CHALMERS

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Master of Science Thesis in the Master´s Programme Infrastructure and Environmental Engineering

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Abstract

Removal of organic compound is a major target for municipal wastewater treatment. Typically, most of the removal takes place in the activated sludge process. The treatment efficiency can change during a year and the reasons for worse performance are not always clear. The purpose of this thesis was to investigate spectrophotometry and HPLC as techniques for characterizing the organic compounds in the influent and effluent from the activated sludge process at Rya wastewater treatment plant (WWTP) in Gothenburg. The analysis was carried out on samples collected after primary sedimentation at the inflow to the activated sludge process and the outflow of the plant. During the sampling period, the WWTP was investigating additional precipitation in the primary sedimentation basins as a method for reducing the organics load to the activated sludge process and thereby improve the effluent quality.

The use of spectrophotometry as a tool to monitor the organic compound in the wastewater could be a cost effective alternative to other methods. In this study, spectrophotometry was used to estimate the total organic carbon (TOC) concentration in both the inflow and the outflow to the Rya wastewater treatment plant (WWTP) in Gothenburg. The a254 (absorbance at 254 nm) parameter and spectral slopes S275-295 were shown useful in estimating TOC concentrations. Correlations, r^2, of 0.84 and 0.90 were reached for a254 and S275-295 respectively. High pressure liquid chromatography showed that humic acid-like compounds were dominating in the effluent from plant. The results even indicate a net production of such compounds in the plant.

Further on, the author suggests that additional studies using more samples taken during similar conditions should be made to more accurately access the changes in the organic compounds.

Key words

Spectrophotometry, wastewater, humic acid, organic compound, high pressure liquid chromatography

List of abbreviations

BOD   -   biochemical oxygen demand

COD   -   chemical oxygen demand

DOC   -   dissolved organic compound

HPLC   -   high pressure liquid chromatography

POC   -   particulate organic carbon

TOC   -   total organic compound

UV   -   ultraviolet

Vis   -   visible

WWTP   -   wastewater treatment plant

Content

1 Introduction

Modern society provides a number of services that makes life for its inhabitants more convenient and comfortable. However, as a result it also generates waste that can be harmful to health and nature if not treated. One such waste is waste water. In the city of Gothenburg, the primary waste water treatment plant (WWTP) is the Rya WWTP, which is owned and operated by Gryaab, a company owned by Gothenburg and the surrounding municipalities. Gryaab is responsible for ensuring that the waste water effluent meets the criteria with regards to BOD, total phosphorous and nitrogen. Over the past years, Gryaab has successfully met the criteria on an annual basis but occasionally failed the BOD requirements on a monthly basis. Waste water treatment efficiency is governed by a number of variables. BOD is primarily removed by biological degradation in the activated sludge process. The capacity of the activated sludge to degrade the incoming BOD load, the composition of the organic compounds and release of organics by the sludge are things that might influence. BOD is a bulk parameter used to describe the concentration of biodegradable organic compounds in the waste water. To examine how different mechanisms affect the BOD concentration in the effluent, more detailed investigations of the organic constituents that make up the BOD are needed.

1.1 Purpose

The purpose of this report is to investigate if data from liquid chromatography and spectrophotometry can be used to characterize the organic compounds in the wastewater. The analyzed samples were collected from the Rya WWTP during a time period when chemical precipitation of the waste water in the primary sedimentation basins was tested as a method to reduce the organic load to the activated sludge process. Organic compounds in samples of pre-settled wastewater going into the activated sludge process and treated effluent going out from the plant were analyzed during periods with and without additional precipitation.

1.2 Scope

This report will give a brief description of the waste water treatment process in a WWTP, focusing on the treatment of organic compounds. The analysis methods high pressure liquid chromatography (HPLC) and spectrophotometry will be explained. Samples from the Rya WWTP in Gothenburg were analyzed and served as input data. This data will be compared to results from related studies and used as a foundation to determine what information the methods give about organic compounds in the wastewater.

2   Literature review

2.1   Wastewater

The composition and flow of wastewater to a treatment plant can vary significantly over time, both short term over the day and long term over the year. This is due to the nature of how waste water is generated. Daily variations of flow are mainly a result of behavior patterns of the population connected to the system. For example, mornings are often characterized by higher flows because of morning activities such as showering. Over the year, heavy rain falls or snow melting can cause higher flows [1].

The content of the wastewater can be divided into the categories micro-organisms, biodegradable organic materials, other organic materials, nutrients, metals, other inorganic materials, thermal effects, odor and radioactivity. Depending on the category, different types of treatment is needed to reduce the concentration to acceptable levels. Municipal WWTPs are typically designed to remove organic material and nutrients [1].

2.2   Wastewater treatment plant

To treat the waste water, it is transported, usually through a sewage system, to a WWTP. The Rya WWTP is the name of the primary wastewater treatment plan in the city of Gothenburg. The wastewater coming to the Rya WWTP is sent through a treatment that roughly can be divided into three steps, mechanical treatment, chemical treatment and biological treatment [2].

2.2.1   Mechanical treatment

The first step for the wastewater is the mechanical treatment. This is where the solids are removed. A coarse screen followed by a fine screen removes solids such as garbage and paper. Gravel and sand is removed in the sand trap. The wastewater is then sent through primary sedimentation tanks where low velocity allows solid particles, heavier than water, to sink to the bottom. The end of the mechanical treatment is a filter where very small particles are removed.

2.2.2   Chemical treatment

The second step, the chemical treatment, is primarily used to remove the nutrient phosphorus. Iron sulphate is added as coagulant to create an iron-phosphorus precipitation. The iron-phosphorus is then flocculated, attached to the biological sludge, and removed through sedimentation.

2.2.3   Biological treatment

The third step is where organic material and nitrogen is removed by biological treatment. An activated sludge process digests the organic material by bacteria in biological sludge. This is done simultaneously with the precipitation of phosphorus in the chemical treatment. The activated sludge tanks consist of an anoxic part (non-aerated, absence of oxygen) and aerobic part (aerated). In the anoxic part of the tanks, absence of oxygen forces the bacteria to use nitrate as electron acceptor instead. By doing so, the nitrate is converted to atmospheric nitrogen and released in the air. The nitrate is produced as a second part of the nitrogen removal process, called nitrification. Nitrification is done in bio films where bacteria use oxygen to convert ammonium to nitrite and then the nitrite to nitrate. In the aerobic part of the tanks, the remaining organic material is supposed to be oxidized aerobically.

2.2.4 Waste water treatment overview

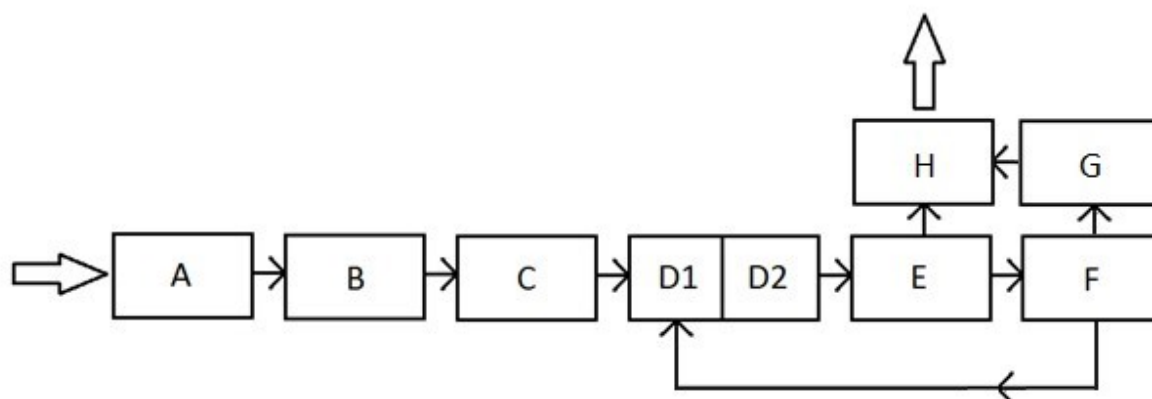A schematic of the Rya WWTP is shown in Figure 1.

- A: Screen, large solids are removed
- B: Sand trap, sand and gravel are removed
- C: Primary sedimentation basins, particles heavier than water are removed. Chemicals can be added to enhance treatment
- D1: Activated sludge anoxic, nitrate is removed
- D2: Activated sludge aerobic, organic carbon is removed
- E: Clarifier, sludge settles and is pumped back to D1
- F: Trickling filters, ammonium is converted to nitrite and pumped back to D1
- G: Post denitrification, nitrate is removed
- H: Disc filters, remaining particles are removed

2.2.5 Effluent requirements

The current effluent requirements to be reached by Gryaab were set by Länsstyrelsen 2011. Table 1 shows annual effluent requirements.

Table 1 - Annual effluent requirements

Phosphorous total	Nitrogen total	Organic material (BOD)
0.4 mg/l	10 mg/l	10 mg/l

Over the years, Gryaab has managed to keep the levels in the effluent within the limits of the requirements on an annual basis. However, on a monthly basis, the level of BOD has during some occasions exceeded 10 mg/l. It is likely that the requirements will be sharpened, demanding lower levels of nitrogen in the WWTP effluent than of today [3].

2.2.6 Treatment efficiency variations

There are a number of factors that can affect the degree to which the wastewater treatment process manages to treat the wastewater before it is being released to the recipient. Variations in flow and concentrations of pollutants require a flexible system that can adapt to the current situation. High

flows during exceptional rain falls can for example be handled by increasing the amount of chemicals added early in the treatment process and allowing excess flow to bypass the biological treatment [2].

The biological treatment process relies on bacteria which efficiency is affected by temperature [4]. The effluent of Rya WWTP does meet the requirement on an annual basis. However, during the winter months, the required level of BOD has been exceeded. The actual reasons for the lowered treatment efficiency during those months are unknown. While future requirements are expected to limit allowed levels even further, it is of high importance to identify the causing factors in order to increase the efficiency of the treatment.

There can be several reasons for treatment efficiency variations. The activity of the sludge can vary, which may cause insufficient oxidation of the incoming organics. The activated sludge also release organic compounds called soluble microbial products, which in previous studies has been found to be the major fraction of the organic compounds in the effluent from biological treatment systems [5]. Toxicity of the influent wastewater could also affect the treatment efficiency. Toxic chemicals are not supposed to be treated through the ordinary wastewater treatment system. However, accidents and illicit disposal of industrial waste can result in toxic chemicals reaching the wastewater treatment plant. An extreme result of such event could be that the bacteria in the biological treatment process are killed, leaving the biological treatment step out of service. Warning systems are in place to avoid that kind of situations.

Small concentrations of substances that might affect the treatment efficiency are harder to detect. If a substance of any kind would have an impact on the biologic process, the organic material would likely also be affected. There are a number of approaches for analyzing fluids in order to characterize the organic compounds. Two of them are ultraviolet-visible spectrophotometry, UV-Vis, and high performance liquid chromatography, HPLC.

2.3   Organic compounds
The wastewater contains a variety of organic compound before and after it is treated. This chapter will describe some of the components of the organic matter and how it is measured.

2.3.1   TOC
The total concentration of organic carbon is called TOC and is measured in milligrams per liter. This can be divided into two groups, representing the two different types of organic carbon. The first type is particulate organic carbon (POC). POC is defined as organic carbon with particles too big to pass through a filter. The common filters are generally within the range 0.22 and 0.7 μm [6]. The second type is the dissolved organic carbon (DOC). DOC is conversely defined as the organic carbon particles that do pass through filter. This definition is not absolute. However, in many studies, a 0.45 μm filter is used to separate POC from DOC [7]. Further on in this report, TOC will be used exclusively throughout the work as a measurement for organic carbon. However, since all used samples are filtered, the proportion of POC is negligible.

2.3.2   Humic acids
Humic acid is an organic compound that can be found naturally as it is created by the microbial degradation of dead plant matter. Humic acid can have an impact on the possibility to treat water. The structure of the humic acid molecules make them susceptible to commonly used chemicals which in combination can create toxic substances. The molecular structure of the humic acid is often

based on combinations of molecular groups where aromatic formations of carbon are grouped together, meaning that the carbon atoms are connected in ring shapes. The mixture of different types of humic acid makes it hard to understand and there are a number of theories explaining it [11].

2.3.3 Absorptivity of organic compounds

The measured UV-light absorbance at certain wavelength, divided by the TOC concentration represents the specific UV absorbance (SUVA). Depending on the molecular structure of the organic compound, the level of absorptivity of UV light differs. The level of absorptivity also differs depending on the wavelength. Different molecular structures have different levels of absorptivity at different wavelengths. Aromatic molecules generally have greatest absorptivity between 200 and 380 nm [8], where molecules with lower weight show higher absorptivity at wavelengths below 240 nm. However, determining a proper wavelength for analyzing the concentration of organic compound of a fluid is not only done by the absorptivity properties of the organic compounds themselves, but also the absorptivity properties of other substances that may also be found in the fluid. If a certain wavelength corresponds well to both organic compound and to an unknown substance, the resulting level of absorbance may be falsely interpreted.

Studies have been done on different factors that may have an impact on the absorptivity in the range of wavelength that would be of interest to analyze organic compounds. The pH of the fluid can affect the UV absorbance. However, pH levels ranging between 2 and 8.6 showed negligible changes in absorptivity [8]. Iron ions, both ferrous (Fe^{2+}) and ferric (Fe^{3+}), can at certain concentration generate considerable absorbance within the same range as aromatic molecules. Iron ions can be present in wastewater, both as a result of the content of the inflow and from the chemicals added during the treatment process. Additionally, nitrate (NO_3^-), which is a present in wastewater, has a maximum absorption at a wavelength of 210 nm. At concentrations higher than 40 mg/L, nitrate can affect the absorption up to a wavelength of 280 nm. However, the level of total nitrogen in the influent to the wastewater treatment plant is seldom higher than 25 mg/L [8].

