more WWTPs, using more data of, for example, multiple years and comparing multiple methods to each other and to reality.

The dendrogram presented in the conclusion of the literature review shows that there are many factors that possibly influence the representativeness of the samples. Further research would be valuable for understanding the effect of these factors. More knowledge in depth on these factors and to what extent they affect the representativeness would result in a more accurate use of WBE.

This research focuses on the application of WBE in the monitoring of the SARS-CoV-2 virus. WBE can also be used for other purposes. For example, in the past, this method has already been used for detecting drugs. Other health indicators like medication use, other pathogens and obesity could also be monitored by WBE. The team of the NRS is currently working on broadening the focus and application of this method.

14.2. Literature review

Besides describing some factors briefly in the literature, this research does, however, not focus on the lab analysis of the virus. Nevertheless, this is an important part of monitoring and challenging since there is a lot of genetic material in the wastewater. Analysis of samples is complicated by inhibitors, fragmentation, presence of other RNA and DNA in large amounts and multiple virus variants and lineages. This can result in low-quality sequence data. Additional testing with, for example, specific qRT-PCR or ddPCR could reduce challenges in the specific analysis.

To ensure that all recent data is included, an additional literature review could be done. It is likely that new studies have been published which provide more insights into the influence of the various factors or models to simulate the effect. The results of the various studies can be compared, to look critically at the different results.

This study was done from the perspective of the NRS's objective to monitor the virus. If WBE is applied to another focus, the results presented in the dendrogram could be different. It is advised to revise the dendrogram when focused on a different objective. Potentially factors should be added, excluded, or neglected.

14.3. Synthesis

The synthesized results created an overview of the effect of the various factors and thereby ensured comparison between the different factors. To provide a complete overview of the effect of all factors affecting representativeness, it is recommended that more research be conducted. This allows to include the quantitative effect of factors that are currently not studied quantitatively. Results can also be obtained for more locations, for example. Hence, the given results could be better validated. This would add value to the reliability of the results presented in the synthesis.

Furthermore, many recommendations are discussed in the synthesis. It is advised to include these recommendations in the methodology of monitoring the virus through WBE. The recommendations also help with the exclusion of samples that are not representative, and therefore should not be added to the trend to monitor the SARS-CoV-2 virus.

14.4. Number of persons shedding to a WWTP by N and P data

The selection of days with dry weather resulted in less data on N and P in summer for both WWTPs. For further research on this method to determine the location of shedders, it is advised to either use other criteria for dry weather days or select the dry weather days on historical data of, for example, the KNMI. It is also possible to use all data if potential delayed delivery of N and P load at WWTPs is included in the methodology.

Not all results obtained on the location of shedders are included in this research. For some WWTPs the frequency of monitoring N and P load in the wastewater was limited. This was only measured once in several weeks. This complicates reliable normalization with these parameters. If N and P data in the future will be used to normalize to the actual number of shedders, it is advisable to analyze the N and P load in wastewater more frequently, especially during holidays for locations with lots of inbound and outbound tourism.

In this research, the assumption has been made that wastewater at every location has the same load for N and P pppd. The load N and P in wastewater is influenced by industries, which may cause a different load per capita to be used. Examples of industries that contribute to the load N and P in wastewater are meat, milk and dairy industries. Further research on the load of N and P in wastewater at different locations in The Netherlands could be performed. This would possibly result in normalization by different loads of N and P pppd for different locations, if the number of persons shedding to a WWTP would be determined by N and P data.

The results of this research on this factor are based on two locations. As discussed before, data analysis on more locations should be carried out and compared with each other in order to validate the results. Also, it is recommended to use data from multiple years to improve the methodology and eliminate the randomness of results.

Besides the determination of the number of people shedding to a certain sewer system by N and P data, other methodologies, like using data based on the CrAssphage system in combination with flow rate, can be used. Also data outside the sewer, for example, cell phone usage and GPS data could be used. Further research could make a comparison of the results and reliability of these various methods.

14.5. Temporary in-sewer storage of the SARS-CoV-2 virus

Further research is also recommended on other possible influences by sedimentation and interaction with biofilm on the virus load. Although analysis concluded that temporary in-sewer storage of the virus is negligible, it is still possible that viral load will remain in the sewer system by these processes. It is also possible these processes accelerate the breakdown of the virus or that detection becomes more difficult.

