

**Master's thesis**

**Monitoring of organic matter removal during wastewater  
treatment using HPSEC-UV-fluorescence**

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## TIIVISTELMÄ

Yhdyskuntien tuottamien jätevesien sisältämä orgaaninen aines on yksi pääasiallisista jätevedenpuhdistamolla poistettavista jäteveden komponenteista. Orgaanisen aineen poistumista jätevedenpuhdistamolla mitataan yleisillä parametreilla, kuten kemiallisella tai biokemiallisella hapenkulutuksella, joiden avulla ei saada tietoa orgaanisen aineen ominaisuuksista. Tämän työn tavoitteena oli karakterisoida orgaanisen aineen koostumusta tulevassa ja lähtevässä jätevedessä Nenäinniemen jätevedenpuhdistamolla. Työssä määritettiin, kuinka hyvin tutkittavat yhdisteet poistuivat jäteveden puhdistamolla, sekä minkä kokoiset osuudet tutkituista yhdisteistä olivat huonosti poistuvia ja kuinka paljon eri kokoisten yhdisteiden poistuminen vaihteli. Lisäksi selvitettiin, millaisia yhdisteitä on peräisin kaatopaikan suotovesistä. Karakterisointimenetelmänä käytettiin korkean erottelukyvyn nestekromatografian ja kokoerottelukolonnin yhdistelmää (HPSEC) UV- ja fluoresenssidetektoilla. UV-detektiota käytettiin aromaattisten yhdisteiden tunnistamiseen ja fluoresenssilla tunnistettiin tyrosiinin, tryptofaanin, fulvon ja humuksen kaltaisia yhdisteitä. Tyrosiinin, tryptofaanin, fulvon ja humuksen kaltaisten yhdisteiden vastaavat keskimääräiset poistumisprosentit jätevedessä olivat  $90 \pm 1$ ,  $77 \pm 3$ ,  $27 \pm 4$  ja  $7 \pm 5$  %. Fulvon ja humuksen kaltaisissa yhdisteissä havaittiin tiettyjen kokoluokkien yhdisteitä, joiden määrä keskimäärin lisääntyi puhdistamolla. Kaatopaikan suotovedet sisälsivät suhteellisen paljon fulvon ja humuksen kaltaisia yhdisteitä. Kirjallisuuden perusteella lähtevässä jätevedessä havaitut yhdisteet ovat todennäköisesti vaikeasti poistuvia yhdisteitä tai muodostuneet puhdistamolla. Tämän työn perusteella HPSEC-UV fluoresenssi on tehokas menetelmä, jonka avulla saadaan perinteisiin parametreihin verrattuna laajempaa tietoa orgaanisen aineen ominaisuuksista sekä jätevedeen jäävistä yhdisteistä.

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## ABSTRACT

Organic matter in municipal wastewaters is one of main pollutants to be removed in wastewater treatment plant. Removal of organic matter is measured by common parameters, such as chemical or biochemical organic matter, which provide no information about characteristics of organic matter. The aim of this study was to characterize organic matter composition in wastewater influent and effluent samples in Nenäinniemi wastewater treatment plant. The removal of detected compound types and different size fractions and variations in their removals were studied. Poorly removable size fractions of each compound type were distinguished. The organic matter composition of landfill leachate was also investigated as one organic matter load source. High-performance size exclusion chromatography with UV and fluorescence detection was used as characterization method. Aromatic compounds were detected with UV detection, and tyrosine-like, tryptophan-like, fulvic-like and humic-like compounds were detected with fluorescence detection. Tyrosine-like compounds accounted for most of organic matter detected in wastewater influent, whereas in effluent fulvic-like compounds dominated. Removal percentages of tyrosine-like and tryptophan-like compounds were highest,  $90 \pm 1$ , and  $77 \pm 3$ , respectively, whereas removals of fulvic-like and humic-like compounds were low,  $27 \pm 4$ , and  $7 \pm 5$  %, respectively. Amount of fulvic-like and humic-like compounds were increased in some size fractions on average, indicating formation of these compounds during the treatment. Organic compounds in wastewater effluent were likely recalcitrant compounds or formed during the treatment. Landfill leachate contained relatively large amounts of fulvic-like and humic-like compounds. Based on this study, HPSEC-UV-fluorescence is an efficient method to provide for valuable information about organic matter characteristics and compounds which remain in wastewater after treatment.

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## **ABBREVIATIONS**

<b>BOD</b>	Biochemical oxygen demand
<b>COD</b>	Chemical oxygen demand
<b>DOC</b>	Dissolved organic carbon
<b>HPSEC</b>	High-performance size exclusion chromatography
<b>NOM</b>	Natural organic matter
<b>SMP</b>	Soluble microbial product
<b>SOC</b>	Synthetic organic compound
<b>TN</b>	Total nitrogen
<b>WWTP</b>	Wastewater treatment plant

## 1 INTRODUCTION

Wastewaters from municipalities contain large amounts of organic matter and nutrients, being major sources of eutrophication in natural waters if discharged untreated (Nathanson and Schneider 2008). In addition, the decomposition of organic matter in aquatic environments consumes oxygen and is one problem resulting from water pollution (von Sperling and de Lemos Chernicharo 2005). Therefore, organic matter and nutrients are among the most important characteristics of wastewater to be removed in a wastewater treatment plant (WWTP). Organic matter in wastewater contains a high variety of compounds, which originate from various sources (Shon *et al.* 2006). In WWTP, organic matter is removed by biological processes, which are efficient for the decomposition of organic compounds. Information about characteristics of organic compounds could be used to enhance wastewater effluent quality by adjusting operational conditions or increasing the removal of specific compounds by advanced treatment methods (Michael-Kordatou *et al.* 2015).

Quantitative parameters, such as biochemical oxygen demand (BOD), and chemical oxygen demand (COD), are commonly used in the evaluation of organic matter removal efficiency in WWTP (Michael-Kordatou *et al.* 2015). These methods provide information about the concentrations of biodegradable and non-biodegradable fractions of organic matter in wastewater. Nevertheless, no information is obtained about the composition of organic compounds in each fraction (Michael-Kordatou *et al.* 2015). For instance, the structure and functional groups of organic compounds affect their behavior in wastewater treatment processes (Jarusutthirak *et al.* 2002, Guo *et al.* 2011, Yang *et al.* 2014). To provide information about composition and



characteristics of organic matter, a variety of non-conventional methods have been used (Her *et al.* 2003, Kim and Dempsey 2012, Yu *et al.* 2013, Yang *et al.* 2014).

Specific information about wastewater organic matter composition can be obtained by the separation of compounds based on their size and shape. High-performance liquid chromatography combined with size exclusion chromatography (HPSEC) is a commonly used method for organic matter characterization (Her *et al.* 2003, Jarusutthirak and Amy 2007, Szabo *et al.* 2016). The method is efficient and compared with quantitative methods, less time consuming (Michael-Kordatou *et al.* 2015). In addition, the use of different detectors for detection of organic compounds after size exclusion enhances the applicability of HPSEC for various study purposes (Her *et al.* 2003, Jarusutthirak and Amy 2007, Guo *et al.* 2011, Keen 2017).

Natural organic matter in waters has been common area of study with HPSEC, but based on variety of studies, the method is also applicable to characterize wastewater organic matter (Zhou *et al.* 2000, Wang and Zhang 2010, Yan *et al.* 2012, Szabo *et al.* 2016). Majority of studies have concentrated on composition of organic matter in wastewater effluent (Her *et al.* 2003, Jarusutthirak and Amy 2007, Szabo *et al.* 2016). In addition, number of studies are found concerning removal or transformations of compounds during treatment of artificial or specific type of wastewater (Wang and Zhang 2010, Fan *et al.* 2011, Guo *et al.* 2011, Kawai *et al.* 2016).