Considering the risk of interfering substances when choosing an appropriate wavelength for analyzing the organic compound, the SUVA at the wavelength of 254 nm, called $SUVA_{254}$, is known to be a good indicator of the humic fraction of the TOC. In fact, studies have shown correlation as strong as $r^2 > 0.97$ between the $SUVA_{254}$ and the concentration of humic substances in fluid [9]. $SUVA_{254}$ is therefore also commonly used to monitor water quality in natural waters regarding natural organic matter (NOM). The previously mentioned possibly interfering factors, iron ions and nitrate, can be considered to have low impact on the resulting absorbance.

3 Methods

This chapter describes the methods used for analysis and the theory behind them.

3.1 Additional precipitation at Rya WWTP

Rya WWTP has a history of exceeding the required effluent levels of BOD7 on a monthly basis during cold months. To avoid this and to evaluate methods for increasing the efficiency of the BOD7 reduction, a full scale test of adding aluminum chloride to achieve additional precipitation in the primary sedimentation basins was carried out. The purpose was to reduce the load on the biological treatment process and thereby reaching lower levels of BOD7 in the WWTP effluent. This additional precipitation was made between January 19th and February 2th. Out of 12 parallel primary sedimentation basins, addition of aluminum chloride was applied in four.

3.2 Sample collection

The samples used in this analysis were collected 18 times at Rya WWTP between January 7th and February 13th 2015. Each sample was collected using flow-proportional sampling for 24 hours. Samples were collected at two points. "Inflow" samples were taken from the effluent of the primary sedimentation basins. "Outflow" samples were taken from the effluent of the plant. All samples were filtered and put in plastic containers and transported to Chalmers where they were frozen until analyzed. Each plastic container was carefully marked with the date the sample was collected and from which treatment step it was collected. In total, 36 samples were collected in pairs during 18 days. Out of the 18 days of sample collection, six days were during the time Rya WWTP had additional precipitation in the primary sedimentation basins, meaning twelve samples were taken during the time of additional precipitation.

3.3 Analysis

The samples were analyzed at Chalmers after defrosting. The plastic containers were put in warm water to decrease the time needed for defrosting. Each sample was carefully filtered manually through a 0.45 μm filter and then put in clean glass containers intended for the analysis equipment. The samples were filtered using syringe with attached syringe filter. This means that the filtration was made under pressure. Between each filtered sample, the syringe was cleaned using distillated water and pre-washed with the following sample. The TOC-concentrations of the filtered samples were analyzed using a TOC analyzer (TOC-V, Shimdazu). All samples were also analyzed both using UV-vis and HPLC. In the HPLC, detection was done at wavelengths 254 nm and 215 nm. In addition to the sample analysis, a separate HPLC analysis of pure humic acid dissolved in water was made for reference.

Figure 2 shows the samples in plastic containers from Rya WWTP.

Figure 2 - Photo of samples

3.4 Absorptivity based analysis methods

The samples were analyzed using two different analysis methods, UV-Vis spectrophotometry and HPLC. Each method will be described in this chapter.

3.4.1 UV-Vis spectrophotometry

A spectrophotometer is used to measure the absorbance spectra of a fluid. The method for using absorbance spectra is based on the Beer-Lambert Law in equation 1.

$$A = \varepsilon \cdot b \cdot c \qquad [1]$$

where A is the absorbance, ε is the molar absorptivity, b is the path length and c is the concentration of the compound in the solution. The path length is 10 mm in the spectrophotometer used.

A sample is put in a container and exposed to a beam of light. A receiver measures the remaining light, determining the transmittance of the sample. The transmittance is defined in equation 2.

$$T = \frac{P}{P_0} \qquad [2]$$

where T is the transmittance, P_0 is the radiant power of the beam directed at the sample and P is the radiant power of the beam leaving the sample.

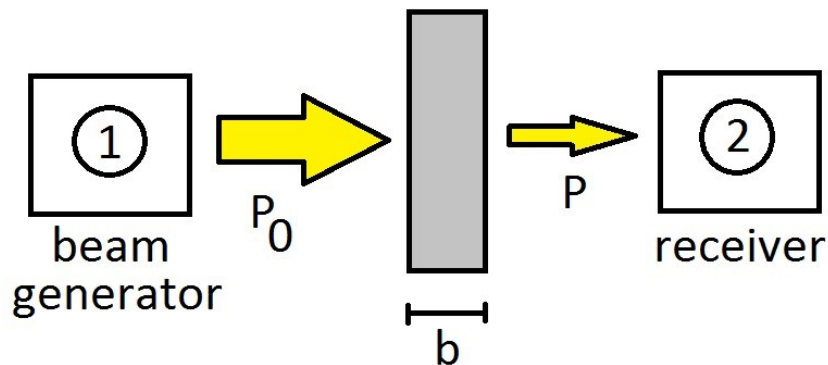Figure 3 shows the process of measuring the transmission.

Figure 3 - UV-Vis system

The transmittance is used to calculate the absorbance. The relationship between absorbance and transmittance is defined in equation 3.

$$A = \log_{10}\left(\frac{1}{T}\right) \qquad [3]$$

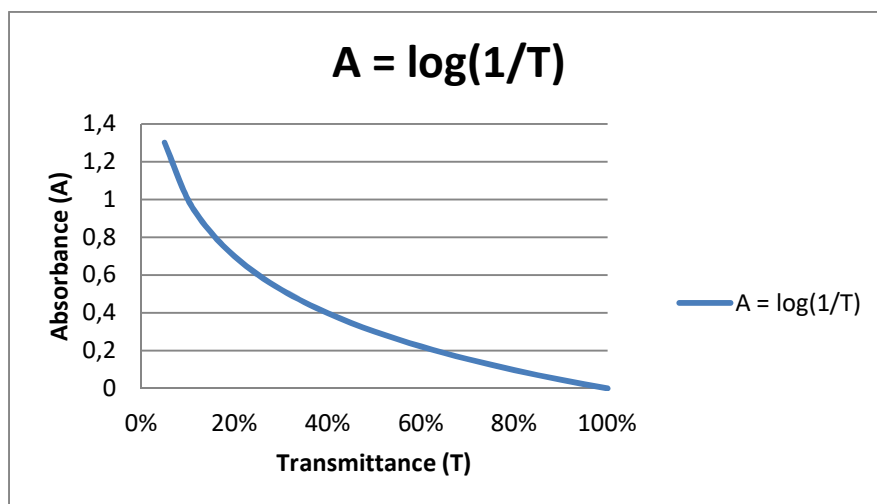Plotted on a graph, the relationship can be seen in Figure 4.

Figure 4 - Relationship between transmittance and absorbance

As can be seen from Beer-Lambert's law, the absorbance is linearly proportional to the concentration. For a compound with a known molar absorptivity at a certain wavelength, it should be possible to determine the concentration in a fluid. However, when used on a fluid containing multiple compounds, the resulting absorptivity for the wavelength of compound in question might be affected by the other compounds. The electronic structures of the exposed determine the level of absorbance. The samples in this study are analyzed using wavelengths between 200 nm and 800 nm using a Shimadzu UV-1800 spectrophotometer.

In this study, four parameters obtained from UV-Vis are used for comparison with the measured TOC concentration and for evaluating changes in the composition of the TOC.

1. a215, the absorbance at a wavelength of 215 nm
2. a254, the absorbance at a wavelength of 254 nm
3. S275-295, the slope of the absorbance curve between wavelength of 275 nm and 295 nm
4. S350-400, the slope of the absorbance curve between wavelength of 350 nm and 400 nm

$SUVA_{215}$ and $SUVA_{254}$ refer to the specific UV absorbance at wavelength of 215 nm and 254 nm, respectively, and are calculated by dividing the absorbance values by the TOC concentration in the sample.

The slopes, S275-295 and S350-400, are calculated by using the value of the linear slope between the points chosen. Both slopes have been tested in an earlier study and S275-295 showed good correlations with the dissolved organic matter [9]. For example the slope S275-295 is calculated as in equation 4.

$$S275\text{-}295 = \frac{a275 - a295}{295 - 275} \qquad [4]$$

3.4.2 HPLC

High pressure liquid chromatography is a method of identifying different compounds in a solvent based on different properties of the molecules. In this study, size-exclusion chromatography (SEC) was used, which means that the molecules were separated based on size. When making a HPLC-analysis, a sample is placed in the HPLC-instrument. A solvent (called eluent) is then mixed with the sample and sent through a column containing small porous particles, in general with a diameter of 3-5 μm. The porosity of the particles leads the molecules as the eluent is pressed through the column. Smaller molecules are led deeper in to the porous system and will therefore have a longer retention time in the column while larger molecules will pass through faster. Figure 5 below describes how the molecules are led through the porous system depending on their size.

After passing through the porous system, the eluent continues through a beam of light. The absorpivity is then measured as described in UV-Vis. However, the difference compared to UV-Vis, where the absorptivities at different wavelengths are measured, the beam is preset to certain wavelengths. The absorptivity at those wavelengths is then measured continuously during the time the eluent is passing through the beam.

Figure 5 shows an HPLC-system in three steps. The first step, marked 1, is where a pump retrieves the sample and injects it into the eluent. The pump sends the eluent mixed sample further to the column (i.e. a tube with a porous filter), marked 2. The red star represents a big particle and the blue star represents a smaller particle. The red arrow shows the path of the big particle and the blue arrow shows the path for the small particle. As the figure illustrates, the small particle is led deeper in to the pores than the big particle, giving the small particle a longer path through the filter. The third part, marked 3, is where the absorptivity is measured. In this study, the eluent contained 5844 mg/L NaCl, 1129 mg/l KH_2PO_4 and 2036 mg/l K_2HPO_4. Two Agilent Bio SEC-5 columns (100Å and 500Å, 30 cm long) were connected in series after a 300Å guard column. The flow rate was 0.5 ml/min
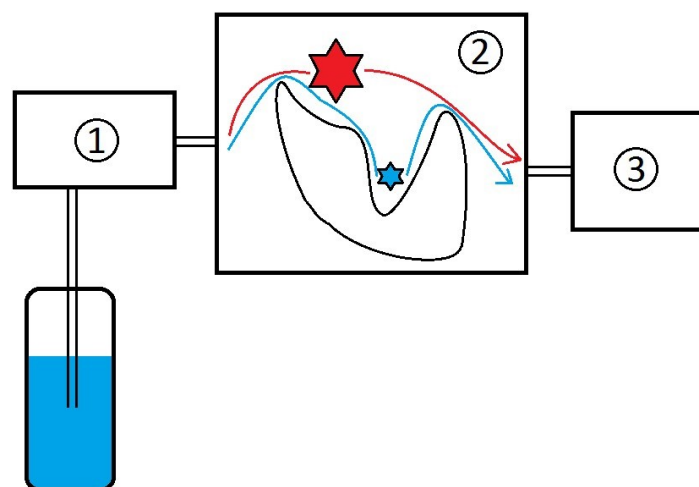

Figure 5 - HPLC system

Figure 6 shows an example of the plotted data from analyzing an inflow sample collected on February 6th. The sample was analyzed using a wavelength of 215 nm.
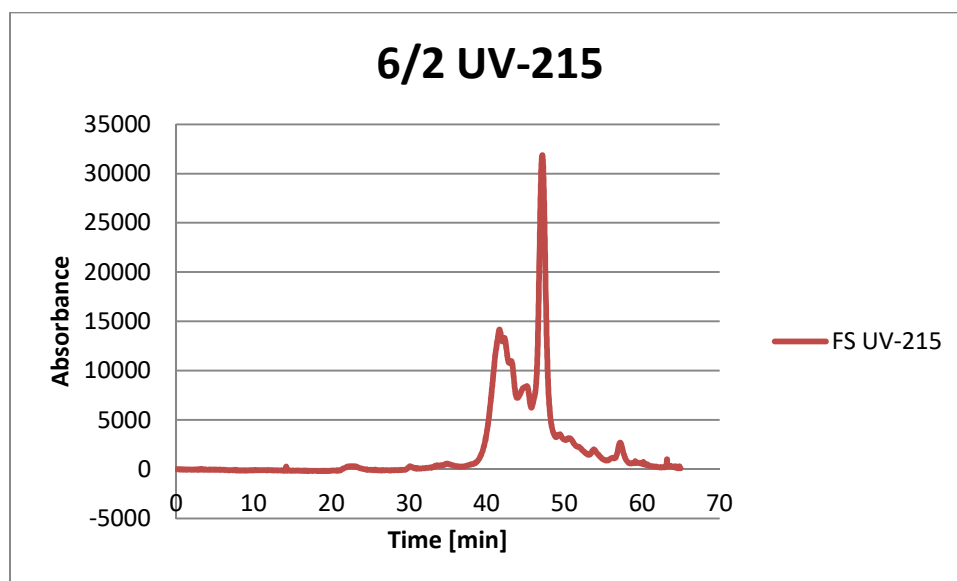

Figure 6 - HPLC result 215 nm wavelength February 6th primary sedimentation basin effluent sample

As can be seen on the graph, the first distinctive peak appears slightly after about 40 minutes and a second right before 50 minutes. Smaller peaks can be distinguished between and after the mentioned ones. Each peak represents a molecular size and the height of the peak indicates the concentration of the molecular size it represents.

The received data can be interpreted in different ways, regarding how to assess the peak values. The purpose of the HPLC-analysis in this study is to compare results from day to day to see if there are differences, as well as to compare inflow and outflow samples. Since one peak represents a certain type of molecule and the absorbance value indicates the concentration of the molecule, the absorbance value in comparison to the measured TOC concentration can show if there are changes in the composition of the TOC. To make the comparison, the absorbance value needs to be evaluated. This can primarily be done in two ways. The first is by picking the peak value and the second by calculating the integral underneath the curve created by the data. The risk by picking the peak value is that the peak might be inaccurately enhanced by occasional larger amounts of molecules passing by at the same time. The risk by calculating the integral is that peak values nearby might be partially included in the integral.