The willingness to get tested, the availability of self-tests and the delay between shedding and testing possibly influenced the number of people who tested positive and consequently influenced the results. This could be partially eliminated by, for example, including the number of tests taken, or the number of hospital admissions. Further research is recommended on the influence of these three factors on the number of persons who tested positive. This further research could include looking at the effect of these factors on the relation between the load of the virus and the registered number of persons who tested positive. Based on those results, it could be recommended for later research to include other parameters that give a better picture of the number of infections and the associated load of virus in wastewater.

14.6. In-sewer decay of SARS-CoV-2 RNA

To determine the relationship between temperature and virus degradation with more certainty, it is recommended to use a larger database. If data availability on data is limited, it is advisable to use more lab experiments. This ensures validation of the relation on the one hand and insight into the standard deviation on the other hand.

This research has focused on the relation between virus decay and temperature. Other factors could possibly also influence the degradation of the virus. Further research on the type of factors and to what extent the decay is influenced is highly recommended. In addition, more profound research on the impact of Dissolved Oxygen (DO), the concentration of solids, the pH, the ionic strength, as well as the organic load and potentially other factors on the decay of the SARS-CoV-2 virus is necessary and highly recommended.

The scope of this research is the NRS and hence The Netherlands. It is clear that the various types of sewer systems, types of substances in the wastewater, and temperatures will vary between countries. Therefore, further research is recommended on the decay in other countries. As a matter of fact, this very research has to provide more profound insight into the effects of the before-mentioned factors on the degradation of the virus. A comparison of the decay between different countries or regions within countries would be more than valuable.

Assumptions are made on the flow velocities in different sewer pipes. In addition, it is assumed that the flow is constant through the pipes. It is, however, recommended to use a model, in which more exact flow velocities and variation are included, to determine the residence time. This would give a more precise approximation of residence times.

Based on figure C.4 elaborated in Appendix C, it can be stated that for the City of The Hague drinking water consumption is much higher compared to produced wastewater. In order to be more precise, it would be advisable to bring more digital water meters to households (as users) in place in respect of supply determination. Consequently, the supply of drink water to industries and their production of wastewater could also be examined. This makes it possible that more knowledge can be developed in respect of the use of water as a resource or for watering crops, which does not end up in the sewer, and how much water is used. In addition. areas to supply drink water and the catchment that produces the wastewater could be compared in more detail.

It is highly suggested to use more data and compare results of different locations. Further on, the distance to the WWTP for different points in The Hague could be estimated for more points. The same methodology which has been in Rotterdam could be applied. Consequently, residence time could be determined for different locations throughout The Netherlands. It is also advised to research the residence time for pressurized sewer systems. In order to estimate the average residence time, it is suggested to use data from multiple years, to get results without randomness. Last but not least, it is suggested to put in use the methodology for determining the dry days compared with data on dry days according to the measured rainfall by KNMI.

14.7. Deviant flow values

A critical warning system for deviant flow values is recommended to exclude the samples taken on days with odd values for flow. To exclude those samples from monitoring, it should be certain that the samples on days with a warning for the flow value are not representative. It is suggested to research which criteria should be included in the warning system in more detail. For a complete warning system, it is recommended to investigate all types of deviant flow rates and their quantitative effects. The warning system should be validated by applying it to multiple WWTPs for the objective of the NRS. The warning system should be checked against historical data on failures and maintenance. The warning system should filter out days with deviant flow values, to ensure the rejection of non-representative samples.

The results on the deviant flow values are based on pumping stations, but the NRS focuses on WWTPs. Therefore, it is recommended that results on deviant flow measurements be obtained for WWTPs. Types of deviant flow values, the occurrence and criteria are possibly different between pumping stations and WWTPs. Further research should also be done on the occurrence of deviant flow rates at WWTPs to determine the urgency of a warning system and how many samples should be excluded.

Furthermore, the results elaborated in this research are just based on data on two pumping stations for approximately 15 months. For further research on the types of deviant flow values and criteria to exclude samples that are not representative, it is recommended to use more data. Due to the focus of the NRS, this data should be on multiple WWTPs and it is advised to use data over a longer period.

The discussion on deviant flow values discusses that results in the relation between flow and precipitation could be influenced by local, intense rainfall events. Therefore, for further research on deviant flow values, it is recommended to use data on precipitation of multiple weather stations close to the WWTP to be researched or validate results with this data of closer weather stations.

14.8. Loss of information due to overflow events

Insight in overflows was determined for a limited number of overflows. It is suggested that the results are validated with data from much more overflows. It would also be valuable to use more historical or modeled data. In addition, it would be beneficial to use data and information from other overflows in different areas of The Netherlands, due to the fact that the type of area and/or size of a city is by definition different and useful as a matter of comparison.