In this study, the first aim was to evaluate the removal of different pollutants on a yearly level in Nenäinniemi WWTP monitored by conventional methods. Second, the removal of different compound types of organic matter was investigated by high-performance size exclusion chromatography with UV and fluorescence detection. Third, the variation in organic matter characteristics between sampling days was evaluated. Finally, the aim of this study was also to investigate landfill leachate as possible source for studied compounds and evaluate the usability of the method for landfill leachate samples.

## 2 THEORETICAL BACKGROUND

### 2.1 Wastewater parameters

#### 2.1.1 Overview

Quality of wastewater is evaluated by a variety of parameters, such as solids, biochemical oxygen demand, chemical oxygen demand, temperature and pH (Metcalf & Eddy 1991). In addition, the amounts of nitrogen and phosphorous are monitored to provide for good wastewater effluent quality. Operation of wastewater treatment plant (WWTP) is evaluated by measuring the amounts of solids, organic matter, and nutrients in wastewater effluent. Measured parameters are connected to each other; part of organic matter and nutrients are bound to solids, and the removal of organic matter is affected by temperature and pH (Metcalf & Eddy 1991).

#### 2.1.2 Solids

Solids are one of the most common parameter monitored in WWTP (von Sperling and de Lemos Chernicharo 2005). Solids can be divided by their filterability; dissolved solids remain in the water after filtration, while suspended solids are retained in the filter (von Sperling and de Lemos Chernicharo 2005). Suspended and dissolved solids can be further divided into fixed and volatile solids based on their behavior in combustion. Fixed solids cover inorganic compounds that remain after ignition, while volatile solids are organic compounds oxidized from the sample (Hammer and Hammer 2001, von Sperling and de Lemos Chernicharo 2005). Solids can also be divided into settleable and non-settleable solids (von Sperling and de Lemos Chernicharo 2005).

### 2.1.3 Organic matter

Biochemical oxygen demand (BOD) is a common parameter used in monitoring the biodegradable organic matter removal in WWTPs (Hammer and Hammer 2001, von Sperling and de Lemos Chernicharo 2005). BOD measures the oxygen consumption in the oxidation of organic carbon by microorganisms in 5 or 7 days (Hammer and Hammer 2001, SFS 5508).

Chemical oxygen demand (COD) includes both biodegradable and recalcitrant fractions of organic matter (von Sperling and de Lemos Chernicharo 2005). COD is determined by the oxidation of organic matter in the sample to carbon dioxide and water by a chemical oxidizer (Hammer and Hammer 2001). The amount of oxygen consumed in oxidation is measured. To oxidize all the organic matter, strong oxidizing agents, usually dichromate, are needed, which reduces the time needed for COD analysis (von Sperling and de Lemos Chernicharo 2005).

BOD/COD ratio is used to describe the fraction of biodegradable organic matter in wastewater (von Sperling and de Lemos Chernicharo 2005). Typically, the BOD/COD ratio of domestic wastewater is 0.4–0.8 (Metcalf & Eddy 1991). The low biodegradability of organic matter or inhibition of biochemical oxygen demand in wastewater results in a low BOD/COD ratio. For example, presence of high amounts of industrial wastewaters decreases the biodegradability. High ratio indicates that biological process is suitable for sufficient removal of organic matter in wastewater (von Sperling and de Lemos Chernicharo 2005).

Total organic carbon (TOC) measures all the organic carbon compounds of the sample (von Sperling and de Lemos Chernicharo 2005). TOC is determined in an instrumental test by the amount of carbon dioxide released from the sample. Inorganic carbon compounds in the sample must be removed prior to analysis to

obtain results only from organic carbon compounds (von Sperling and de Lemos Chernicharo 2005).

#### 2.1.4 Nitrogen

Nitrogen in domestic wastewater occurs in forms of organic nitrogen and ammonia (von Sperling 2005 and de Lemos Chernicharo 2005). After the aerobic decomposition of ammonia, nitrite and eventually nitrate is formed. Therefore, wastewater effluent contains ammonia when nitrification is not included in the wastewater treatment, and nitrate, when nitrification is included. Bacterial metabolism in biological treatment requires sufficient amount of nitrogen as nutrient (Hammer and Hammer 2001). Removal of nitrogen in WWTP is important to prevent oxygen consumption in receiving water body due to nitrification process (Hammer and Hammer 2001).

#### 2.1.5 Phosphorous

Phosphorous in wastewater includes inorganic phosphates, such as polyphosphates and orthophosphates, and organic phosphates in organic compounds (Hammer and Hammer 2001, von Sperling and de Lemos Chernicharo 2005). Detergents containing inorganic phosphorous are one source of phosphorous in wastewaters. Organic phosphates are mainly attached to particulate organic matter, whereas inorganic phosphorous occurs in soluble form in wastewaters. Phosphorous is also required in the growth of microorganisms in biological treatment. Sufficient amount of phosphorous is available in domestic wastewaters, but WWTP receiving large amounts of industrial wastewaters might need addition of phosphorous (Hammer and Hammer 2001, von Sperling and de Lemos Chernicharo 2005).

### 2.1.6 Temperature

Temperature is also an important parameter monitored during biological wastewater treatment (Hammer and Hammer 2001). Temperature range of 25–35 °C is optimal for microorganisms. In addition, the solubility of oxygen in water is affected by wastewater temperature. In high temperature, the solubility of oxygen is decreased (Metcalf & Eddy 1991).

### 2.1.7 pH and alkalinity

The hydrogen ion concentration of the solution is represented by pH (Metcalf & Eddy 1991). Alkalinity, on the other hand, represents the ability of water to resist pH changes when acid is added. Suitable pH level for microorganisms in biological treatment is important for efficient removal of organic matter (Metcalf & Eddy 1991). Optimal pH for biological treatment is between 6–8, which is usually achieved with domestic wastewaters (Gray 2004).

## 2.2 Wastewater treatment

### 2.2.1 Treatment methods

Wastewater treatment processes consist of preliminary, primary, secondary, and tertiary treatment (Metcalf & Eddy 1991). Treatment processes utilize physical, biological and chemical methods. Physical methods used in wastewater treatment include screening, mixing, flocculation, sedimentation, flotation, filtration and gas transfer (Metcalf & Eddy 1991, von Sperling and de Lemos Chernicharo 2005). Biological methods utilize the biological activity of organisms to remove most of the biodegradable fraction of organic matter, suspended solids, and, additionally, nitrogen and phosphorous (Metcalf & Eddy 1991, Hammer and Hammer 2001). Chemical reactions are utilized in chemical methods, such as precipitation,

adsorption and disinfection (Metcalf & Eddy 1991, von Sperling and de Lemos Chernicharo 2005).

### 2.2.2 Preliminary and primary treatment

In preliminary treatment, coarse solids are removed, usually by screening, to prevent possible operational failures in the treatment system (Metcalf & Eddy 1991). Thereafter, screening and sedimentation are used in primary treatment to remove settleable solids, mainly sand, and part of organic matter. In addition, the collection of floating materials, such as oil and grease, takes place in primary treatment (Metcalf & Eddy 1991, von Sperling and de Lemos Chernicharo 2005). As large amount of wastewater organic matter is in soluble form, only a small fraction of organic matter is removed in these processes (Metcalf & Eddy 1991, Michael-Kordatou *et al.* 2015). Additionally, chemical precipitation can be used in the removal of phosphorous and enhancement of suspended solids removal (Metcalf & Eddy 1991).

### 2.2.3 Secondary treatment

Major fraction of biodegradable organic matter and suspended solids are removed by secondary treatment, which is brought about by biological methods (Metcalf & Eddy 1991, von Sperling and de Lemos Chernicharo 2005). Most commonly, the activated sludge process is used (Gray 2004). In addition, nitrification and denitrification processes can be utilized to remove nitrogen from the wastewater (Metcalf & Eddy 1991). The end-products of secondary treatment are gaseous compounds and microbial biomass removed by settling. The removal of phosphorous can be enhanced by addition of metal salts in the aeration tank of biological treatment (Metcalf & Eddy 1991).