In this study, three different aspects of the HPLC spectra area were analyzed:

1. Area HPLC, refers to the total area under the HPLC spectrum
2. HPLC 40-45, refers to the peak value obtained with a retention time between 40 and 45 min
3. HPLC 45-50, refers to the peak value obtained with a retention time between 45 and 50 min

For each parameter listed above, results were obtained for spectra obtained at both 215 nm and 254 nm. Retention time in the HPLC corresponds to molecular size. A calibration with polyethylene glycol standards showed that molecules smaller than about 500 Da will have a retention time longer than 45 min while molecules with a size of about 500-4000 Da will have peaks in the span 40-45 min.

3.5 Data evaluation

When performing any kind of research where data is gathered from tests or analyses, it is important to interpret the data correctly. This can for example mean to determine if there is a relationship between two or more observed sets of data. After identifying relationships, statistical models can be used to evaluate the likelihood that the observed relationship is not just due to chance.

3.5.1 Linear correlation

The analysis of the linear correlation shows if one set of data is related to another. The degree of correlation between two sets of data samples is shown using the correlation coefficient, r, which is calculated in equation 5.

$$r = \frac{\sum x \times y}{\sqrt{\sum x^2 \times \sum y^2}} \qquad [5]$$

where x and y are calculated in equation 6 and 7.

$$x = x_i - \bar{x} \qquad [6]$$

$$y = y_i - \bar{y} \qquad [7]$$

Where $\bar{x}$ and $\bar{y}$ are the mean values of the two data samples respectively. The correlation coefficient ranges between -1 and 1, where -1 means a perfect negative correlation and 1 means a perfect positive correlation. 0 means no correlation at all. The correlation coefficient squared, r^2, is called the coefficient of determination which is a statistical measure of how well the regression line fits the measured data.

3.5.2 Significance

Once the correlation coefficient is calculated, it needs to be determined whether the correlation is significant or not. This is tested by evaluating if the correlation is significantly different from 0. By establishing the null hypothesis as the correlation is not significantly different from 0, the probability falsely rejecting the null hypothesis can be calculated. If this probability is lower than 5%, the correlation is considered significant.

Significance is also used to determine if there are differences between two groups of samples. The null hypothesis is set to no differences between the two groups, or that the groups are from the same population. This is written as equation 8.

$$H_0 : \mu_1 = \mu_2 \qquad [8]$$

where H_0 represent null hypothesis, μ_1 represent group one and μ_2 represent group two.

This is controlled by calculating two values from each group, the mean value and the variance. Those values are used to calculate the t-value. The t-value is calculated in equation 9.

$$t = \frac{M_1 - M_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \qquad [9]$$

where M is the mean value of the group, S^2 is the variance and n is the number of samples in the group.

To decide if the null hypothesis should be rejected, the critical t-value is picked from a t-value table. If the critical t-value is larger than the calculated t-value, the null hypothesis can be rejected. The critical t-value is chosen depending on the α-level and the degrees of freedom. The α-level is set to 0.05 as the limit for correlation. The degrees of freedom are calculated in equation 10.

$$df = n_1 + n_2 - 2 \qquad [10]$$

where df is the degrees of freedom and n is the number of observations in each sample group.

All t-tests in this study were done using a two tailed t-test.

4 Results and discussion

4.1 Goals of analysis

4.1.1 Comparing spectral parameters and TOC concentration
Spectrophotometric methods are often faster, simpler and less expensive than TOC analyses and are therefore often used as proxies for the TOC concentration. UV absorbance at 254 nm is most commonly used and has been found to be highly correlated with the concentration of dissolved organic compounds in natural waters [10]. In this study, the correlations between TOC concentration and several other spectral parameters (a215, a254, Area HPLC 215 nm, Area HPLC 254 nm, S275-295 and S350-400) were investigated. The goal was to investigate how well the different spectral parameters describe the TOC concentration in the wastewater.

4.1.2 Determining differences between inflow and outflow
Some spectral parameters could be used to characterize the composition of the organic compounds in the samples. The characteristics of the samples taken from the effluent of the primary sedimentation basins (inflow) and the effluent of the WWTP (outflow) were compared using statistical methods. The characterization was done by comparing the ratio between HPLC-results and the TOC concentration from the inflow and outflow.

4.1.3 Effect of additional precipitation
During part of the time when the samples were taken, the treatment process was enhanced by additional precipitation in the primary sedimentation basins. The results were analyzed on the basis of determining whether there are any significant changes between the time with additional precipitation and the time without. To evaluate the effect of additional precipitation, the samples are also analyzed by comparing groups of samples taken during conditions with high or low wastewater flows to the plant. High flows are defined as > 6.0 m^3/s and low flows as < 4.9 m^3/s. In total, half of the samples are defined as high flow samples and the other half is defined as low flow samples.

4.2 Comparing spectral parameters and TOC concentration
This chapter describes the comparison between the chosen spectral parameters and the measured TOC concentrations. Correlations are made with the TOC concentrations in both inflow and outlow samples. Both the strength and significance of the correlation between each spectral parameter and the TOC concentration is investigated.

The linear correlation between TOC concentration and absorbance at 254 nm (Figure 7), absorbance at 215 nm (Figure 8), S275-295 (Figure 9), S350-400 (Figure 10), area under the HPLC spectrum at 215 nm (Figure 11), area under the HPLC spectrum at 254 nm (Figure 12) and the HPLC 40-45 at 254 nm (Figure 13) are shown below. Absorbance at 254 nm, S275-295 and area under the HPLC spectrum at 254 nm show the strongest correlations with coefficients of determinations of 0.64-0.90. The S275-295 has the strongest correlation with TOC concentration.
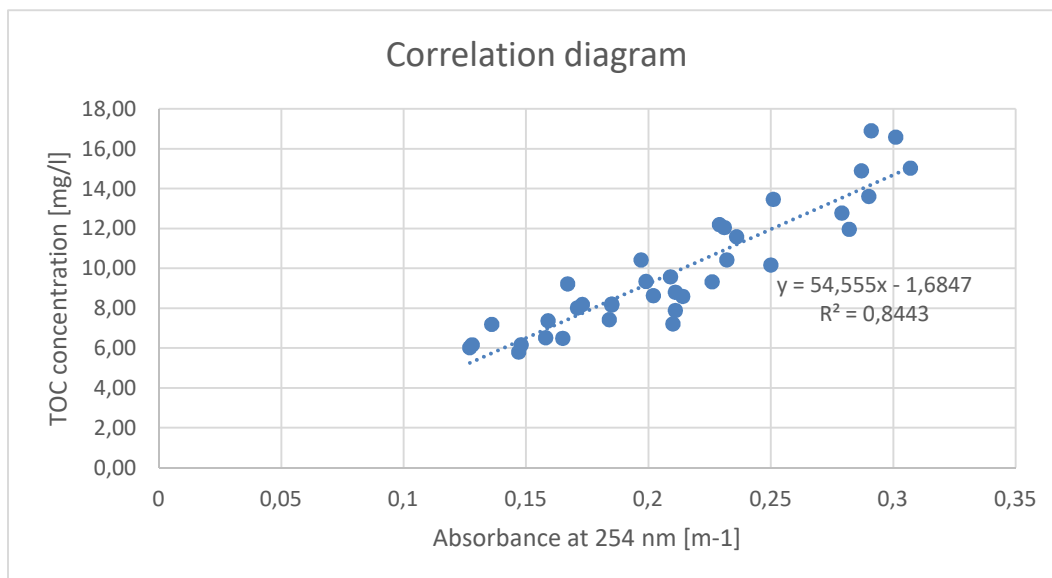
Figure 7 - Correlation between absorbance at 254 nm wavelength and TOC concentration
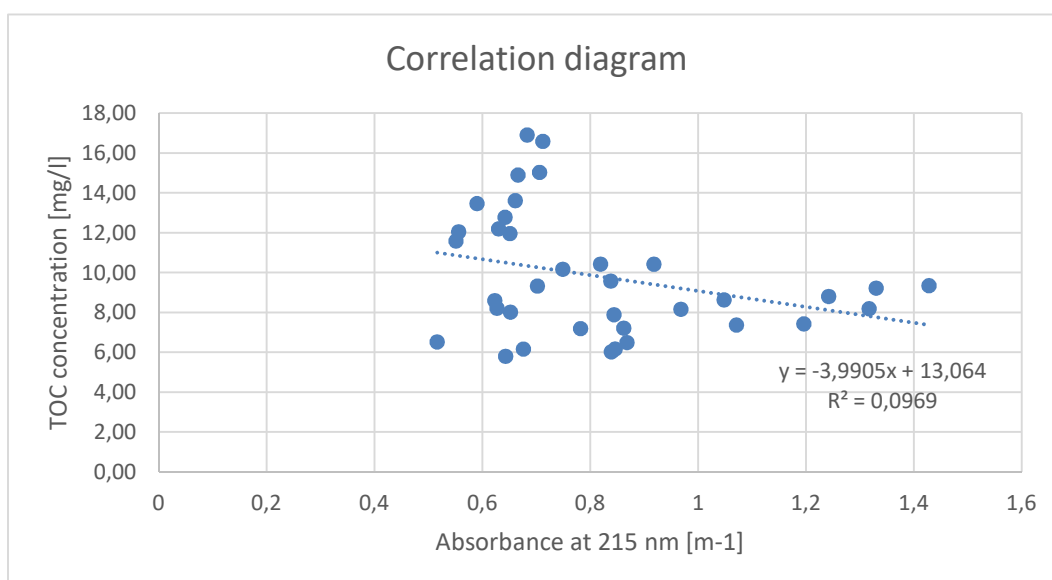
Figure 8 - Correlation between absorbance at 215 nm wavelength and TOC concentration
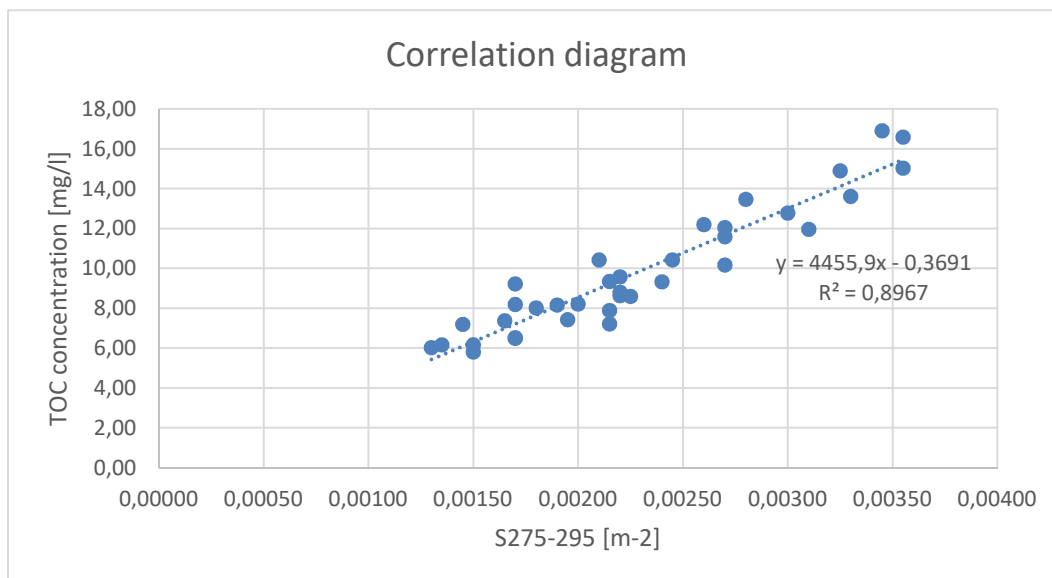
Figure 9 - Correlation between S275-295 and TOC concentration
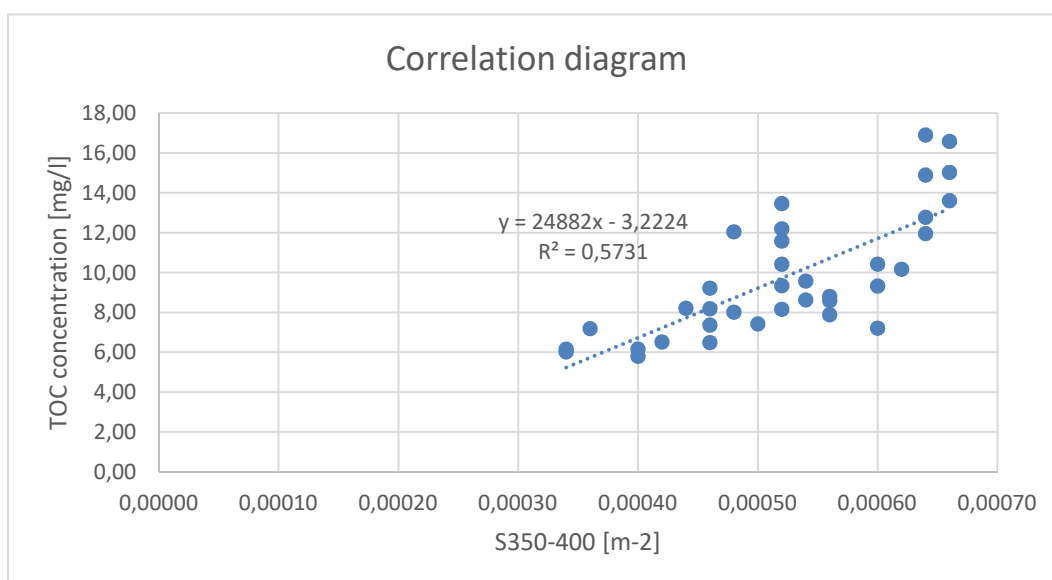