Furthermore, it is recommended that more insight will be obtained into the fraction of wastewater (possibly containing viral load) that is discharged during an overflow event.

14.9. Automated samplers

The results on the occurrence of different rejections of samples ordered could be checked by available data on temperature, volumes of aliquots and pulses. Samplers from different water labs could also be interviewed about how they record sample rejection. This could eliminate the bias of arrangement among the before-mentioned 13 errors. Based on these interviews, types of errors could be adjusted if any types are missing or redundant.

It is highly advisable for the various water labs and water authorities to use the same notation for the explanation of the rejection. This makes documenting and analyzing the rejected samples much easier and more reliable. Furthermore, for some water labs and water authorities, the notation is currently done manually. It would be time-saving to use a unified system with the possibility of placing comments.

Since the analysis of the data was mostly done manually, it was quite time-consuming. Therefore, only a limited period of data was used. It is recommended to perform further analysis with more data from different seasons. This would exclude the randomness from the results.

To validate the results of the histogram on the 7.5% deviation more data should be used. I.e. all data on rejections that have a greater deviation should be included. Further research on the deviation for flooded collection tanks would be valuable to understand how much deviation applies to this type of error. It would be beneficial to use data from different water labs and - authorities.

It is recommended to investigate the influence of the number of rejected samples by different adjustments made to samplers, locations of sampling as well as settings. This could result in possible adjustments for the location with difficulties in the supply of samples. Also, checking out locations from which relatively few accepted samples are supplied would be valuable. This could eliminate errors in sampler location and/or settings.

It is strongly suggested to investigate the differences in sampling effectiveness between water authorities and different WWTP’s. For example, it is suggested to develop a method to see immediately which WWTP’s have difficulties with sampling (plotting the number of samples) compared to the number of samples rejected, say once a month. This allows observing whether one water authority or WWTP is doing better than the other. The reason for performing less should be figured out too. It could also be examined whether the problems occur for multiple water authorities and for what reason. If there have been adjustments in the past at certain locations that experienced the same problems, these could be applied to the other sites as well. This would possibly result in fewer sample failures.

14.10. Flow rate measurements

It can be stated that errors in the measured flow rate can significantly affect the representativeness of the monitored load of the virus. Consequently, it is recommended to quantitatively estimate the water recoil on week- and weekend days and dry- and wet days. Since this provides insight into the amount of water flowing back due to water recoil.

Furthermore, it is advised to educate operators on water recoil. This would result in a better understanding and interpretation of flow measurements. It would also provide awareness of the factors that may cause the measured flow rate to not match the actual flow rate. This in turn could ensure that operators are aware of the importance of the calibration and the accuracy of different types of calibrations. Last but not least, it is highly advisable that the settings of the flow measurements should be checked to ensure that the returning water is included correctly and the low flow cut-off does not ignore water recoil.

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A
Number of persons shedding to a WWTP using N and P data

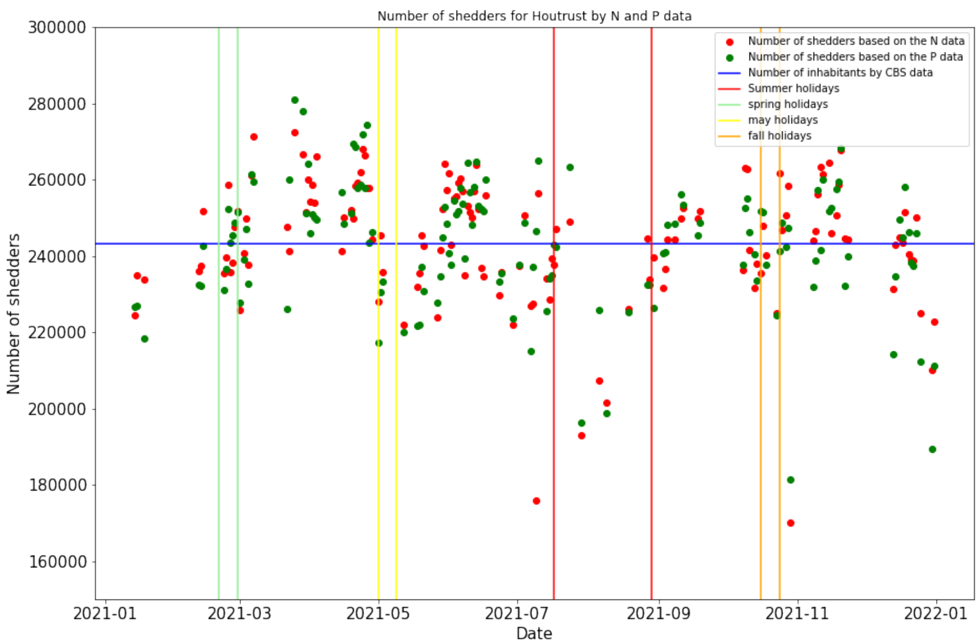

Figure A.1: Number of shedders using data on total N (red dots) and P (green dots) for WWTP Houtrust. The horizontal blue line represents the relative number of inhabitants by the CBS and the colored vertical lines are highlighting the holidays.