#### 2.2.4 Tertiary treatment

Tertiary treatment can be applied to remove toxic or non-biodegradable compounds, to enhance the removal of nutrients and suspended solids, or for wastewater hygienisation (Metcalf & Eddy 1991, von Sperling and de Lemos Chernicharo 2005). Commonly used tertiary treatment methods include filtration, flocculation, and adsorption (Metcalf & Eddy 1991, Gray 2004). Organic compounds not removed by secondary treatment can be removed by adsorption and hygienisation can be used to destruct disease-causing organisms. Wastewater hygienisation methods include, among others, ultraviolet radiation, chlorine and ozone (Metcalf & Eddy 1991).

### 2.3 Activated sludge process

#### 2.3.1 Overview

The activated sludge process is one of the most efficient and therefore among most commonly used biological treatment methods (Metcalf & Eddy 1991). In this process, culture of microorganisms is formed to degrade organic pollutants from wastewater (Metcalf & Eddy 1991, Hammer and Hammer 2001). Microorganisms use organic matter and nutrients in wastewater for growth, producing biomass, CO<sub>2</sub> and organic by-products. Aeration is used to provide the system with sufficient concentration of oxygen for aerobic microorganisms (Hammer and Hammer 2001).

#### 2.3.2 Removal of organic matter

Organic matter is removed from the wastewater by oxidation and biosynthesis (Gray 2004). Oxidation converts the organic compounds to end-products, whereas in the process of biosynthesis, new cellular material is formed from the organic matter in wastewater. As part of the organic matter in wastewater is transformed into microbial biomass, the biomass containing sludge must be separated from

treated water before discharge (Metcalf & Eddy 1991, Gray 2004). A fraction of sludge separated from the water is returned to the aeration tank to maintain microbial population (Hammer and Hammer 2001). Removal of excess sludge enhances the growth of bacteria and the removal of organic matter from wastewater (Metcalf & Eddy 1991, Hammer and Hammer 2001).

### 2.3.3 Floccs

Bacteria in the activated sludge form a floc, which reduces the number of free bacteria in the water (Metcalf & Eddy 1991, Hammer and Hammer 2001). Floc is a cluster of microbial cells attached to microbial material, adsorbed organic matter and non-reactive compounds in the wastewater (von Sperling and de Lemos Chernicharo 2005). Formation of floc is required for sufficient settling of solids from the water in activated sludge process (Metcalf & Eddy 1991, Gray 2004). As most of organic compounds in the wastewater are in particulate and colloidal form, the removal of this fraction is essential in the biological treatment (Gray 2004). Microorganisms cannot utilize organic matter in its particulate form, and therefore these compounds are adsorbed into the floc for metabolization (Gray 2004, von Sperling and de Lemos Chernicharo 2005). Before absorption into the floc, particulate BOD is hydrolyzed by extracellular enzymes to convert these compounds into soluble form and available for microorganisms (Gray 2004, von Sperling and de Lemos Chernicharo 2005).

### 2.3.4 Sludge settleability and sludge problems

The sufficient settleability of floc is brought about by both filamentous and floc-forming organisms in the floc (von Sperling and de Lemos Chernicharo 2005). Pinpoint floc with poor settleability occurs when the amount of filamentous organisms is lower relative to floc forming organisms (Gray 2004). As a result, flocs are small-sized and possess weaker structure. Reduced adhesion between flocs



results in sludge bulking and is caused by high number of filamentous organisms in the floc (Gray 2004, von Sperling and de Lemos Chernicharo 2005). Because of bulking, higher amount of sludge remains in the effluent. In addition, the quality of return sludge is reduced, which decreases the amount of microorganisms in the aeration tank (Gray 2004). Other problems related to sludge settling include, among others, dispersed bacterial growth with no formation of flocs, and floating of flocs caused by nitrogen gas formed in denitrification (Gray 2004).

## **2.4. Organic matter in wastewater**

### 2.4.1 Overview

The main components of organic matter in wastewater are biodegradable compounds, such as carbohydrates, proteins, and fats (Metcalf & Eddy 1991, Hammer and Hammer 2001). Smaller quantities of other organic compounds, such as urea, and synthetic organic compounds (SOCs) are present in wastewater (Hammer and Hammer 2001, von Sperling and de Lemos Chernicharo 2005, Shon *et al.* 2006). SOCs include synthetically produced compounds, such as detergents, surfactants and pharmaceuticals. Major fraction of SOCs are non-biodegradable in biological treatment, whereas large fractions of proteins and carbohydrates are degradable (Shon *et al.* 2006). In addition, microorganisms are also one constituent of organic matter in wastewater (Shon *et al.* 2006).

### 2.4.2 Proteins

Proteins are composed of amino acids, which contain mainly carbon, hydrogen, oxygen and varying amount of nitrogen (Hammer and Hammer 2001). Proteins in wastewaters are mostly from animal origin and food sources. As nitrogen is one component in proteins, they are one of the major sources of nitrogen in wastewater. (Metcalf & Eddy 1991). Proteins occur in both soluble and insoluble forms in

wastewater. Major fraction of proteins are easily degraded in biological treatment (Metcalf & Eddy 1991).

#### 2.4.3 Carbohydrates

Carbohydrates are composed of carbon, hydrogen and oxygen containing sugar units (Hammer and Hammer 2001). Carbohydrates in wastewaters include sugars, starches, and cellulose, and are mainly derived from food processing and lumber industries (von Sperling and de Lemos Chernicharo 2005, Shon *et al.* 2006). Sugars are soluble in water and easily degraded. Starches are insoluble but still degradable by microorganisms. Cellulose is non-biodegradable in the biological process timescale (Metcalf & Eddy 1991).

#### 2.4.4 Fats, oils and grease

Fats and oils are composed of fatty acids and glycerol (Metcalf & Eddy 1991). Fats, oil and grease, mainly derived from food products, can cause problems to wastewater treatment processes because of their low solubility in water (Metcalf & Eddy 1991, Hammer and Hammer 2001). In addition, mineral oils, such as road oils, are a source of greasy compounds (Metcalf & Eddy 1991). The biological treatability of wastewater can be decreased by presence of large amounts of fats (Hammer and Hammer 2001).

#### 2.4.5 Synthetic organic compounds

Synthetic organic compounds (SOCs) include surfactants, detergents, endocrine-disrupting chemicals, pharmaceuticals and personal care products (Metcalf & Eddy 1991, Hammer and Hammer 2001). These compounds are synthetically produced and derived from industries and households (Metcalf & Eddy 1991, Hammer and Hammer 2001). SOC's can be degraded during the wastewater treatment, adsorbed to sludge, or remain unchanged in the wastewater effluent (Metcalf & Eddy 1991).

Concentrations of SOCs are quite low but the removal of these compounds is difficult because of high number of a variety of compounds (Metcalf & Eddy 1991).

## **2.5 Organic matter in wastewater effluent**

### 2.5.1 Overview

Organic matter in wastewater effluent can be divided into major fractions of particulate and dissolved organic matter (Shon *et al.* 2006). Particulate organic matter in wastewater effluent consists of cells, bacterial flocs and organic debris (Shon *et al.* 2006). Dissolved effluent organic matter is mainly composed of natural organic matter (NOM), soluble microbial products (SMP), synthetic organic compounds, and disinfection by-products (Shon *et al.* 2006, Michael-Kordatou *et al.* 2015). Dissolved organic matter comprises organic compounds that are passed through 0.45  $\mu\text{m}$  filter. As dissolved organic matter in wastewater consists of a variety of compounds, the removal of this fraction is difficult with conventional methods, and a large fraction is found in wastewater effluent (Shon *et al.* 2006, Michael-Kordatou *et al.* 2015).