Figure 10 - Correlation between S350-400 and TOC concentration
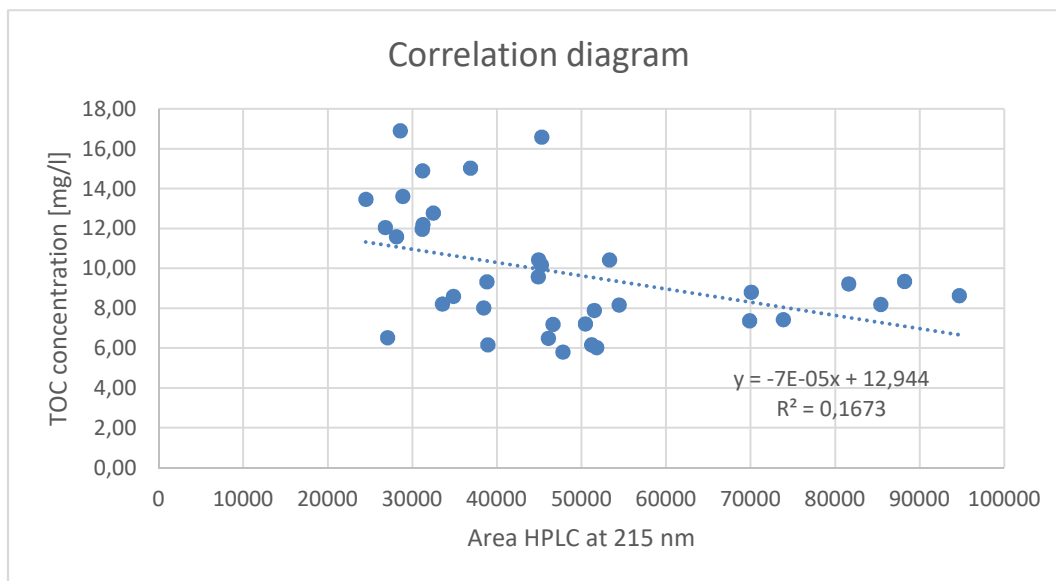
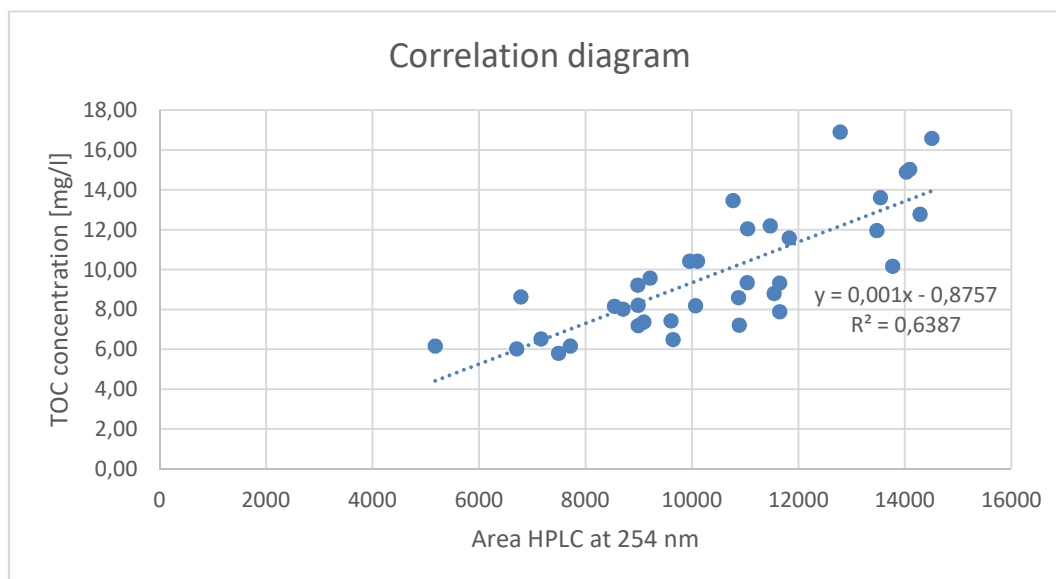**Figure 11 - Correlation between Area HPLC at 215 nm and TOC concentration**

Figure 12 - Correlation between Area HPLC at 254 nm and TOC concentration
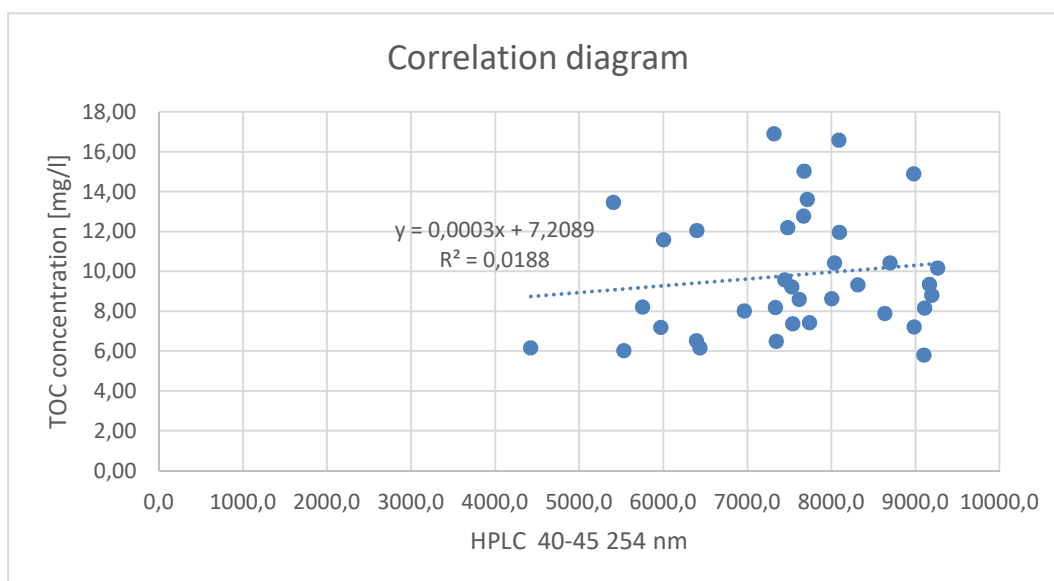
Figure 13 - Correlation between HPLC 40-45 at 254 nm and TOC concentration

A summary of the statistical analysis is shown in Table 2 and Table 3. A t-test was carried out to determine if the linear slopes were significantly different from zero. All spectral parameters except the absorbance at 215 nm had slopes significantly different from zero suggesting that they had some correlation with the TOC concentration.

Table 2 – Spectral parameter comparison. Note that SUVA = absorbance/TOC.

Inflow and outflow	$SUVA_{254}$	$SUVA_{215}$	S275-295/TOC	S350-400/TOC
Mean value, μ	0.0220	0.0927	0.000235	0.0000560
Standard deviation, σ	0.0027	0.0395	0.000024	0.0000107
Ratio σ/μ	0.124	0.426	0.104	0.191
Correlation, r^2	0.84	0.10	0.90	0.57
p-value	2.7×10^{-15}	0.064	2.5×10^{-18}	9.1×10^{-8}
Significant correlation	yes	no	yes	yes

Table 3 - Spectral parameter comparison

Inflow and outflow	Area HPLC 215/TOC	Area HPLC 254/TOC	HPLC 40-45 254/TOC
Mean value, μ	5 475	1 102	831.3
Standard deviation, σ	2 833	200	252.7
Ratio σ/μ	0.517	0.182	0.304
Correlation, r^2	0.17	0.64	0.02
p-value	0.013	5.1×10^{-9}	0.425
Significant correlation	yes	yes	no

The S275-295 shows the strongest correlation to TOC concentration. It even has stronger correlation than the absorbance at 254 nm which is a commonly used parameter. The correlation between the

area HPLC at 254 nm and the TOC concentration shows that most of the content in the TOC is included in the HPLC analysis, which covers content with molecular size < 4000 Da. However, the lack of correlation between HLC 40-45 at 254 nm and the TOC concentration shows that the composition of the TOC might not be constant.

4.3   Determining differences between inflow and outflow
The sample parameters were compared to the TOC concentration by separating the inflow and the outflow. All samples were treated equally, regardless if they were taken during the time of additional precipitation or not.

4.3.1   Comparing test values between inflow and outflow
As an initial comparison between the inflow and outflow, the test values are compared to determine if there are significant differences between the groups generated by the inflow and the group generated by the outflow. The test values are shown in Table 4.

Table 4 - Comparison of test values

Test value type	Inflow $\mu \pm \sigma$	Outflow $\mu \pm \sigma$	p-value	Significant difference - inflow outflow
TOC	11.97 ± 2.76	7.63 ± 1.32	2.7×10^{-8}	Yes
a215 nm	0.681 ± 0.087	0.956 ± 0.265	0.0005	Yes
a254 nm	0.251 ± 0.037	0.170 ± 0.027	1.3×10^{-11}	Yes
SUVA215	0.0604 ± 0.019	0.1250 ± 0.025	3.3×10^{-8}	Yes
SUVA254	0.0214 ± 0.0027	0.0225 ± 0.0028	0.132	No
S275-295	0.00279 ± 0.0005	0.00178 ± 0.0003	5.8×10^{-11}	Yes
S350-400	0.00058 ± 6.80 $\times 10^{-5}$	0.00046 ± 7.5 $\times 10^{-5}$	4.6×10^{-8}	Yes
S275-295/S350-400	4.771 ± 0.586	3.839 ± 0.178	3.1×10^{-7}	Yes
HPLC40-45	7587 ± 1092	7490 ± 1371	0.693	No
HPLC45-50	4390 ± 1387	1605 ± 469	3.6×10^{-7}	Yes
HPLC45-45/HPLC45-50	1.886 ± 0.598	4.952 ± 1.47	1.1×10^{-7}	Yes

All test values showed significant differences between the inflow and the outflow group except SUVA$_{254}$ and HPLC40-45. Since the reduction of TOC concentration is part of the WWTP process, it was expected to find significant differences between the inflow and the outflow. The fact that SUVA$_{254}$ did not show significant differences, indicate that the proportion between a254 and the TOC concentration is stable. In studies of the SUVA$_{254}$ using different sources for example dark waters and swamps, the results have varied as a result of varying chemical properties of the content [8]. The variation in the wastewater does not seem to be of similar magnitudes as the variations in the mentioned sources. The HPLC40-45 shows no significant changes which indicates that the level of humic acid in the inflow over time is similar to the outflow.

4.3.2   Spectrophotometry
The comparison of spectral parameters is repeated with the samples divided in groups of inflow and outflow. The highest correlations with TOC were generated by a254 and S275-295. To determine if the spectral parameters need to be adjusted for inflow and outflow, the correlations were compared.

Figure 14 and Figure 15 shows the correlation between TOC concentration and absorbance at 254 nm and the slope S275-295 respectively. For both parameters, the slope of the regression line differs between the inflow and the outflow.

The difference in slope of the regression line could mean that the parameters have to be calibrated depending on if they are used to measure TOC in the inflow or the outflow. To determine if the difference in slope is significant, the inflow data were compared to the outflow data using a t-test. The null hypothesis was that there are no differences between the inflow and the outflow and the alternate hypothesis that there is a difference. The t-test was carried out for both a254 and S275-295. The values of the sample parameters a254 and S275-295 were divided by the corresponding TOC. For the parameter a254, this gives the $SUVA_{254}$ value. The p-values were calculated to 0.13 for $SUVA_{254}$ and 0.84 for S275-295/TOC. This means that the null hypothesis of no differences during the treatment cannot be rejected based on any of the parameters $SUVA_{254}$ and S275-295/TOC when comparing inflow and outflow.
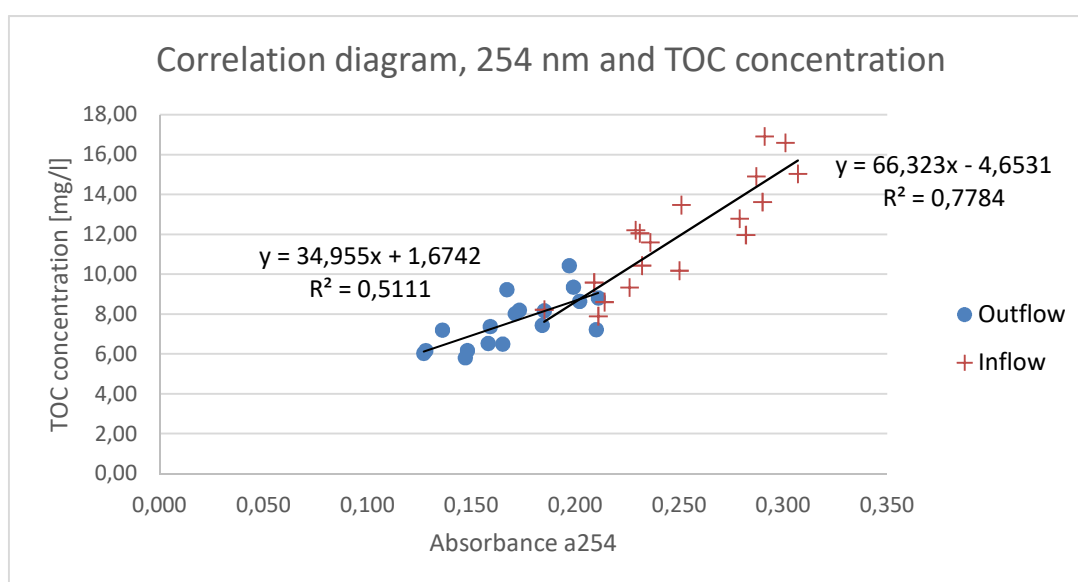
Figure 14 - Correlation between absorbance at 254 nm and TOC concentration
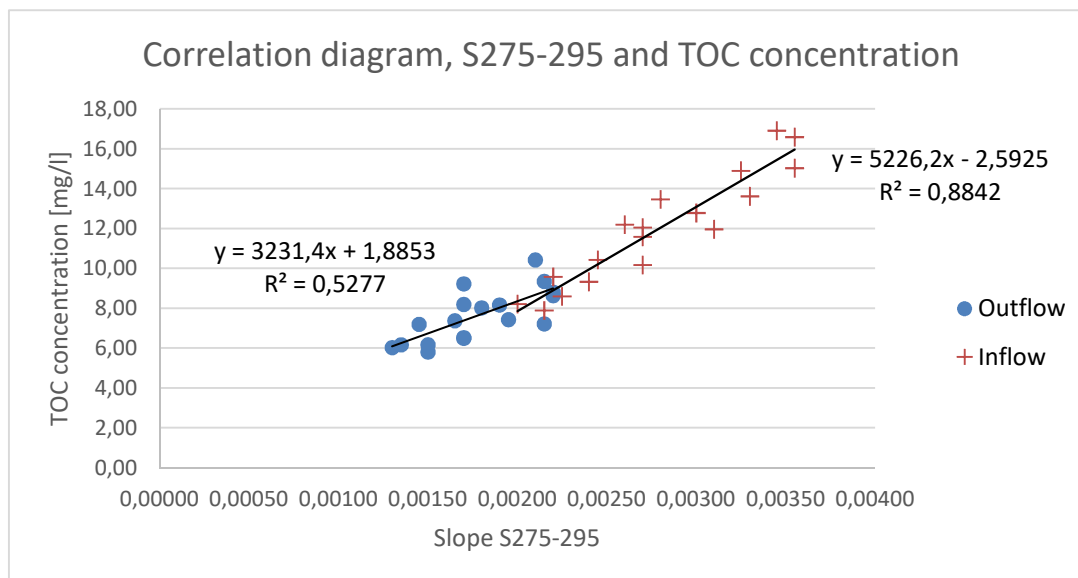
Figure 15 - Correlation between slope S275-295 and TOC concentration

The results from the inflow are shown in Table 5 and Table 6. The results from the outflow are shown in Table 7 and Table 8.