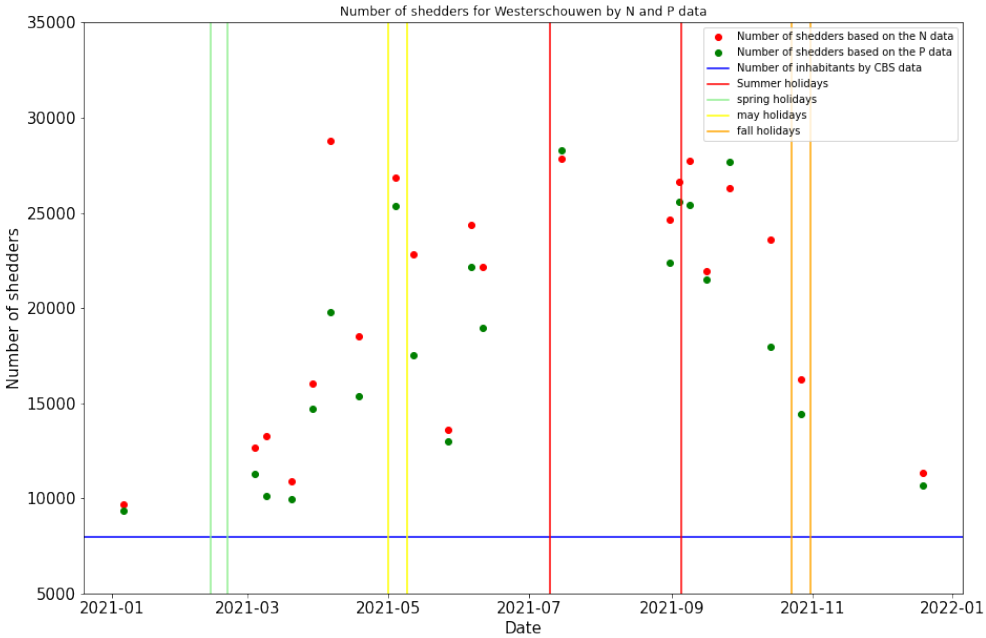

Figure A.2: Number of shedders using data on total N (red dots) and P (green dots) for WWTP Westerschouwen. The horizontal blue line represents the relative number of inhabitants by the CBS and the colored vertical lines are highlighting the holidays.

B

Temporary in-sewer storage of the SARS-CoV-2 virus

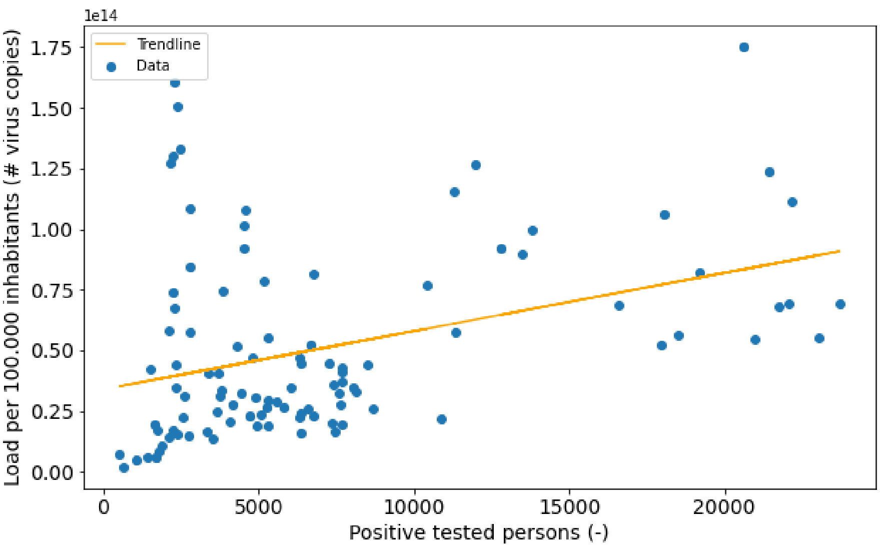

Figure B.1: Normalized load of the SARS-CoV-2 virus plotted against the registered number of infected persons for WWTP Amsterdam West. The $R^2 - value$ for the trendline is 0.14.