Characteristics of organic matter that remains in the effluent depends on WWTP conditions, treatment processes and wastewater origin (Her *et al.* 2003, Shon *et al.* 2006, Guo *et al.* 2011). For example, disinfection by-products can be formed in the reactions between disinfectant and dissolved organic matter in WWTPs where disinfection is applied (Michael-Kordatou *et al.* 2015). In addition, as SMPs are components of effluent organic matter formed during the biological process, the purification efficiency of wastewater can be influenced by adjusting process conditions suitable for microbes (Wang and Zhang 2010, Michael-Kordatou *et al.* 2015, Yu *et al.* 2015).

### 2.5.2 Natural organic matter

All the natural organic compounds in natural waters are referred to as natural organic matter (Michael-Kordatou *et al.* 2015). For example, humic acids, fulvic acids, low molecular weight (MW) organic acids, carbohydrates, polysaccharides and proteins are components of NOM (Michael-Kordatou *et al.* 2015, Sillanpää 2015). A fraction of NOM from drinking water source, majorly humic compounds, is poorly removed during WWTP, and therefore it remains in wastewater effluent (Shon *et al.* 2006, Nam and Amy 2008).

Compounds of NOM vary on their chemical structures, molecular weight and charge (Michael-Kordatou *et al.* 2015, Sillanpää 2015). Variation in NOM characteristics is caused by environmental conditions and NOM sources (Leenheer and Croué 2003). NOM can be divided into fractions of hydrophilic and hydrophobic compounds (Brezonik and Arnold 2011). The hydrophilic fraction consists of proteins, carbohydrates, and compounds with aliphatic structures, whereas the hydrophobic fraction contains humic compounds with aromatic structures (Brezonik and Arnold 2011, Sillanpää *et al.* 2015). Humic compounds can be divided into humic acids, fulvic acids and humins (Brezonik and Arnold 2011). Humic acids are insoluble in strong acids, whereas fulvic acids are soluble within the whole pH range. Humins are not soluble in water. Humic acids are usually larger in size and more aromatic than fulvic acids (Brezonik and Arnold 2011).

### 2.5.3 Soluble microbial products

SMPs are compounds, such as proteins, polysaccharides and humic compounds, that are produced by microorganisms in the biological treatment of wastewater (Shon *et al.* 2006, Michael-Kordatou *et al.* 2015). SMPs can be divided into utilization associated products (UAPs) and biomass associated products (BAPs) (Shon *et al.* 2006, Michael-Kordatou *et al.* 2015). Utilization associated products are formed

during bacterial metabolism, whereas biomass associated products originate from biomass due to the lysis of bacterial cells. As the composition of SMPs varies between different WWTP operating conditions and wastewater characteristics, the exact constituents of SMPs have not been identified (Shon *et al.* 2006, Michael-Kordatou *et al.* 2015).

Conditions that are harmful for microbes in the biological treatment process can increase the lysis of cells and affect the characteristics of SMPs (Wang and Zhang 2010). Different types of SMPs are produced under different kinds of harmful conditions, such as low pH, high temperature, and high salinity (Wang and Zhang 2010). Thus, by modifying the operational conditions of WWTP more suitable for microbes, the characteristics of SMPs can be altered or amount of these compounds can be decreased (Wang and Zhang 2010, Yu *et al.* 2015). In addition, an increase in sludge retention time has been observed to decrease the formation of SMPs in the process (Guo *et al.* 2011, Yu *et al.* 2015).

## **2.6 Non-conventional methods used in characterization of organic matter**

### 2.6.1 Overview

A variety of non-conventional methods, such as fractionation, chromatographic, and spectroscopic methods, are used for the characterization of organic matter in water (Michael-Kordatou *et al.* 2015, Sillanpää *et al.* 2015). These methods provide qualitative information about organic matter components, such as information about size, charge or polarity (Chow *et al.* 2005, Vitha 2017). Characterization of NOM has been utilized in a number of studies to provide information about its behavior in drinking water treatment processes (Chow *et al.* 2004, Zhao *et al.* 2009, Peleato and Andrews 2015). In addition, use of these methods have been reported on a variety of studies to characterize organic matter composition in various types of wastewater (Imai *et al.* 2002, Her *et al.* 2003, Janhom *et al.* 2011, Keen 2017).

### 2.6.2 Fractionation methods

Fractionation is used to divide dissolved organic matter into groups with specific chemical or physical characteristics (Chow *et al.* 2005). Physical and chemical fractionation methods include, for example, precipitation, solvent extraction, reverse osmosis, electrophoresis, ultrafiltration, and resin fractionation (Chow *et al.* 2005, Michael-Kordatou *et al.* 2015). In addition, chromatographic methods, such as size exclusion chromatography, or reversed-phase high-performance liquid chromatography, can be used for fractionation (Chow *et al.* 2005, Stenson 2008).

The most commonly used fractionation method is resin fractionation, which is a method used to divide organic matter components into hydrophobic and hydrophilic fractions (Leenheer 1981, Imai *et al.* 2002, Abbt-Braun *et al.* 2004, Chow *et al.* 2005). XAD resin fractionation, which utilizes commercially available Amberlite XAD resins in various pH conditions, has frequently been used (Leenheer 1981, Kim and Dempsey 2012, Xing *et al.* 2012). In this fractionation method, hydrophobic fractions are adsorbed onto XAD resins, whereas the hydrophilic fraction, not adsorbed onto resins, can be separated with cation and anion exchange resins (Leenheer 1981). This method is used by International Humic Substances Society (IHSS) as a standard method for fulvic acid and humic acid isolation (Brezonik and Arnold 2011).

Fractionation provides an isolation method of organic matter from water (Chow *et al.* 2005). In some cases, concentration of water samples with fractionation methods prior to analysis is needed when using advanced methods for analysis (Chow *et al.* 2005). Despite being used in a variety of studies, resin fractionation methods are laborious and rather expensive, and the use of strong acids and bases can alter the structure of organic matter (Peuravuori and Pihlaja 1997, Leenheer and Croué 2003, Song *et al.* 2009, Xing *et al.* 2012). In addition, the yield is quite low, because part of organic matter might be retained in the resins (Esteves *et al.* 1995, Santos *et al.* 2009).

In physical fractionation methods, such as membrane filtration, observed molecular weight might be different than that obtained with other methods (Schäfer *et al.* 2002, Schwede-thomas *et al.* 2005). Furthermore, the accumulation of molecules to the filter and variations in operational conditions affect the results of membrane filtration (Song *et al.* 2009, Kruger *et al.* 2011).

### 2.6.3 Chromatographic methods

Chromatographic methods are based on the separation of molecules in a column by intermolecular interactions (Vitha 2017). Depending on the type of chromatography, either gas or liquid is used as mobile phase to transport analyte molecules through the column. After separation, different methods can be used to identify and quantify separated components. Chromatographic methods are widely used in studies on organic matter because of possibility to provide qualitative or quantitative information (Vitha 2017).

Majority of organic compounds can be analyzed by liquid chromatography, whereas smaller fraction of organic compounds are volatile which can be analyzed by gas chromatography (Vitha 2017). Most columns used in liquid chromatography contain porous particle filling (Vitha 2017). Liquid chromatography is most commonly used with high pressure and columns with small particles to enhance the separation of components, in which case the method is referred to as high-performance liquid chromatography (HPLC) (Lough and Wainer 1996). The common methods of high-performance liquid chromatography used for organic matter characterization or detection of organic compounds in water environments include reversed-phase high-performance liquid chromatography (RPHPLC), high-performance size exclusion chromatography (HPSEC), and high-performance liquid chromatography mass spectrometry (HPLC-MS) (Leenheer and Croué 2003, Sillanpää *et al.* 2015).