Table 5 – Spectral parameter comparison, inflow. Note that SUVA = UV/TOC.

Inflow	SUVA$_{254}$	SUVA$_{215}$	S275-295/TOC	S350-400/TOC
Mean value, μ	0.0214	0.0604	0.000236	0.0000506
Standard deviation, σ	0.0027	0.0190	0.000021	0.0000102
Ratio σ/μ	0.124	0.315	0.087	0.201
Correlation, r^2	0.78	0.06	0.88	0.31
p-value correlation	1.3×10^{-6}	0.32	6.7×10^{-9}	0.015
Significant correlation	yes	no	yes	yes

Table 6 – HPLC area comparison, inflow

Inflow	Area HPLC 215/TOC	Area HPLC 254/TOC
Mean value, μ	3 193	1 043
Standard deviation, σ	1 296	191
Ratio σ/μ	0.406	0.183
Correlation, r^2	0.15	0.43
p-value correlation	0.11	0,0033
Significant correlation	no	yes

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Table 7 – Spectral parameter comparison, outflow. Note that SUVA = UV/TOC.

Outflow	$SUVA_{254}$	$SUVA_{215}$	S275-295/TOC	S350-400/TOC
Mean value, μ	0.0225	0.1250	0.000235	0.0000613
Standard deviation, σ	0.0028	0.0254	0.000028	0.0000084
Ratio σ/μ	0.122	0.203	0.121	0.137
Correlation, r^2	0.51	0.40	0.53	0.42
p-value correlation	0.00085	0.0045	0.00064	0.0035
Significant correlation	yes	yes	yes	yes

Table 8 - HPLC area comparison, outflow

Outflow	Area HPLC 215/TOC	Area HPLC 254/TOC
Mean value, μ	7 757	1 161
Standard deviation, σ	1 955	196
Ratio σ/μ	0.252	0.169
Correlation, r^2	0.33	0.32
p-value	0.012	0.015
Significant correlation	yes	yes

The $SUVA_{254}$ shows consistency when it comes to mean values and the standard deviations based on the data gained from both outflow and inflow separately and combined. The correlation in the outflow is slightly lower but is still significant. Similar results were shown by the HPLC area at 254 nm. S275-295 shows slightly better correlation, both in terms of lower relative standard deviation and lower p-value than $SUVA_{254}$. The S350-400 shows significant but weaker correlation. Judging by the results, the $SUVA_{215}$ does have a significant but low correlation with regards TOC concentration in the outflow. However, the results from the inflow show insignificant and low correlation. However, the absorbance levels at 215 nm were higher in the outflow, despite lower TOC concentrations. This indicates that substances, other than organic compounds, that interfere with the absorbance at 215 nm have a higher concentration in the outflow than the inflow. The HPLC area at 215 nm showed similar results compared to $SUVA_{215}$. This means that the HPLC analysis in total is able to detect the substances giving the single absorbance value from the UV-Vis.

It should be noted that both a215 and the HPLC area at 215 nm generated negative slopes on the regression line. When separated into inflow and outflow, only the inflow slope was negative. As can be seen in Figure 16, the negative slope is generated by a dense cluster of data. The results from the HPLC area at 254 nm and the HPLC 40-45 at 254 nm can be seen in Figure 17 and Figure 18 respectively.

Figure 16 - Correlation between Area HPLC at 215 nm and TOC concentration, inflow and outflow separate

Figure 17 - Correlation between Area HPLC at 254 nm and TOC concentration, inflow and outflow
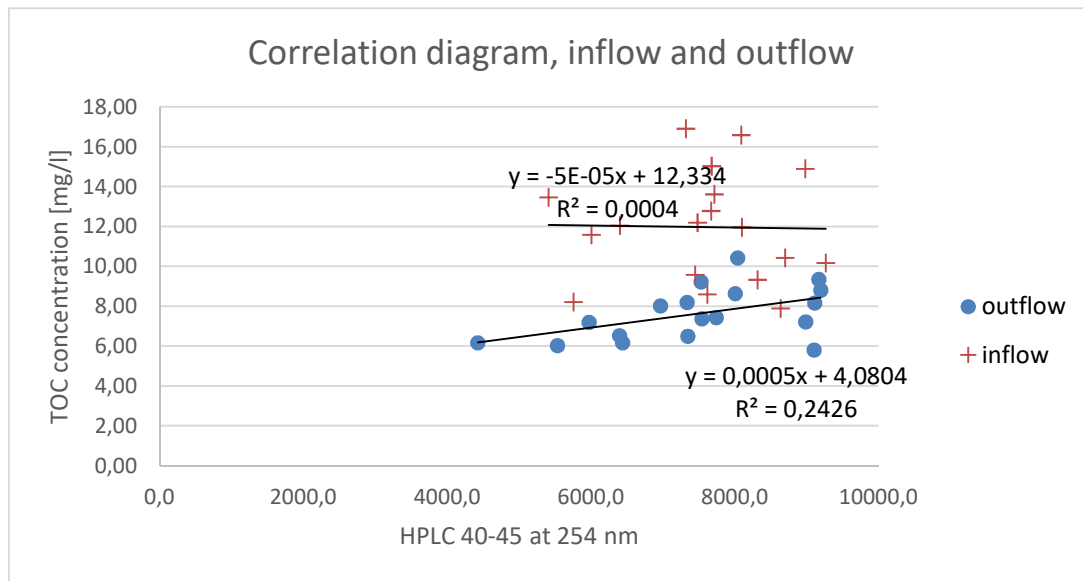
Figure 18 - Correlation between HPLC 40-45 at 254 nm and TOC concentration, inflow and outflow
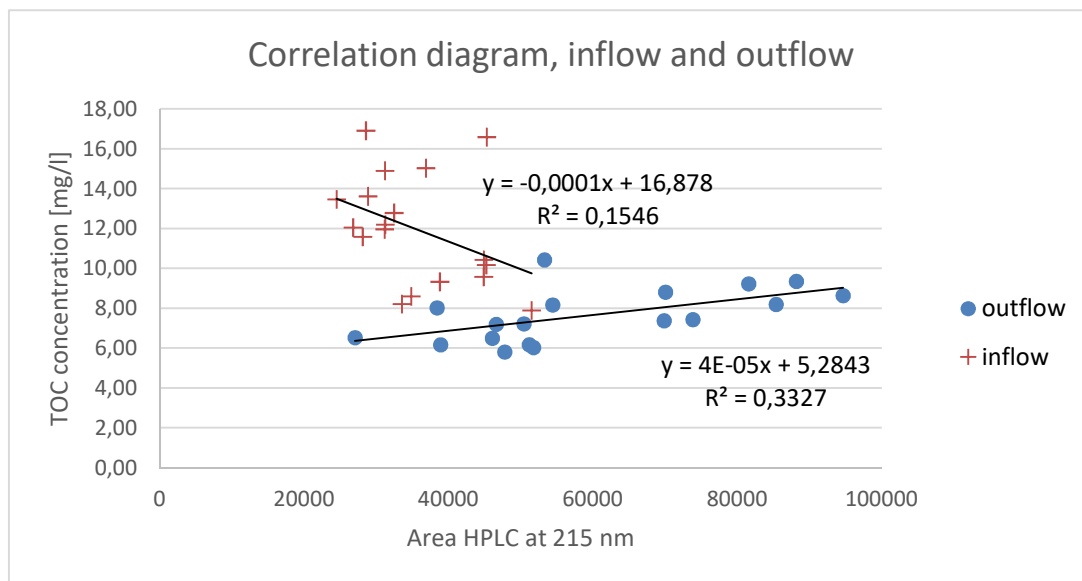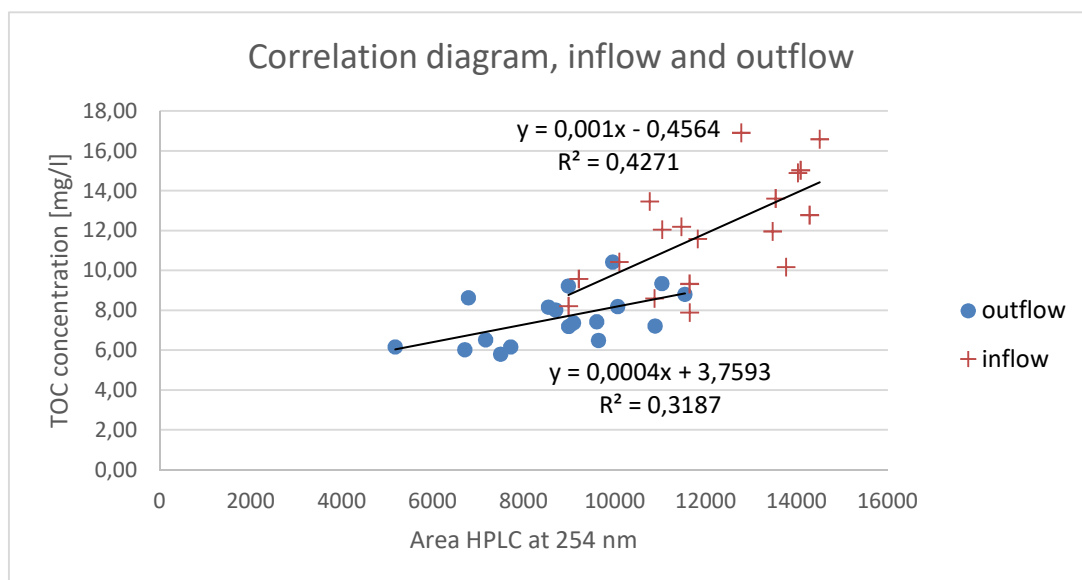
4.3.3 HPLC analysis

HPLC is used to detect differences between two samples. As described earlier, a peak value received in HPLC indicates the concentration of a molecule with a certain size. If two or more types of molecules have similar size and therefore similar retention times, the absorbance at certain wavelengths could interfere. To assure that the given peak values are reliable, HPLC analyses were performed on laboratory produced samples with known properties. Both acetate and humic acid samples were analyzed using 215 and 254 nm wavelength. For 215 nm wavelength, acetate and humic acid generated peak values corresponding to similar retention times. This means that any content of acetate in the wastewater might interfere with the measurement of humic acid when using 215 nm wavelength. At 254 nm wavelength, the acetate has negligible impact on the HPLC analysis. However, the humic acid sample gives a clear peak after about 40-45 minutes retention time.

Figure 19 shows the result of an analysis made on the laboratory produced humic acid sample with 40 mg/l humic acid using a wavelength of 254 nm. As can be seen, the peak value occurs slightly after 40 minutes. This peak value will be used further on and be called 40-45.
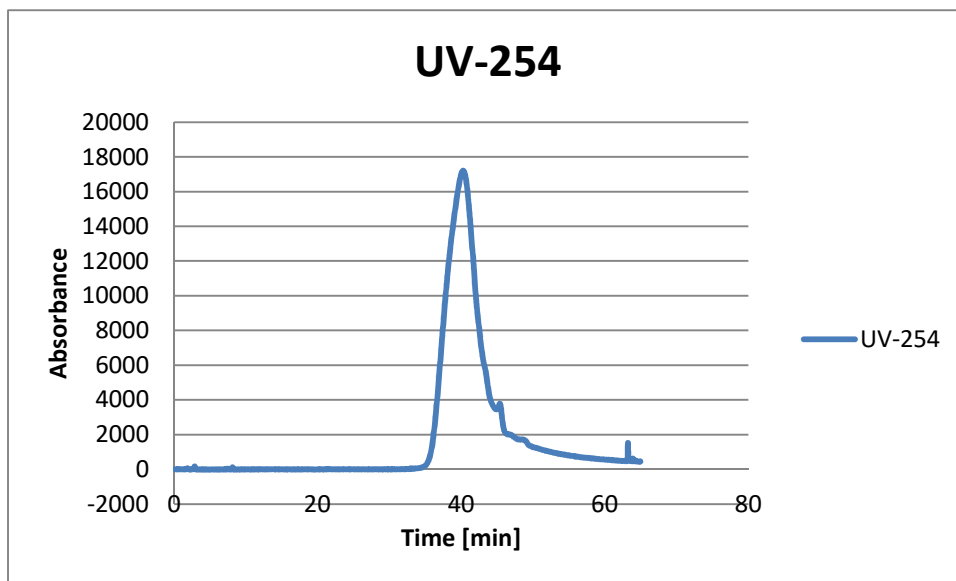
Figure 19 - HPLC result 254 nm wavelength on humic acid sample
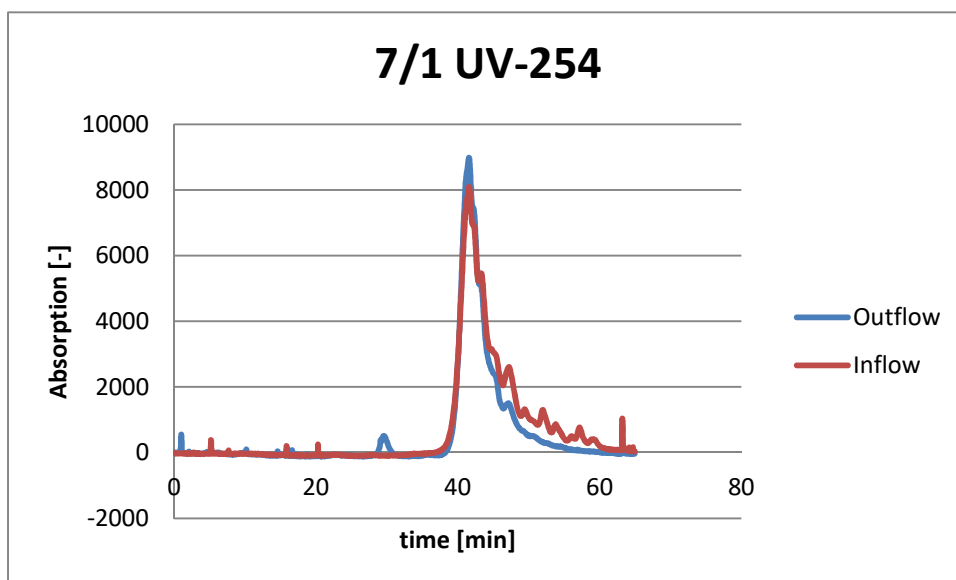
Figure 20 - HPLC result 254 nm January 7th samples

Figure 20 shows the result of HPLC analysis of the specimen taken on January 7th and is typical for most samples with a distinctive peak after slightly 40 minutes. The inflow has several lower peaks later which show that different compounds, likely organic, have been removed during the process. It is also common that the peak slightly after 40 minutes is higher in the outflow than in the inflow.