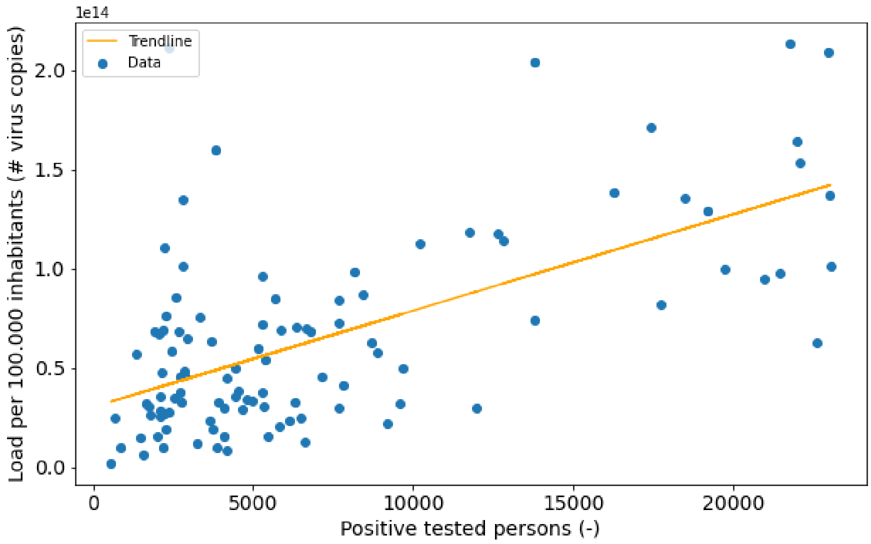

Figure B.2: Normalized load of the SARS-CoV-2 virus plotted against the registered number of infected persons for WWTP Houtrust. The $R^2 - value$ for the trendline is 0.39.

C

In-sewer decay of SARS-CoV-2 RNA

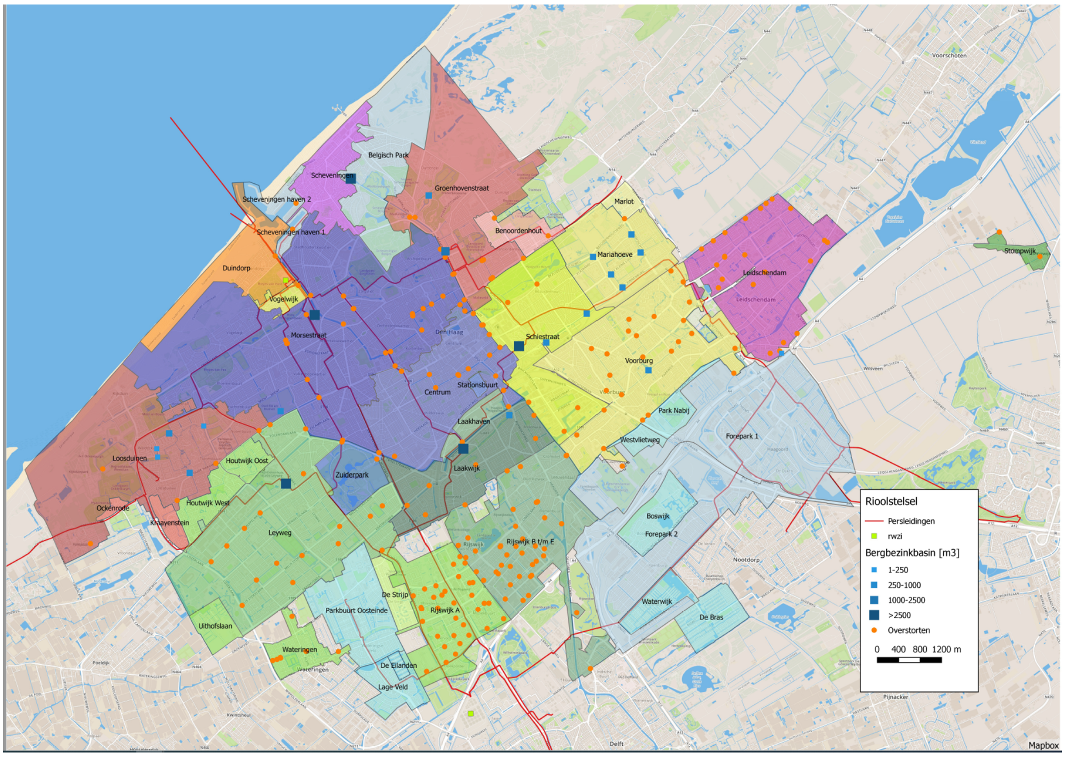

Figure C.1: Overview on structure of pressurized pipes in The Hague to WWTP Houtrust.