In RPHPLC, the separation is based on the polarity of molecules and the method can also be used for fractionation (Stenson 2008, Vitha 2017). HPSEC, on the other hand, separates molecules based on their size and shape, rather than interactions (Vitha 2017). HPSEC has been used in various studies on wastewater organic matter (Her *et al.* 2003, Jarusutthirak and Amy 2007, Guo *et al.* 2011, Huang *et al.* 2016). In HPLC-MS, molecules are ionized after the LC column and information about the chemical constituents of analytes is provided based on their mass spectrum in mass spectrometry (Vitha 2017). Various LC-MS techniques have been used for the detection of pharmaceutical compounds and removal of a variety of pollutants in wastewaters (Li *et al.* 2000, Gebhardt and Schröder 2007, De Sena *et al.* 2009).

Gas chromatography is used to analyze volatile and semi-volatile compounds (Vitha 2017). High-pressured mobile phase, usually He, N<sub>2</sub> or H<sub>2</sub> gas, is provided to the column. Separation of the compounds is based on their structural characteristics. Unlike in liquid chromatography, the column in gas chromatography does not contain a particle filling, as analyte molecules interact with column-wall coating (Vitha 2017).

Gas chromatography mass spectrometry is among common methods used for the identification and quantitative analysis of organic compounds (Sparkman *et al.* 2011). For example, pharmaceuticals and antibiotics in wastewaters have been analyzed by GC-MS (Jones *et al.* 2007, De Sena *et al.* 2009). Pyrolysis gas chromatography-mass spectrometry (Py-GC-MS) is a method where high temperature is applied to degrade analyte compounds to smaller volatized compounds before the GC column and detection by MS (Wampler 2012). Py-GC-MS has been used to identify the components of wastewater effluent organic matter and organic matter in natural waters (Schulten and Gleixner 1999, Berwick *et al.* 2010, Greenwood *et al.* 2012, Chon *et al.* 2013).



Chromatographic analyses are rather inexpensive and efficient to characterize and identify organic compounds (Vitha 2017). Most detectors are easily available and applicability of a variety of detectors enhances the flexibility of chromatographic methods for different purposes (Her *et al.* 2003, Jarusutthirak and Amy 2007, Chon *et al.* 2013). On the other hand, results are dependent on the type of column used in separation and the mobile phase conditions (Lough and Wainer 1996, Vitha 2017). The characteristics of analyte compounds, such as polarity and structure, need to be considered when choosing the column, the mobile phase, and the detector to provide good separation and resolution of compounds (Lough and Wainer 1996, Vitha 2017).

#### 2.6.4 Spectroscopic methods

Spectroscopic methods used for the characterization of dissolved organic matter include Ultraviolet and visible light (UV-Vis) absorption spectroscopy and fluorescence spectroscopy (Michael-Kordatou *et al.* 2015, Sillanpää *et al.* 2015). UV-Vis absorption is used to detect light-absorbing structures, which are referred to as chromophores, in organic matter (Lambert *et al.* 1998). Chromophores in organic matter are, for example, double bonds between carbon atoms or carbon and oxygen atoms (Lambert *et al.* 1998).

Wavelength range or a single wavelength can be used for absorbance measurement (Sillanpää *et al.* 2015). The aromatic content of organic matter is measured by absorbance at 254 nm (Sillanpää *et al.* 2015). Specific UV absorbance (SUVA) is another commonly used method that provides information about the aromaticity of dissolved organic matter. The SUVA value of a sample is determined by dividing the UV absorbance at 254 nm by DOC concentration (Michael-Kordatou *et al.* 2015). High amount of aromatic compounds results in high SUVA value. Additionally, information about NOM characteristics has been provided by ratios of absorbance at different wavelengths (Hur *et al.* 2006, Li *et al.* 2009, Xu-Jing *et al.* 2011). For

example, Xu-Jing *et al.* (2011) used ratios of  $A_{250}/A_{365}$  and  $A_{253}/A_{203}$  to determine fulvic-acids content of organic matter and types of substituents in aromatic compounds in lake water samples.

Fluorescence is a phenomenon where energy absorbed by a molecule is emitted as light (Lakowicz 2006). First, irradiation at a certain wavelength provides energy that is absorbed by an electron in the molecule. This results in the excitation of the electron to a higher energy level. Collision and non-radiative decay reduce the energy of the electron before it returns to its ground state of energy and emits the energy by radiation at a certain wavelength. Therefore, the emission wavelength is different from the excitation wavelength (Lakowicz 2006). Excitation and emission wavelengths vary depending on the molecule (Hudson *et al.* 2007). Commonly, the fluorescence of a compound is caused by aromatic structure (Lakowicz 2006). In fluorescence spectroscopy, different types of fluorescent compounds, fluorophores, can be observed using different excitation-emission wavelength combinations for the detection of fluorescence (Hudson *et al.* 2007). Environmental conditions, such as pH, metal ions and temperature, can affect the wavelengths at which a compound is detected and observed fluorescence intensity (Hudson *et al.* 2007).

Fluorescence spectroscopic methods have been used to evaluate wastewater quality and the methods are suitable for such purposes (Hudson *et al.* 2008, Cohen *et al.* 2014, Goffin *et al.* 2018). In fluorescence spectroscopy, single fluorophore can be studied with specific excitation emission wavelength pair (Hudson *et al.* 2007). However, if multiple fluorophores are studied, other methods are more efficient. Information about a number of fluorophores can be obtained with excitation emission matrix fluorescence spectroscopy (EEMS) (Hudson *et al.* 2007, Carstea *et al.* 2016). In EEMS, the fluorescence intensity is scanned over a range of excitation emission wavelengths. Three-dimensional excitation-emission matrix (EEM) obtained by this method represents the excitation wavelength, the emission

wavelength and the fluorescence intensity (Hudson *et al.* 2007, Carstea *et al.* 2016). EEMS is commonly used for fluorescence studies on wastewater (Her *et al.* 2003, Hudson *et al.* 2008, Yu *et al.* 2015).

Despite its applicability for water quality monitoring, problems, such as biofilm formation on the instrument and effects of environmental conditions hinders the use of fluorescence spectroscopy for real-time monitoring of wastewater (Carstea *et al.* 2016). In addition, organic compounds with a variety of physico-chemical properties and similar fluorescence cannot be distinguished by fluorescence spectroscopy (Li *et al.* 2014, Yang *et al.* 2015a). Therefore, possible limitations need to be considered when applying fluorescence spectroscopy as a monitoring technique for wastewater quality (Carstea *et al.* 2016).

## **2.7 High-performance size exclusion chromatography**

### 2.7.1 Method description

High-performance size exclusion chromatography introduces qualitative information about the size of organic matter (Vitha 2017). HPSEC provides a rapid analysis of organic matter and only simple pretreatment of the sample is required (Chin *et al.* 1994, Her *et al.* 2002). In size exclusion chromatography, liquid sample moves in the column with mobile phase (Striegel *et al.* 2009). The column is a packing of porous particles that contain non-mobile liquid phase. Depending of pore size of particles, molecules of certain size range can migrate to the liquid phase inside a particle. As smaller molecules can permeate into particles, molecules with larger size are eluted first from the column. Based on this phenomenon, molecules can be divided into different fractions based on their elution time. Detection of the molecules takes place after the size exclusion column (Striegel *et al.* 2009).

### 2.7.2 Operational conditions

In HPSEC analysis of organic matter, operational conditions need to be considered in order to obtain valid results. As with other chromatographic methods, column, detector and mobile phase are chosen based on the type of sample (Vitha 2017). For example, the pore size of the column particles must be chosen based on the size of analyte molecules (Vitha 2017). Interactions between the sample and the stationary phase of the column will affect the retention time of sample molecules (Lough and Wainer 1996, Vitha 2017). Most commonly, silica-based and polymer-based columns are used in studies on organic matter in water environments (Her *et al.* 2002, Szabo *et al.* 2016, Chon *et al.* 2017). In silica-based columns, interactions between column and molecules are caused by hydrogen bonding and dipole-dipole interactions (Vitha 2017). Interactions between both column types and organic compounds have been observed (Hongve *et al.* 1996, Specht and Frimmel 2000). However, these columns provide good separation of organic compounds (Hongve *et al.* 1996, Szabo *et al.* 2016).