Figure 21 shows the linear correlation between the HPLC peak values at 254 nm wavelength and the TOC concentrations for the inflow and the outflow.

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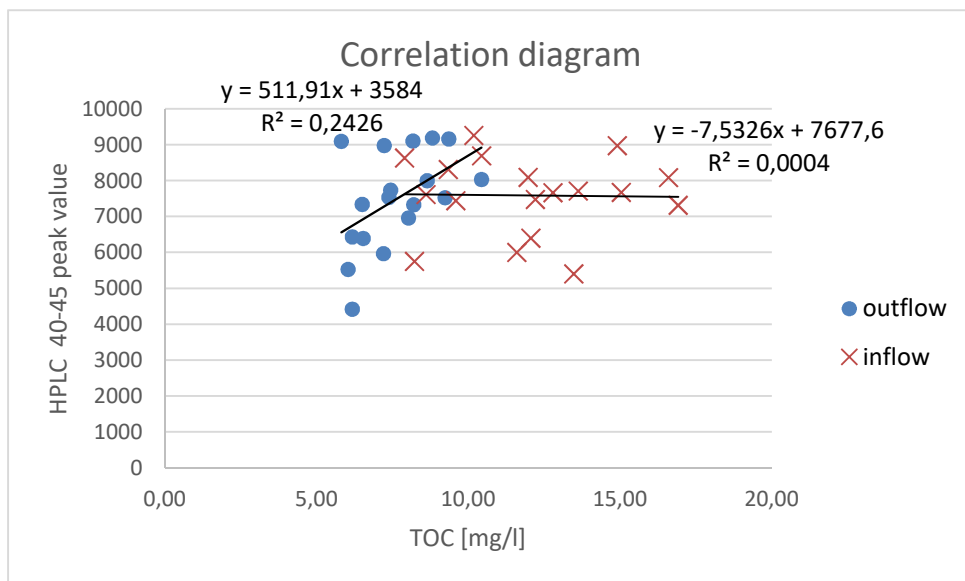
Figure 21 - Correlation between HPLC 40-45 at 254 nm and TOC concentration

As can be seen, the regression line is close to flat on the inflow diagram but rising on the outflow diagram.

Figure 22 shows the linear correlation between the HPLC peak values and the spectral slope S275-295 for the inflow and the outflow.
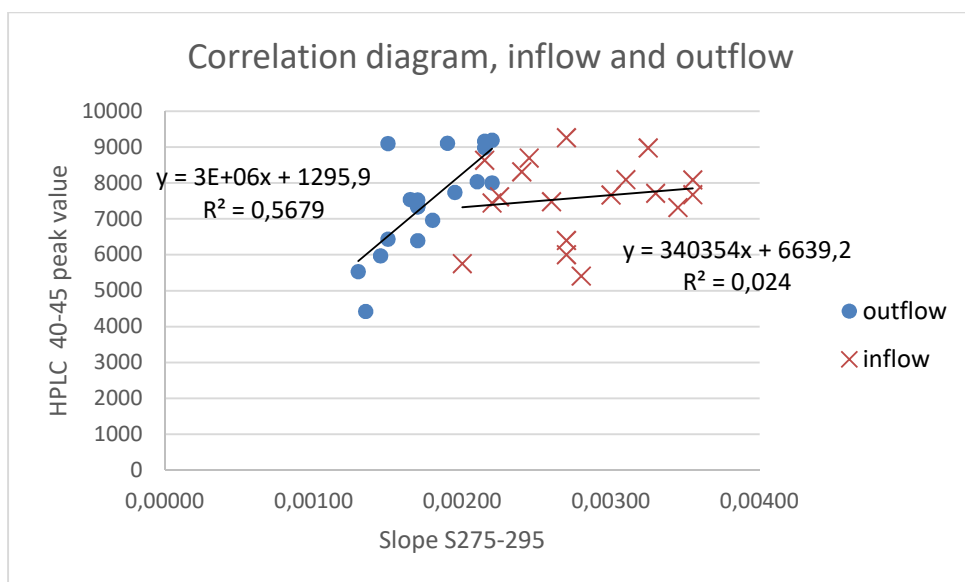
Figure 22 - Comparison HPLC peak value 40-45 and S275-295

Similar to the comparison to TOC, the spectral slope S275-295 also shows tendencies of having close to flat regression line representing the inflow but rising on the outflow diagram.

Figure 23 shows the linear correlation between the measured peak values and the absorbance at 254 nm.
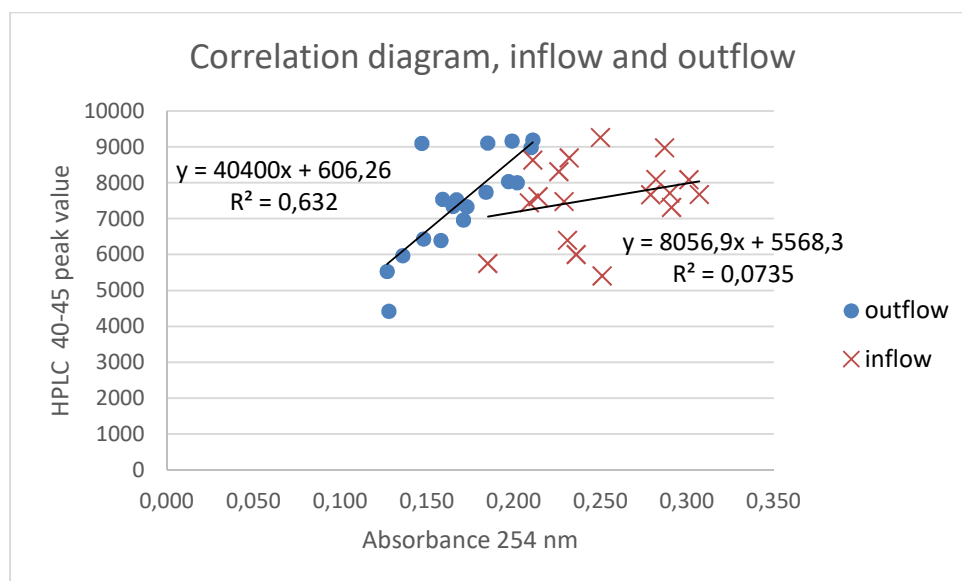
Figure 23 - Comparison HPLC peak value 40-45 and absorbance at 254 nm wavelength of inflow

The third comparison with the absorbance level at 254 nm wavelength follows the same pattern as previous comparisons. A difference is a slightly increased correlation. The summary of the correlation analysis of the inflow is found in Table 9.

Table 9 - Evaluation of HPLC 40-45 peak value at 254 nm wavelength, inflow

Inflow	40-45/TOC	40-45/S275-295	40-45/a254
Ratio σ/μ	0.287	0.218	0.178
Correlation, r^2	0.00	0.02	0.07
p-value	0.940	0.539	0.276
Significant correlation	No	no	no

All p-values are too high to determine significant correlation. In fact, the p-value where the HPLC peak values are compared to the TOC levels is high enough to suspect that the level of humic acid and the TOC level are independent for the inflow values. However, the values change when analyzing the outflow. As can be seen in Table 10, all three comparisons show significant correlation and the correlations are stronger. The ratio σ/μ is reduced in the outflow, indicating a lower spread in the outflow.

Outflow	40-45/TOC	40-45/S275-295	40-45/a254
Ratio σ/μ	0.197	0.137	0.125
Correlation, r^2	0.24	0.57	0.63
p-value correlation	0.038	3.05×10^{-4}	8.05×10^{-5}
Significant correlation	yes	yes	yes

To investigate further weather the measured correlation in the comparisons are of any value, Figure 24 shows the HPLC peak values plotted with inflow values on the x-axis and outflow values on the y-axis.

Comparison HPLC peak values 40-45

y = 0,8424x + 1097,9
R² = 0,4502
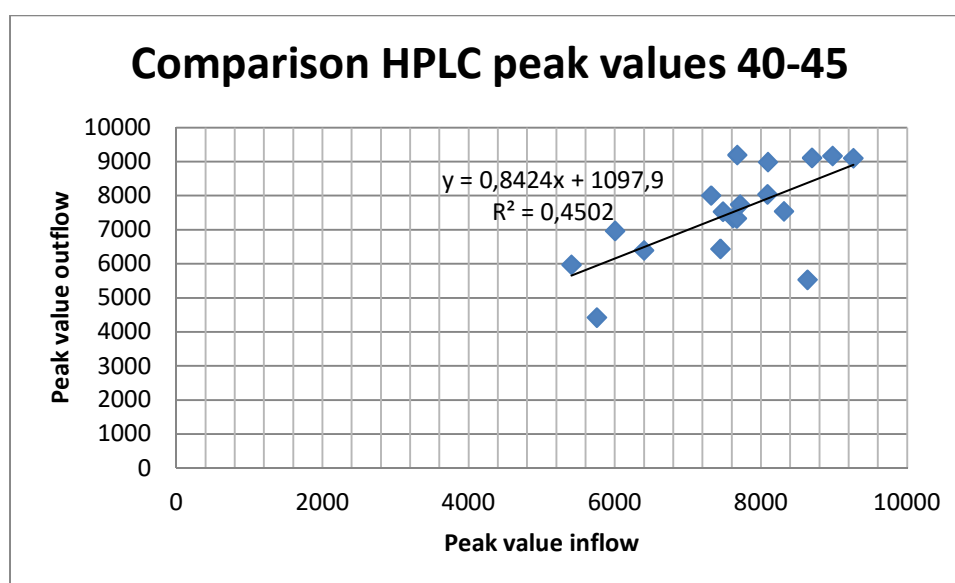
Figure 24 - HPLC 40-45 peak value 254 nm wavelength

The measured peak values have a coefficient of determination of 0.45. As can be seen by the regression line, the slope is almost one to one but has a non negligible constant meaning that the regression line is far from crossing origo. By using the regression line to evaluate the treatment of humic acid, the fact that the slope is slightly lower than one can mean that some of the humic acids are removed during the treatment process. The constant could represent humic acid that is added during the process as a result of the biological material in the biological treatment part of the process. The increase in correlation when comparing the outflow with the inflow can be explained by humic acids representing a larger portion of the TOC in the outflow when other carbon components are removed. Another theory considering the strong correlation and significance measured between S275-295 and the HPLC peak values is that the levels of humic acids in the inflow determines the WWTPs capacity to treat the TOC. It should be noted that the actual TOC has a lower level of correlation with the HPLC peak values than what the S275-295 has. However, the TOC values are produced by the Rya WWTP and the S275-295 are calculated by results generated from the same samples that have been analyzed in the HPLC. Assuming that the S275-295 is a good parameter for measuring TOC, the comparison between S275-295 and the HPLC peak values might be more accurate.

The differences in the composition of the TOC between the inflow and outflow can thereby be assumed to be small throughout the process. The rather consistent levels of humic acids throughout the treatment process combined with the fact that the final levels of TOC in the outflow tend to correlate rather well with the humic acid levels raises the question if the humic acids are a limiting factor when it comes to the WWTPs capacity to treat TOC.

4.4 Effect of additional precipitation

During the time of additional precipitation, the capacity of the WWTP to treat organic compound should have been increased. The average TOC concentration in the inflow sample (the effluent from the primary sedimentation) was 10.47±2.25 mg/L during the time of additional precipitation and 12.72±2.77 mg/L during the time without. The average changes in TOC concentration between inflow and outflow samples were 6.69±0.78 during the time with additional precipitation and 8.10±1.30 during the time without. Based on this, it is difficult to say if the additional precipitation had any effect on the capacity of the activated sludge to remove organic compounds. However, the composition of the organic compounds might also be affected. This is analyzed by comparing $SUVA_{254}$ as a spectral parameter and HPLC peak values with the TOC concentration for the time with additional precipitation and without separately. Changes in $SUVA_{254}$ will indicate if the organic composition has changed as a result of the additional precipitation. The HPLC comparison will show to what extent the humic acid-like substances are removed in comparison to the TOC.