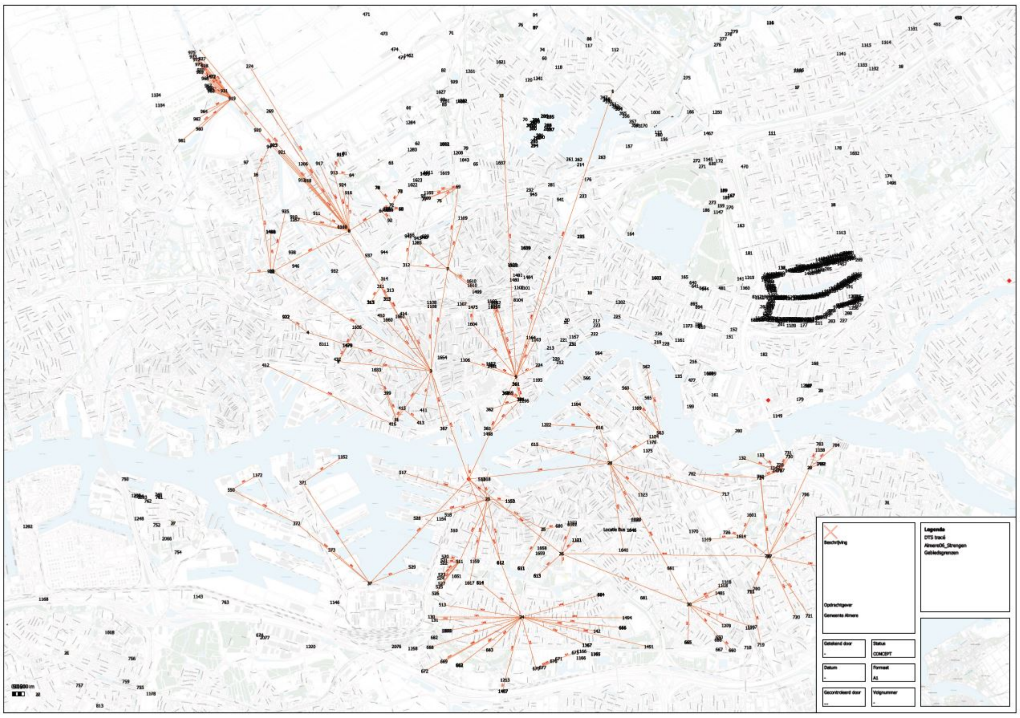

Figure C.2: Overview on Euclidean distances from draining pumping stations to WWTP Dokhaven for Rotterdam.

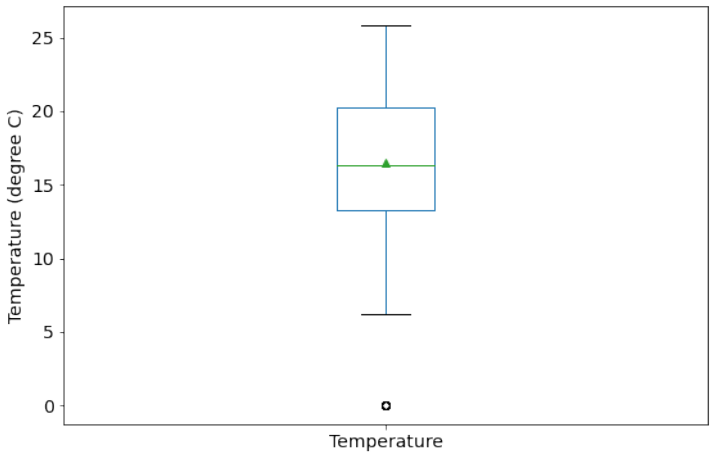

Figure C.3: Boxplot on the data of the temperature of the activated sludge of 20 WWTPs. The green line represents the median temperature, the green triangle the mean temperature.