Ionic strength and pH of the mobile phase have effect on the behavior of organic molecules and the observed results (Hongve *et al.* 1996, Specht and Frimmel 2000, Szabo *et al.* 2016). Peak resolution decreases with lower ionic strength of the mobile phase, as repulsion between the column and organic matter is increased (Specht and Frimmel 2000). On the other hand, hydrophobic interactions with the column are enhanced in higher ionic strength (Hongve *et al.* 1996). Phosphate and acetate buffers with adjusted ionic strength have been used as mobile phase in analysis of organic matter in water (Hongve *et al.* 1996, Her *et al.* 2003, Szabo *et al.* 2016). Szabo *et al.* (2016) considered the effects of different eluent conditions on the separation of wastewater effluent organic matter with SEC using silica-based column and acetate eluent. They observed that neutral or slightly basic pH and low ionic strength of eluent was suitable for these study purposes (Szabo *et al.* 2016).

### 2.7.3 Different detectors used with HPSEC

HPSEC can be used with a variety of on-line detectors, and therefore the method is applicable to studies with different objectives (Her *et al.* 2003, Kawasaki *et al.* 2011). For example, DOC analyzer, UV/UV-Vis and diode array detectors, excitation-emission fluorescence detection, and combinations of these have been used for the characterization of organic matter in wastewaters (Her *et al.* 2003, Jarusutthirak and Amy 2007, Guo *et al.* 2011, Szabo *et al.* 2016, Keen 2017). Use of multiple detectors on-line provides more information about compounds comprising organic matter and their structures (Her *et al.* 2003, Jarusutthirak and Amy 2007, Guo *et al.* 2011, Szabo *et al.* 2016).

UV-Vis and diode array detectors are most commonly used for organic matter detection with HPSEC because of their good availability (Her *et al.* 2003, Jarusutthirak and Amy 2007, Guo *et al.* 2011, Szabo *et al.* 2016). UV-Vis detection provides mainly information about organic molecules with high MW because these compounds have more likely aromatic structures compared with low MW compounds (Sillanpää *et al.* 2015).

Fluorescence detection with a specific excitation emission wavelength provides information about specific fluorophore (Vitha 2017). By using a variety of excitation-emission wavelength combinations, a higher range of compounds can be detected compared with UV-Vis (Her *et al.* 2003, Guo *et al.* 2011, Szabo *et al.* 2016). For example, protein-like compounds can be targeted based on information from literature or results of EEM (Her *et al.* 2003, Guo *et al.* 2011, Szabo *et al.* 2016). Limitation of fluorescence is that it only provides information about compounds which fluoresce with the specific wavelength used for detection (Hudson *et al.* 2007).

Information about concentration of dissolved organic compounds in the sample is provided by on-line DOC detection (Her *et al.* 2002). Compounds lacking aromatic

or fluorescent structures are not visible by UV or fluorescence detection, and therefore DOC is good method for detection of other types of organic compounds (Her *et al.* 2002). On the other hand, DOC does not provide information about other characteristics of OM than size, and therefore this detector is useful when used in combination with other detectors (Her *et al.* 2003).

#### 2.7.4 Use of HPSEC with different detectors in studies on DOM

Her *et al.* (2003) used HPSEC with UVA-fluorescence-DOC detection to determine MW of different components of DOM from ground water, surface water and wastewater secondary effluent. Protein-like and fulvic-like substances were differentiated with fluorescence, and when comparing fluorescence results to DOC and SUVA values, characteristics of compounds could be concluded. For example, an increase in DOC concentration and protein-like fluorescence intensity of compounds with low MW indicated presence of protein-like substances, whereas an increase in DOC without response in other detectors was identified as aliphatic organic compounds (Her *et al.* 2003). Similarly, by using UV and DOC detectors, Jarusutthirak and Amy (2007) concluded that soluble microbial products formed in bench-scale sequencing batch reactors using artificial wastewater were hydrophilic compounds. In this study, high MW compounds (>10 000 Da) were observed to have an increase in DOC response but no response in UV absorbance, indicating presence of hydrophilic compounds (Jarusutthirak and Amy 2007).

## 2.8 Fluorescent compounds in water

Humic substances and amino acids are naturally occurring fluorophores and most frequently studied fluorescent compounds in natural waters (Hudson *et al.* 2007). Humic substances contain high amount of aromatic carbon structures, such as quinones, which cause their fluorescence (Brezonik and Arnold 2011). Only three amino acids, tyrosine, tryptophan, and phenylalanine, are fluorescent amino acids,

as they contain chemical structure suitable for excitation (Lakowicz 2006, Hudson *et al.* 2007). In tyrosine, aromatic ring structure containing electrons available for excitation causes the fluorescence of the amino acid (Fig. 1a) (Hudson *et al.* 2007). In tryptophan, functional group causing the fluorescence is indole group (Fig. 1b). Indole group consists of a benzene ring fused to a heterocyclic aromatic ring containing nitrogen (Hudson *et al.* 2007). Fluorescence from proteins is most frequently detected from tryptophan and tyrosine, because emission from phenylalanine is not usually observed (Lakowicz 2006).

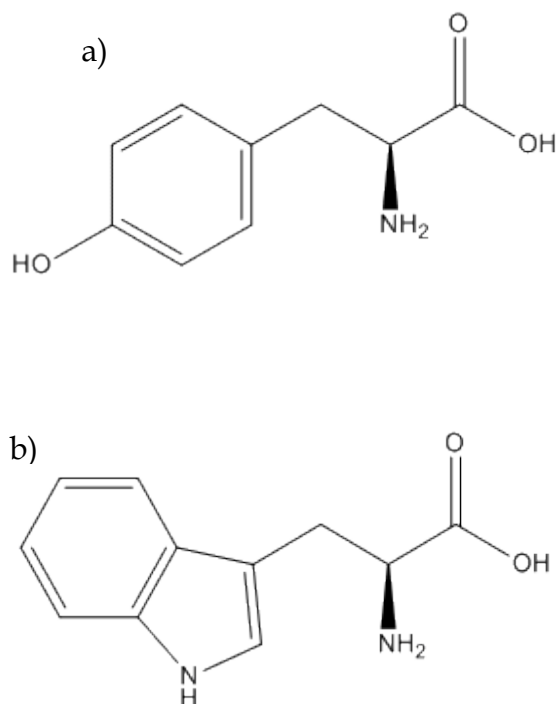


Fig. 1. Chemical structures of a) tyrosine and b) tryptophan.

Humic and fulvic compounds and proteins detected by fluorescence are called humic-like, fulvic-like, tyrosine-like and tryptophan-like compounds, as the identification of a specific fluorescent compound in waters is problematic (Hudson *et al.* 2007). Due to the influence of environmental conditions, the excitation emission

wavelengths at which these compounds fluoresce detected in various studies are different (Table 1) (Coble 1996, Marhaba *et al.* 2000). For example, the wavelength range where tryptophan-like fluorescence is detected depends on the solvent conditions, such as polarity and pH (Lakowicz 2006). In addition, energy absorbed by tyrosine can be transferred to tryptophan in the same protein, and therefore excitation and emission wavelengths at which protein-like fluorescence is observed might be different in different studies (Lakowicz 2006, Goffin *et al.* 2018).

Table 1. Excitation emission wavelengths for common fluorophores detected in water environments.