4.4.1 Spectrophotometry

The $SUVA_{254}$ was calculated for the inflow and outflow and compared for the time with and without additional precipitation. The comparison was made to determine if the $SUVA_{254}$ parameter is significantly different in any of the two situations. The correlation between $SUVA_{254}$ for the inflow and the outflow is shown in Figure 25.
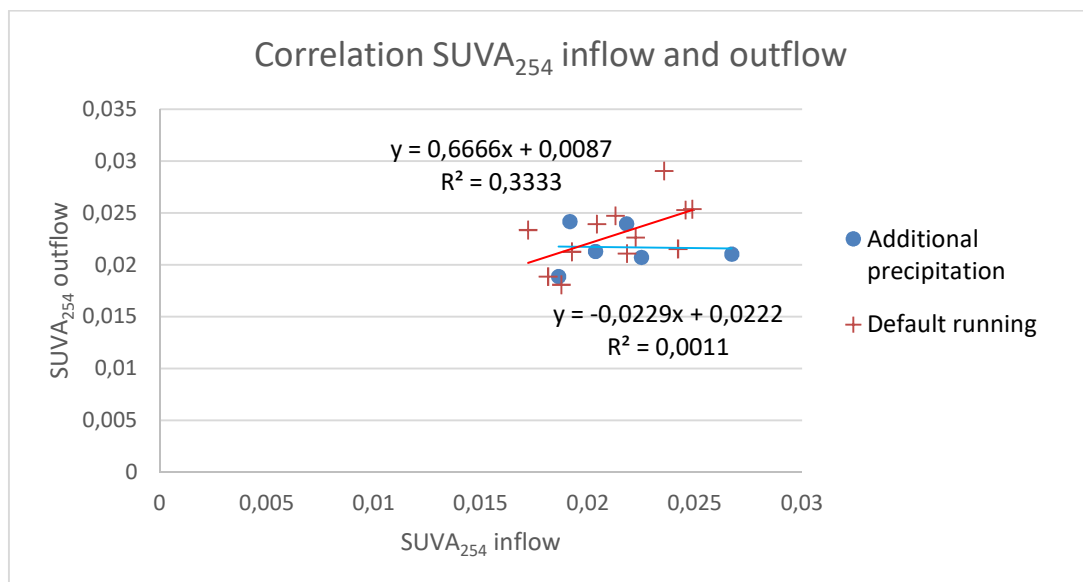
Figure 25 - Correlation between $SUVA_{254}$ inflow vs outflow, with and without additional precipitation

To be able to relate to the differences in the $SUVA_{254}$ parameter based on the separation between additional precipitation and default running, a second comparison was made by separating high flows and low flows. Low flows were defined as < 4.9 m^3/s and high flows as > 6.0 m^3/s. The

correlation between the SUVA$_{254}$ for the inflow and the outflow with flow separation is shown in Figure 26.

When determining if there are differences in the inflow and the outflow, the null hypothesis for the comparison between the groups is set to no differences with the alternate hypothesis that there are differences between the groups. Paired two tailed t-tests were performed to calculate the p-value. The calculated results for adding precipitation and flow variations are shown in Table 11 and Table 12 respectively.
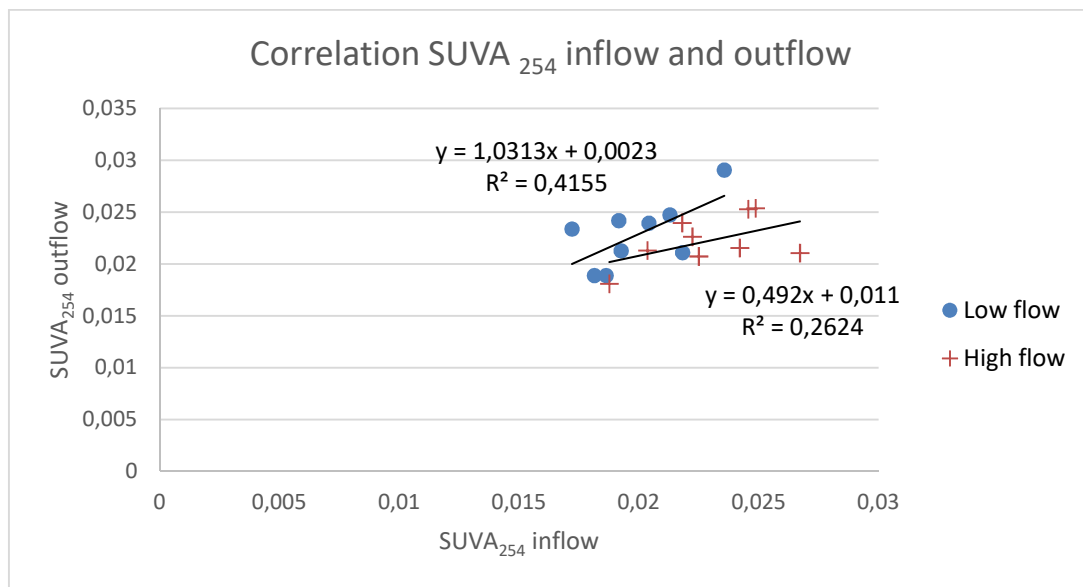
Figure 26 - Correlation between SUVA$_{254}$ inflow vs outflow, low and high flows

Table 11 - Effect of additional precipitation on SUVA$_{254}$

SUVA$_{254}$	Additional precipitation		Default running	
	Inflow	Outfow	Inflow	Outflow
Mean value, μ	0.0215	0.0216	0.0213	0.0229
Standard deviation, σ	0.00294	0.00203	0.00263	0.00303
Ratio σ/μ	0.136	0.094	0.123	0.132
Correlation, r^2	0.0011		0.33	
p-value correlation	0.95		0.049	
Significant correlation	no		yes	
p-value null hypothesis	0.917		0.061	
Reject null hypothesis*	no		no	
*The null hypothesis being no differences				

Table 12 - Effect of flow variation on SUVA$_{254}$

SUVA$_{254}$	Low flow (< 4.9 m^3/s)		High flow (> 6.0 m^3/s)	
	Inflow	Outfow	Inflow	Outflow
Mean value, μ	0.0199	0.0228	0.0229	0.0222
Standard deviation, σ	0.00200	0.00321	0.00246	0.00236
Ratio σ/μ	0.101	0.141	0.107	0.106
Correlation, r^2	0.42		0.26	
p-value correlation	0.06		0.16	
Significant correlation	no		no	
p-value null hupothesis	0.008		0.426	
Reject null hypothesis*	yes		no	
*The null hypothesis being no differences				

As the results suggest, there are no significant changes between inflow and outflow depending on additional precipitation. However, during low flows, the difference is significant. It should be noted that the p-value for default running was very close to suggested significant differences. A rejection of the nullhypothesis indicates that the organic composition that generates the SUVA$_{254}$ changes during the treatment process. This means that the opposite is that the organic composition does not change during the treatment process. High flows can be assumed to be a result of rainfalls. The organic material brought by the rain can be found naturally in the environment. If organic material brought by rain is increased in proportion to total TOC and there is a biological process creating organic material in the treatment process, it could be assumed that the composition of organic material in the inflow is more similar to the outflow during high flows caused by rainfall.

4.4.2 HPLC analysis

To evaluate if there are changes in the treatment process with regards to the humic acid content of the TOC concentration, the relative amount of humic acid is compared using data from the inflow and the outflow for the time periods with and without additional precipitation separately. In addition, the HPLC peak values are compared to evaluate if there are any changes in the treatment of humic acid regardless of the TOC concentration. For reference, the changes between high and low flows are added to the comparison.

The first comparisons between the time with and without additional precipitation are shown in Figure 27 and Figure 28. The reference comparisons are shown in Figure 29 and Figure 30.
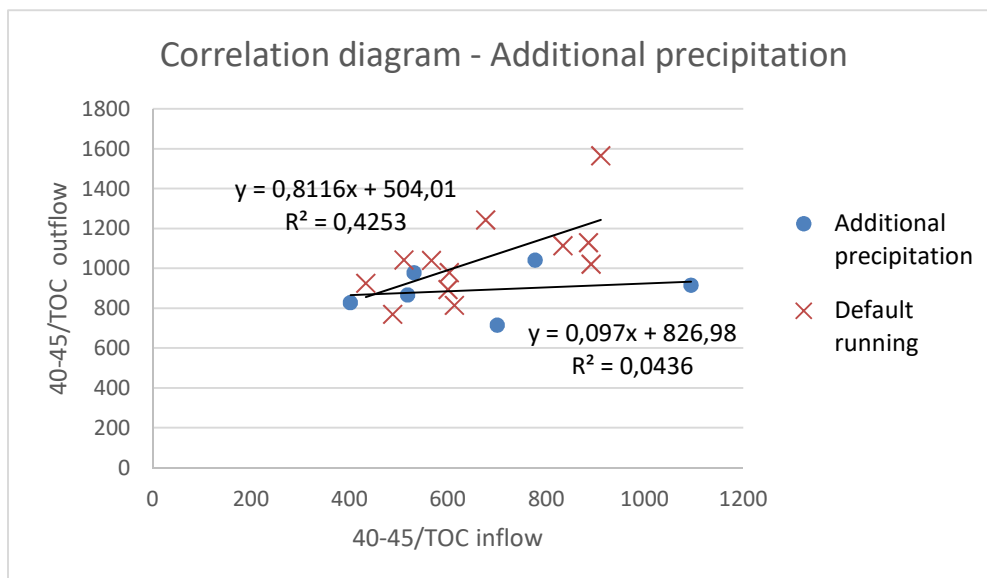
Figure 27 - Comparison between humic acid concentration of TOC in inflow vs outflow, with and without additional precipitation
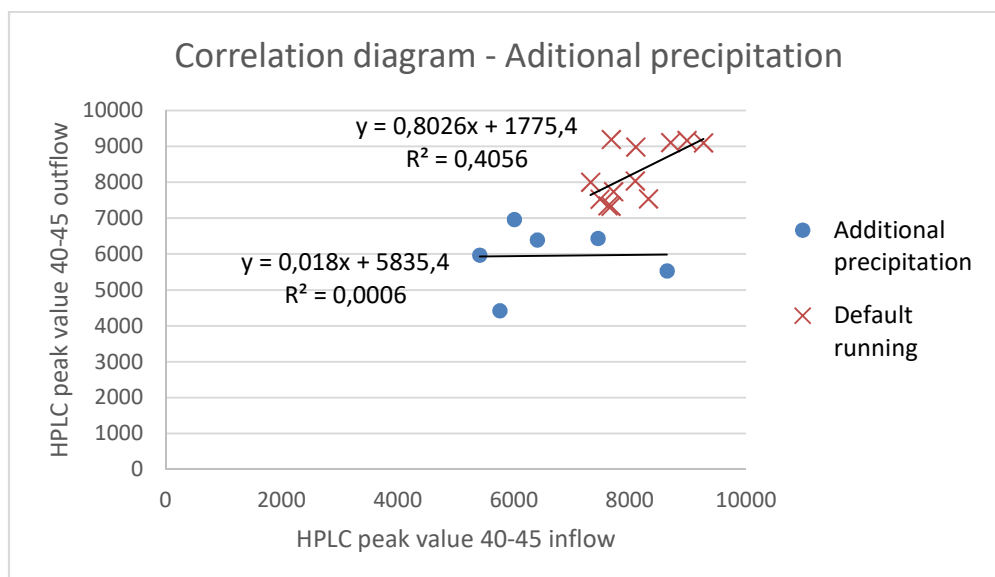
Figure 28 - Comparison between HPLC peak values in inflow vs outflow, with and without additional precipitation

The humic acid content stays on a similar level when comparing the inflow and outflow during the default running, as can be seen on the slope of the regression line. The humic acid content in the outflow seem to be less affected by the humic acid content in the inflow when adding additional precipitation. The slope of the regression line is small compared to the default running without additional precipitation. The summary of the comparisons are shown in Table 13 and Table 14.

Table 13 - Summary comparison 40-45/TOC additional precipitation

40-45/TOC	Additional precipitation		Default running	
	Inflow	Outfow	Inflow	Outflow
Mean value, μ	725	1090	612	899
Standard deviation, σ	154.5	219.3	216.9	113.9
Ratio σ/μ	0.213	0.201	0.355	0.127
Correlation, r^2	0.044		0.43	
p-value correlation	0.69		0.022	
Significant correlation	no		yes	
p-value nullhypothesis	0.082		6.4×10^{-6}	
Reject nullhypothesis*	no		yes	
*The nullhypothesis being no differences				

Table 14 - Summary comparison 40-45 peak additional precipitation

40-45 peak	Additional precipitation		Default running	
	Inflow	Outfow	Inflow	Outflow
Mean value, μ	8200	8368	6975	6612
Standard deviation, σ	654.4	886.8	1124.0	1215.4
Ratio σ/μ	0.0798	0.106	0.161	0.184
Correlation, r^2	0.0006		0.41	
p-value correlation	0.96		0.026	
Significant correlation	no		yes	
p-value nullhypothesis	0.33		0.33	
Reject nullhypothesis*	no		no	
*The nullhypothesis being no differences				

During the time of additional precipitation and without, the humic acid level of the outflow seem to be unaffected by the humic acid level in the inflow. This can be assumed based on the significant correlation between the peak values in the inflow and the outflow during default running. There were also no significant differences between the mean values of the peak values in the inflow and the outflow regardless of additional precipitation or not.

The analysis showed that the level of humic acid relative to TOC concentration in the outflow seem to have no correlation with the humic acid level relative to TOC concentration in the inflow just like the absolute level of humic acid. However, the mean value of the humic acid relative to TOC concentration showed significant difference during the time of additional precipitation. Since there was no significant difference for the absolute level of humic acid, it can be assumed that the additional precipitation had a larger impact on other organic compounds than the humic acid.