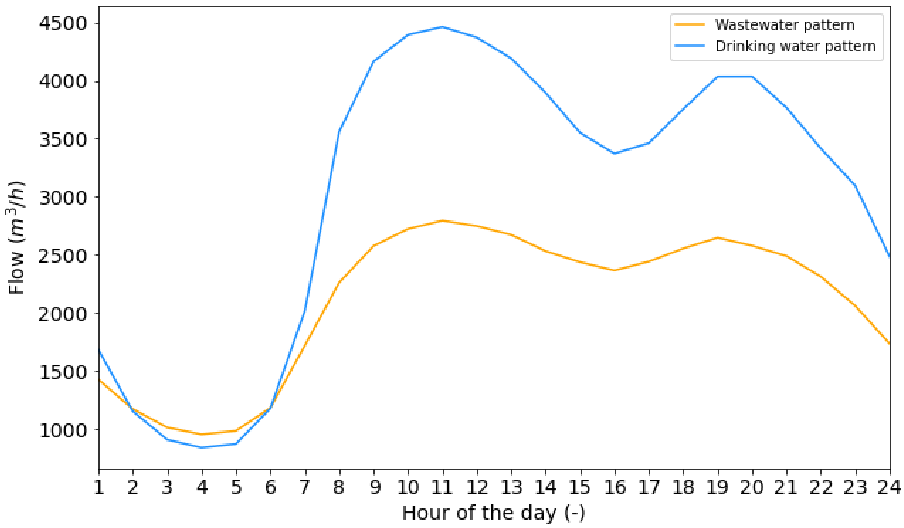

Figure C.4: Drinking water and wastewater patterns for The Hague to estimate the average residence time of wastewater in the sewer system.

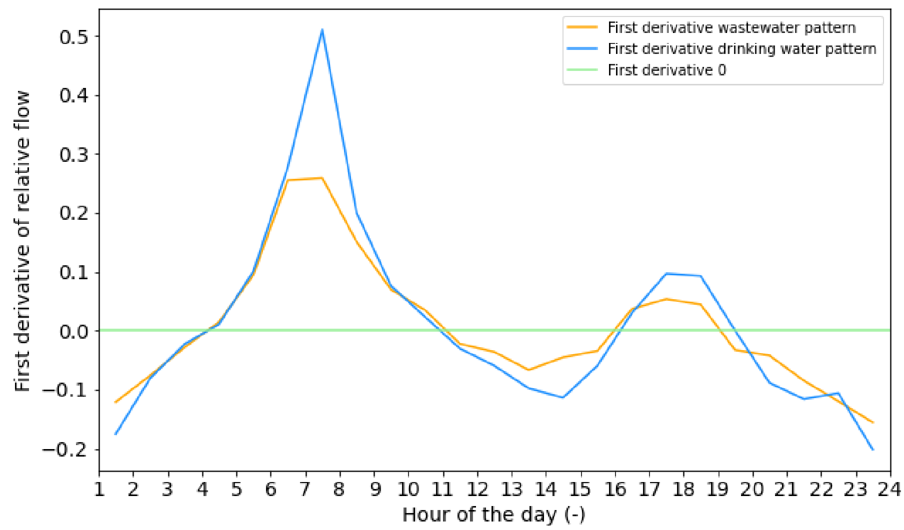

Figure C.5: First derivatives of drinking water and wastewater patterns for The Hague to estimate the average residence time of wastewater in the sewer system.

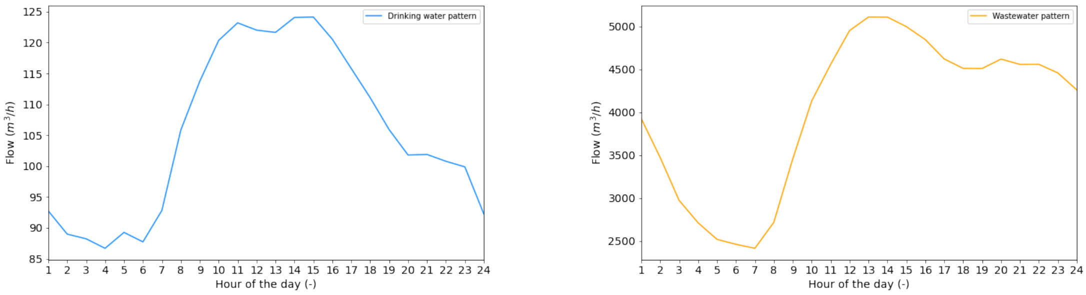

Figure C.6: Drinking water pattern (left) and wastewater pattern (right) for Rotterdam to estimate the average residence time of wastewater in the sewer system.