Compound type	Ex./Em. wavelength (nm)	Author
Tryptophan-like	275/310	(Coble 1996)
	225–237/345–381	(Coble 1996, Marhaba <i>et al.</i> 2000)
Tyrosine-like	275/340	(Coble 1996)
	225–237/309–321	(Marhaba <i>et al.</i> 2000)
Fulvic-like	260/380–460	(Coble 1996)
	237–249/417–429	(Marhaba <i>et al.</i> 2000)
Humic-like	350/420–480	(Coble 1996)
	297–309/417–429	(Marhaba <i>et al.</i> 2000)

Humic-like fluorescence is the most abundant in natural waters, whereas protein-like fluorescence is dominant in wastewaters (Yang *et al.* 2015b). Humic-like compounds in water are derived from both terrestrial and microbial sources (Ishii



and Boyer 2012, Yang *et al.* 2015b). In several studies conducted on wastewater fluorescent compounds, lower removal of humic-like and fulvic-like compounds has been observed in WWTP compared with that of protein-like compounds, indicating poor degradability of this fraction (Yu *et al.* 2013, Yang *et al.* 2014, Cohen *et al.* 2014). In addition, Yang *et al.* (2014) observed that humic-like components were increased during WWTP.

Tyrosine-like and tryptophan-like components are largely derived from microbial activities and for most part removed during biological treatment in WWTP (Yu *et al.* 2013, Yang *et al.* 2014, Cohen *et al.* 2014, Yu *et al.* 2015). In addition, tryptophan-like fluorescence has been observed to correlate with wastewater monitoring parameters, especially BOD (Hudson *et al.* 2008, Yang *et al.* 2014). Yu *et al.* (2015) observed that tyrosine-like components were produced by microbial activity in sequencing batch reactors. Compounds from industrial sources influence the fluorescence properties of wastewater, and therefore variation between wastewaters originating from different source types can be observed (Baker and Curry 2004, Cohen *et al.* 2014, Yang *et al.* 2015a).

## 3 MATERIALS AND METHODS

### 3.1 Nenäinniemi wastewater treatment plant

#### 3.1.1 Process overview

Nenäinniemi WWTP is owned by Jyväskylän Seudun Puhdistamo Oy. Wastewaters from municipalities of Jyväskylä, Laukaa, Muurame and Uurainen are treated in Nenäinniemi WWTP. Treatment of wastewater is based on activated sludge process with parallel chemical precipitation. (JS-Puhdistamo 2018)

In the wastewater treatment process, part of dissolved phosphorous is precipitated by adding ferrous sulfate before preliminary treatment of wastewater (Hynynen 2017). Thereafter, coarse particles are removed by coarse screening, and, in addition, grease is removed (Hynynen 2017). Preliminary treatment includes also sand settling, which takes place in two V-shaped basins with aeration. After sand removal, smaller particles are removed from the wastewater by fine screening followed by primary clarifiers. Organic matter is washed from the screening waste and directed to primary clarifiers with wastewater (JS-Puhdistamo 2018). Primary clarification takes place in three circular tanks where precipitated phosphorous and part of solids are removed. Settled sludge is collected by scraper to the bottom of tank and directed to thickening (JS-Puhdistamo 2018).

In secondary treatment, wastewater is aerated in four activated sludge basins containing fine bubble aerators in the bottom (JS-Puhdistamo 2018). Majority of organic matter and part of soluble phosphorous and nitrogen compounds in wastewater is converted into microbial biomass of the activated sludge (Hynynen 2017, JS-Puhdistamo 2018). Sludge containing water from aeration basins is directed to final clarifiers where activated sludge is removed by settling. Most of settled sludge is returned to aeration basins and the rest is directed to sludge treatment.

Polyaluminium chloride, polymer and ferrous sulfate are added to enhance precipitation of phosphorous and sludge settling. After secondary settling, wastewater effluent is discharged into Lake Päijänne (JS-Puhdistamo 2018).

In sludge treatment, water is removed from the sludge by gravity thickening and mechanical thickener. Sludge is stabilized in three digesters in 38°C and anoxic conditions. In this process, digestion by anaerobic microorganisms takes place, and, as a result, methane and carbon dioxide are formed. After 16 days of anaerobic digestion, formation of biogas is halted by introducing oxygen to sludge by aeration. Biogas is used to produce energy in combined heat and power plant for use of the treatment plant. After aeration sludge dewatering takes place in centrifuges and is enhanced by addition of polymer. Thereafter sludge is stored and transported for composting in Mustankorkea waste treatment plant. (JS-Puhdistamo 2018)

### 3.1.2 Environmental permission

Environmental permission of Nenäinniemi WWTP valid until 31.12.2017 set only concentration limits for BOD, COD, phosphorous, and solids (Table 2). In addition, target value for ammonium nitrogen removal was 80 % in a year level. According to review decision of environmental permission granted by Supreme Administrative Court, limits of maximum concentrations in wastewater effluent are lowered and removal efficiencies increased. New limits also include nitrification of ammonium nitrogen. In addition, removal of fecal coliforms and enterococcus in a 90 % level is obligated during 1.4.-30.11. New limits were put into operation in 1.1.2018. (KHO 2013:164)

Table 2. Maximum concentrations and minimum removal efficiencies of monitored parameters according to previous (valid until 31.12.2017) and present (valid since 1.1.2018) environmental permissions. The values are calculated as quarter average.

Parameter	Environmental permission valid until 31.12.2017		Environmental permission valid since 1.1.2018	
	Concentration (mg/l)	Removal efficiency (%)	Concentration (mg/l)	Removal efficiency (%)
BOD <sub>7ATU</sub>	12 <sup>1</sup>	92	10 <sup>1</sup>	96
Total phosphorous	0.5	92	0.3	96
Solids	30	-	10	90
COD <sub>Cr</sub>	125	75	80	90
Ammonium nitrogen	-	-	4	80 <sup>2</sup>

<sup>1</sup> mg O<sub>2</sub>/l

<sup>2</sup> Nitrification level

To meet the new limits of environmental permission, enlargement of WWTP has taken place since 2016, and new processes are introduced in the wastewater treatment in summer 2018. As nitrification and hygienisation were not included in the wastewater treatment before, additional treatment techniques have been introduced during the enlargement. The volume of aeration basins of biological process has been enlarged from existing 12 000 m<sup>3</sup> to 29 000 m<sup>3</sup>, which enables nitrification process to occur. In addition, mixers for summer-time nitrogen removal has been installed to first aeration basins. To enhance solid removal, an additional final clarification basin has been constructed and disc filtration with micro filters as a tertiary treatment method is introduced after final clarifiers. Phosphorous is also

removed along with solids by microfiltration. A new tertiary method also includes hyginisation by UV radiation to remove pathogens. (JS-Puhdistamo 2016)

### 3.1.3 Wastewater quality in Nenäinniemi WWTP

Wastewaters from approximately 160 000 residents are treated in Nenäinniemi WWTP. In addition, wastewaters from various industrial sources account for large fraction of wastewater loading. Producers of industrial wastewaters causing major loading are food processing plants, waste treatment facilities, and machinery industries. (JS-Puhdistamo 2017)

In Nenäinniemi WWTP, poor settleability of sludge have occurred during autumn in 2012-2014 and 2016. This has resulted in reduced effluent quality, as increased amounts of phosphorous, organic matter and solids have been resulted due to sludge in the effluent. The cause of disturbance in the treatment plant has been investigated and it seems to originate from industrial or external source. (JS-Puhdistamo 2016, JS-Puhdistamo 2017)

## 3.2 Monitoring data

Monitoring data of water quality in Nenäinniemi WWTP in 2015–2017 was used to calculate averages and standard deviations for treatment efficiencies of solids, BOD, COD, total nitrogen and total phosphorous, and nitrification level. Averages and standard deviations were calculated for timescales of 2015, 2016, 1.1.-27.9.2017, and 29.6.-21.9.2017.