Figure 29 - Comparison between humic acid concentration of TOC in inflow vs outflow, low and high flow

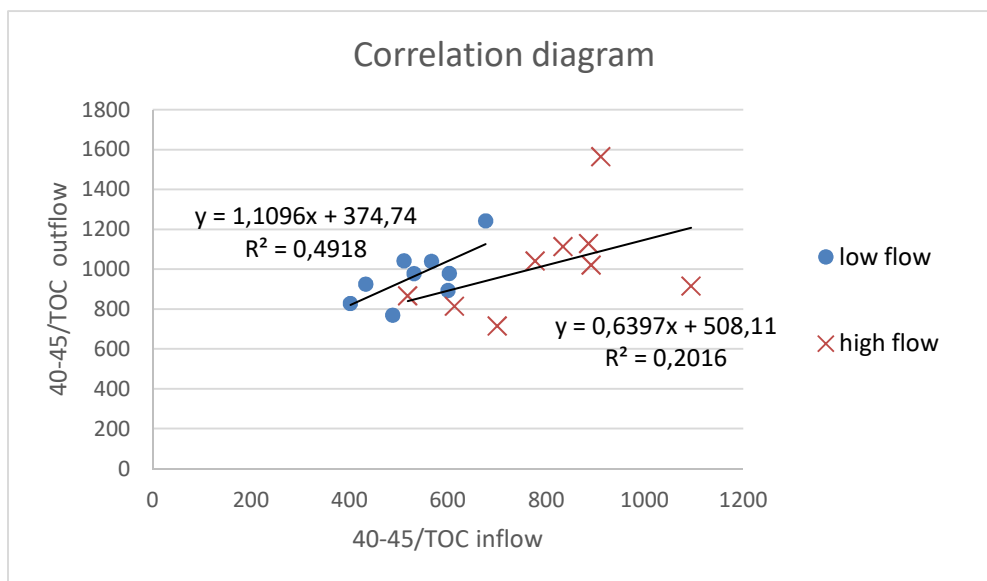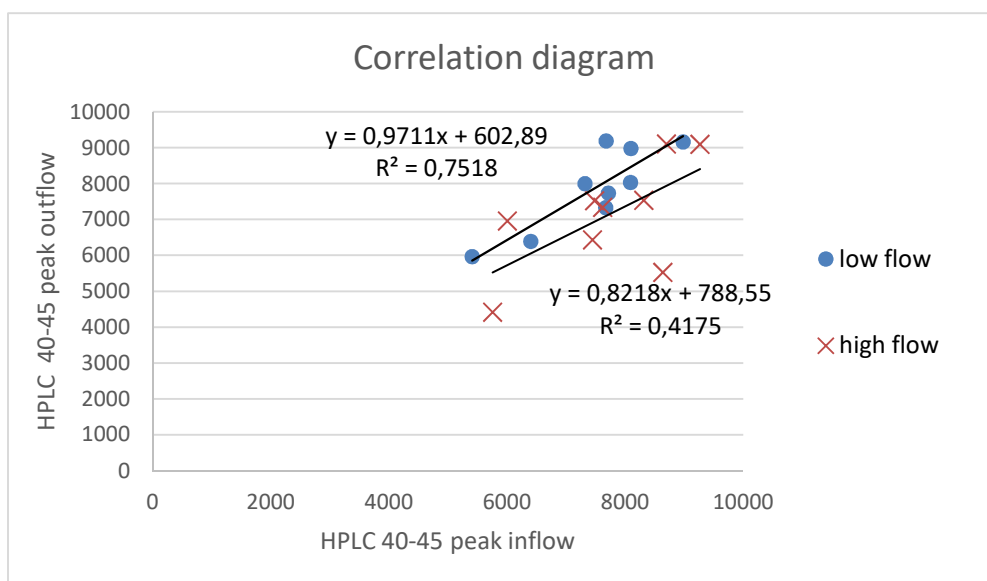**Figure 30 - Comparison between HPLC peak values in inflow vs outflow, low and high flows**

The summary of the comparisons between high and low flows for the HPLC analysis are found in Table 15 and Table 16.

Table 15 - Summary comparison 40-45/TOC low and high flow

40-45/TOC	Low flow (< 4.9 m³/s)		High flow (> 6.0 m³/s)	
	Inflow	Outfow	Inflow	Outflow
Mean value, μ	534	967	802	1021
Standard deviation, σ	87.3	138.1	173.2	246.8
Ratio σ/μ	0.163	0.143	0.216	0.242
Correlation, r^2	0.49		0.20	
p-value correlation	0.035		0.23	
Significant correlation	yes		no	
p-value nullhypothesis	1.1×10^{-6}		0.021	
Reject nullhypothesis*	yes		yes	
***The nullhypothesis being no differences**				

Table 16 - Summary comparison 40-45 peak low and high flow

40-45 peak	Low flow (< 4.9 m³/s)		High flow (> 6.0 m³/s)	
	Inflow	Outfow	Inflow	Outflow
Mean value, μ	7484	7870	7691	7109
Standard deviation, σ	1038	1162	1198	1523
Ratio σ/μ	0.139	0.148	0.156	0.214
Correlation, r^2	0.75		0.42	
p-value correlation	0.0025		0.06	
Significant correlation	yes		no	
p-value nullhypothesis	0.081		0.18	
Reject nullhypothesis*	no		no	
***The nullhypothesis being no differences**				

On the correlation diagrams, all comparisons show correlation. However, during high flows the correlations are not significant. It should be noted that the p-value for correlation between peak values during high flows is very close to show significant correlation. During both high and low flows, there were no significant differences between the mean values of peak values in the inflow and the outflow. However, there was a significant difference between the mean values of humic acid relative to TOC concentration. This indicates that for both low and high flows, the treatment process treats other organic compounds to a different degree than the humic acid. As can be seen by the mean values of the concentration of humic acid in comparison to the TOC concentration, the value is higher in the outflow than in the inflow.

5 Conclusion

Both SUVA$_{254}$ and S275-295 can be confirmed to be good parameters for evaluating the TOC. This was expected since it has been the conclusion of earlier studies. The HPLC analysis detected a large part of the organic compounds as indicated by the correlation between the area under the HPLC curve and the TOC concentration.

The peaks in the HPLC spectra obtained at 254 nm and having a retention time of 40-45 min where assumed to correspond to humic acids because a standard humic acid sample showed a distinct peak with that retention time. Previous research has also used the spectral parameter SUVA$_{254}$ as an indicator of the humic acid content of natural organic matter. Previous research also recommended S275-295 for measuring the concentration of organic compound. The analysis of comparing correlations between the amount of humic acid and the TOC concentration showed varying results depending on the method used for evaluating TOC concentration. The WWTP effluent showed more consistency in giving results indicating a stable relationship between the humic acid level and the TOC concentration from day to day than what the primary sedimentation basins effluent did. The level of humic acid in the primary sedimentation basins effluent also showed a correlation with the level of humic acid in the WWTP effluent. This indicates that the treatment process might be affected by the level of humic acid in the inflow.

When analyzing the effect of additional precipitation, it seemed like the spectral parameters were more affected by the flow than if additional precipitation was used or not. It was only during low flows that there was a significant difference between SUVA$_{254}$ in the inflow and outflow. For the levels of humic acid, it seems as f the additional precipitation affects the other organic compounds more than the humic acid. In fact, the levels of humic acid in comparison to the TOC concentration were significantly higher in the outflow than the inflow for all cases, except during the time of additional precipitation. However, even the samples taken during additional precipitation were not far from also showing significantly higher levels of humic acid in comparison to the TOC in the outflow than the inflow. The absolute levels of humic acid were close to be significantly higher in the outflow than in the inflow during low flows. If this would be correct, humic acid would not just be hard for the WWTP to treat, but it would also be a net production of humic acid during the process. Since the p-value vas very close to suggesting this, additional studies with increased number of samples could be made to give more accurate estimates.

Over all, additional studies could be made on particularly focusing on the humic acid, both in comparison to TOC concentration and absolute levels, in the inflow using larger amount of samples in order to increase the accuracy of the comparison between inflow and outflow. This study used a rather small number of samples. The effects assumed to be a result of high and low flows could have been affected by the additional precipitation since additional precipitation was added both during low and high flows.

6   References

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7 Appendix

Table 17 shows the data used for the calculation based on the effluent of the primary sedimentation basins. The FS added to the description means that the data refers to the effluent of the primary sedimentation basins.

Collection day	TOC FS [mg/l]	254 FS	SUVA$_{254}$	215 FS	SUVA$_{215}$
07-jan	11.97	0.282	0.0236	0.651	0.0544
08-jan	12.21	0.229	0.0188	0.63	0.0516
09-jan	9.34	0.226	0.0242	0.702	0.0752
13-jan	10.44	0.232	0.0222	0.819	0.0785
14-jan	10.18	0.25	0.0246	0.749	0.0736
16-jan	8.60	0.214	0.0249	0.623	0.0724
20-jan	11.60	0.236	0.0204	0.551	0.0475
21-jan	12.06	0.231	0.0192	0.556	0.0461
23-jan	13.47	0.251	0.0186	0.59	0.0438
27-jan	8.22	0.185	0.0225	0.627	0.0763
28-jan	9.59	0.209	0.0218	0.838	0.0874
30-jan	7.90	0.211	0.0267	0.844	0.1069
03-feb	12.79	0.279	0.0218	0.642	0.0502
04-feb	14.91	0.287	0.0193	0.666	0.0447
06-feb	15.04	0.307	0.0204	0.706	0.0469
10-feb	13.62	0.29	0.0213	0.661	0.0485
11-feb	16.91	0.291	0.0172	0.683	0.0404
13-feb	16.59	0.301	0.0181	0.712	0.0429

Table 17 - Data of primary sedimentation basins effluent

Table 18 shows the data used for the calculation based on the WWTP effluent. The UT added to the description means that the data refers to the effluent of the WWTP.

Collection day	TOC UT [mg/l]	254 UT	SUVA$_{254}$	215 UT	SUVA$_{215}$
07-jan	7.22	0.210	0.0291	0.862	0.1193
08-jan	9.23	0.167	0.0181	1.33	0.1441
09-jan	7.38	0.159	0.0215	1.071	0.1451
13-jan	8.17	0.185	0.0226	0.968	0.1185
14-jan	5.81	0.147	0.0253	0.643	0.1106
16-jan	6.50	0.165	0.0254	0.868	0.1335
20-jan	8.03	0.171	0.0213	0.652	0.0812
21-jan	6.53	0.158	0.0242	0.516	0.0790
23-jan	7.20	0.136	0.0189	0.782	0.1086
27-jan	6.17	0.128	0.0207	0.676	0.1095
28-jan	6.17	0.148	0.0240	0.846	0.1370
30-jan	6.03	0.127	0.0210	0.839	0.1390
03-feb	8.20	0.173	0.0211	1.317	0.1606
04-feb	9.35	0.199	0.0213	1.428	0.1527
06-feb	8.81	0.211	0.0239	1.242	0.1409
10-feb	7.44	0.184	0.0247	1.196	0.1608
11-feb	8.64	0.202	0.0234	1.048	0.1213
13-feb	10.43	0.197	0.0189	0.918	0.0880

Table 18 - Data of the WWTP effluent

Table 19 shows the data used for the calculation based on the effluent of the primary sedimentation basin. The slope values are divided with the TOC concentration. shown in the columns as for example S275-295/TOC representing the slope value S275-295 divided by the TOC concentration.

Collection day	TOC [mg/l]	S275-295	S275-295/TOC	S350-400	S350-400/TOC
07-jan	11.97	0.00310	0.000259	0.00064	0.0000535
08-jan	12.21	0.00260	0.000213	0.00052	0.0000426
09-jan	9.34	0.00240	0.000257	0.00060	0.0000643
13-jan	10.44	0.00245	0.000235	0.00060	0.0000575
14-jan	10.18	0.00270	0.000265	0.00062	0.0000609
16-jan	8.60	0.00225	0.000262	0.00056	0.0000651
20-jan	11.60	0.00270	0.000233	0.00052	0.0000448
21-jan	12.06	0.00270	0.000224	0.00048	0.0000398
23-jan	13.47	0.00280	0.000208	0.00052	0.0000386
27-jan	8.22	0.00200	0.000243	0.00044	0.0000535
28-jan	9.59	0.00220	0.000230	0.00054	0.0000563
30-jan	7.90	0.00215	0.000272	0.00056	0.0000709
03-feb	12.79	0.00300	0.000235	0.00064	0.0000501
04-feb	14.91	0.00325	0.000218	0.00064	0.0000429
06-feb	15.04	0.00355	0.000236	0.00066	0.0000439
10-feb	13.62	0.00330	0.000242	0.00066	0.0000485
11-feb	16.91	0.00345	0.000204	0.00064	0.0000378
13-feb	16.59	0.00355	0.000214	0.00066	0.0000398

Table 19 - Data of primary sedimentation basin effluent

Table 20 shows the data used for the calculation based on the outflow.

Collection day	TOC [mg/l]	S275-295	S275-295/TOC	S350-400	S350-400/TOC
07-jan	7.22	0.00215	0.000298	0.00060	0.0000830
08-jan	9.23	0.00170	0.000184	0.00046	0.0000498
09-jan	7.38	0.00165	0.000224	0.00046	0.0000623
13-jan	8.17	0.00190	0.000232	0.00052	0.0000636
14-jan	5.81	0.00150	0.000258	0.00040	0.0000688
16-jan	6.50	0.00170	0.000262	0.00046	0.0000708
20-jan	8.03	0.00180	0.000224	0.00048	0.0000598
21-jan	6.53	0.00170	0.000260	0.00042	0.0000643
23-jan	7.20	0.00145	0.000201	0.00036	0.0000500
27-jan	6.17	0.00135	0.000219	0.00034	0.0000551
28-jan	6.17	0.00150	0.000243	0.00040	0.0000648
30-jan	6.03	0.00130	0.000215	0.00034	0.0000563
03-feb	8.20	0.00170	0.000207	0.00046	0.0000561
04-feb	9.35	0.00215	0.000230	0.00052	0.0000556
06-feb	8.81	0.00220	0.000250	0.00056	0.0000635
10-feb	7.44	0.00195	0.000262	0.00050	0.0000672
11-feb	8.64	0.00220	0.000255	0.00054	0.0000625
13-feb	10.43	0.00210	0.000201	0.00052	0.0000498

Table 20 - Data of WWTP effluent