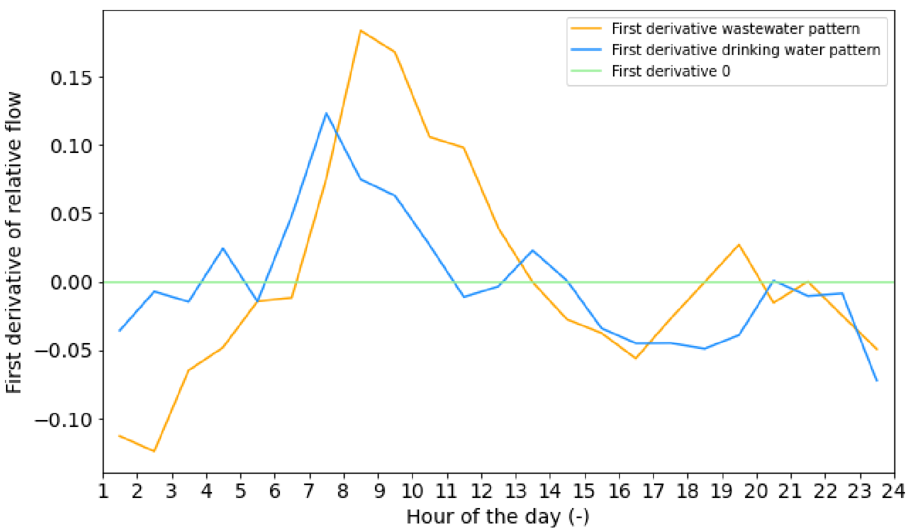

Figure C.7: First derivatives of drinking water and wastewater patterns for Rotterdam to estimate the average residence time of wastewater in the sewer system.

D

Automated samplers

Table D.1: The 13 types of error messages with an overview of the explanations given by the samplers - part 1.

Type of error	**Explanation**
Deviation > 7.5% (NEN6600-1)	Maximum number of pulses reached
	Too many pulses missed
	Not enough water in the collection tank
Temperature (NEN6600-1)	Empty glycerol bottle
	Glycerol bottle filled
	Too high temperature sample
	Too low temperature sample
Not enough volume or pulses (NEN6600-1)	Not enough pulses
	New sampler is not yet working properly
Not meeting the standard NEN6600-1 (others)	No 24-hour composite sample
	Sampler starts sampling too late (8.11 am instead of 8.00 am)
	Mistake from sampler
	Rejection sample without a reason
	Rejection without information
	Sampler is still sampling
	Aliquot cannot be determined
	Sampled wastewater too long in the collection tank
Too much volume and/or many pulses	Collection tank flooded
	Collection tank flooded because of rainfall
	Too much stormwater flow (RWA)
	All collection tanks were overfull
Too much volume (others)	Collection tank flooded due to maintenance of the system
	Difficulties with switching to the next vessel
	Double sampling in the weekend with rainfall
	Collection tank is not emptied
	Two days of sampling in the same tank
Empty collection tank (others)	Unknown reason
	Sampling is not planned / Sampler is not switched on
	Collection tank already emptied
	Still on the option 'taking aliquot manually'
	Effluent is not started
	Error in planning / communication
	Mistake by operator

Table D.2: The 13 types of error messages with an overview of the explanations given by the samplers - part 2.

Type of error	**Explanation**
Technical error sampler	Software control is not working correctly
	Outage (of control system / PLC)
	Error in installation by sampler
	Power failure
	Program stopped (LF 2 active)
	Sampler not properly programmed (new sampler)
	Pump did not work
	Error in software control
	Arm stands above full tank
	Information on number of pulses not available
	Information on flow not available because of outage
	No power on sampler
	Error message device
	Reboot program
Mechanical error sampler	Broken sampler (new sampler)
	Problems with the carousel
	Sampler rejection
	Nipple supply broken off
	Pipe from flask to supply not connected (correctly) / stuck
	Maintenance sampler
	Plunger broken
	No suction
	Wire breakage (indicated by electrode)
	Samplers were not connected correctly after maintenance
	Outage day before
Blockage	Flask outlet clogged
	Wipe in the supply pipe (or other cause of blockage)
	Frost
	Blockage of an other component
Maintenance (excluding sampler maintenance)	Storage of the influent is drained
	Week of other measurements (no sampling)
Pre-announced cancelled sampling	Because of effluent
	Cancelled by operator
	Cancelled due to failure of other sample (mixed samples)
Others	The cabinet around the sampler was locked
	Sampler is not used for sampling anymore
	Wind (fallen sampler)
	Access gate defective
	Wrongly rejected by sampler
	No reason