### 3.3 Samples

#### 3.3.1 Wastewater samples

Wastewater influent and effluent samples from Nenäinniemi WWTP were analyzed weekly during 30.6.-22.9.2017. Three additional samples were also analyzed during 5.-7.9.2017. Samples had been taken as 24-h aggregate samples. Samples were obtained from Nab Labs Oy and held in refrigerator before pretreatment. As a pretreatment, each sample was centrifuged in 50 ml sample tube for 15 min with 6000 rpm (Centrifuge: Harrier 18/80 MSE Refrigerated MSE, SANYO). After centrifugation, samples were filtered through 0.45 µm cellulose filter (VWR International) for HPSEC analysis. In addition, about 20 ml of filtered sample were stored in freezer for dissolved organic carbon (DOC) and total nitrogen (TN) analysis.

#### 3.3.2 Landfill leachate samples

Total of six landfill leachate samples were taken from Mustankorkea Oy in 18.10.-19.10.2017. Four samples were collected in 18.10.; samples from two collection wells receiving leachate from closed landfill sites (V1, V2), sample from a collection well receiving leachate from landfill site currently in use (V5) and sample from a well that collects all leachate from the area (V7). After collection, samples were filtered and frozen for both HPSEC, and DOC and TN analysis. Only sample V7 was centrifuged same way as wastewater samples before filtering. Samples from a collection well (V3) and a stabilization pond (V4) both receiving leachate from old landfill sites were collected in 19.10. Thereafter, samples were filtered and HPSEC analysis were run to all leachate samples the same day. Samples for DOC and TN analyses were frozen.

### 3.4 HPSEC-UV-fluorescence analyses

HPSEC analyses were conducted using high performance liquid chromatography (Shimadzu) with Phenomenex Yarra 3000x silica-based column. The mobile phase was 5 M phosphate buffer (2.5 M Na<sub>2</sub>HPO<sub>4</sub> + 2.5 M NaH<sub>2</sub>PO<sub>4</sub>). Injection volume of sample was 30 µl for effluent and 15 µl for influent. Flow rate was 1 ml/min. Diode array detector (SPD-M20A, Shimadzu) was used for UV absorbance with detection wavelength of 254 nm. Eight excitation/emission wavelength sets for fluorescence detection (Prominence RF-20Axs, Shimadzu) were used for tyrosine-like detection (Ex./Em. 220/310 nm and 270/310 nm), tryptophane-like detection (Ex./Em. 230/355 nm and 270/355 nm), fulvic-like detection (Ex./Em. 240/440 nm and 330/425 nm) and humic-like detection (Ex./Em. 270/500 nm and 390/500 nm). The first excitation/emission wavelength set for each compound was based on results from EEM and the second one was based on previous studies. The column was calibrated with polystyrene sulfonate standards (Sigma-Aldrich, Germany) with MWs of 210, 1 600, 3 200, 4 800, 6 400, 17 000, and 32 000 Da, and acetone with MW of 58 Da.

### 3.5 DOC and TN analyses

DOC and TN concentrations were measured using a TOC analyzer (Shimadzu TOC-L) equipped with total nitrogen measuring unit (Shimadzu TNM-L, Ordior). Samples were acidified with 80 µl of 2 M HCl prior to analysis. Standard solutions for carbon and nitrogen with concentrations of 2 mg/l, 10 mg/l and 100 mg/l were used. Three parallel measurements were conducted for each sample and average values of measurements were used.

### 3.6 Processing of fluorescence chromatograms

Fluorescence chromatograms from HPSEC analysis were combined and analyzed using MATLAB R2017a program. Fluorescence chromatograms of landfill leachates were normalized by wastewater flow from landfill site divided by average flow of WWTP:

$$F_{landfill} = F \times \frac{Q_{landfill}}{Q_{WWTP}}, \quad (1)$$

in which  $F$  is the fluorescence intensity per retention time,  $Q_{landfill}$  is average wastewater flow from landfill and  $Q_{WWTP}$  is average wastewater flow of Nenäinniemi WWTP. Wastewater flow through each leachate collection well V1–V5 was estimated to be 1/5 of total flow from landfill, and flow through collection well V7 was estimated to be total WW flow of landfill, as all leachates are collected to this well.

Fluorescence chromatograms from one wavelength combination for each compound were further processed. Wastewater influent and effluent chromatograms were divided to seven areas representing different peaks between retention time of 4.5–30 min. Integration of total fluorescence chromatograms and peaks was conducted. Data of total area of fluorescence chromatogram and peak areas were obtained and collected to Microsoft Excel 2016 software. Areas of total fluorescence chromatograms were normalized by wastewater flow as follows:

$$F_{flow} = \frac{F}{Q_{WWTP}}. \quad (2)$$

Data of wastewater flow was obtained from monitoring data. Areas of total fluorescence chromatograms were normalized also by measured DOC concentration of the sample:



$$F_{DOC} = \frac{F}{C_{DOC}}, \quad (3)$$

in which  $C_{DOC}$  is DOC concentration of the sample. Removal percentage for each type of fluorescent compound was calculated as follows:

$$Removal \% = \frac{F_{inf} - F_{eff}}{F_{inf}} \times 100 \%, \quad (4)$$

in which  $F_{inf}$  is the area of total fluorescence chromatogram in wastewater influent sample and  $F_{eff}$  is the corresponding area in wastewater effluent sample. removal percentage was calculated for results normalized by DOC concentration in a similar way. In addition, removal percentages for peak areas was calculated. Boxplots for peak removals of each compound type were created using IBM SPSS Statistics 24 software to represent differences in removal percentages of peaks between different days.

### 3.7 Processing of UV absorbance chromatograms

UV absorbance chromatograms were processed manually using LabSolutions Postrun Analysis software. Chromatograms were divided to seven areas representing different peaks between retention time of 4.3–14.2 min. Integration of total UV absorbance chromatograms and peaks was conducted using Manual Integration tool, and results were collected to Microsoft Excel 2016 software.

## 4 RESULTS

### 4.1 Treatment efficiencies of monitoring parameters

Most of monitored parameters in Nenäinniemi WWTP had over 90 % treatment efficiency (Table 3). The removal of solids from wastewater was the highest and had least variation in 2017 ( $97 \pm 1$  %), whereas in 2015 and 2016 removal of solids was slightly lower and had more variation ( $96 \pm 2$  % and  $96 \pm 4$  %, respectively). Nitrification level was lower than removal of other measured parameters, and the removal of total nitrogen was the lowest.

Table 3. Treatment efficiencies of different parameters in 2015, 2016, 29.6.-21.9.2017 and 1.1.-27.9.2017. Table represents average and standard deviation of removal efficiencies in the form of  $\bar{x} \pm SD$ .

Parameter	Removal (%) 2015	Removal (%) 2016	Removal (%) 29.6.-21.9.2017	Removal (%) 1.1.-27.9.2017
Solids	$96 \pm 2$	$96 \pm 4$	$97 \pm 1$	$97 \pm 1$
COD <sub>Cr</sub>	$93 \pm 2$	$93 \pm 3$	$94 \pm 3$	$93 \pm 2$
BOD <sub>7ATU</sub>	$97 \pm 1$	$97 \pm 2$	$95 \pm 11$	$97 \pm 6$
Total nitrogen	$27 \pm 7$	$26 \pm 9$	$28 \pm 6$	$26 \pm 7$
Total phosphorous	$96 \pm 3$	$95 \pm 4$	$96 \pm 2$	$96 \pm 1$
Nitrification level	$57 \pm 9$	$57 \pm 10$	$61 \pm 8$	$48 \pm 16$

## 4.2 Dissolved organic carbon and total nitrogen

Concentration of DOC in influent water samples varied between 70–80 mg/l in most samples, but in 6.9.-7.9. the concentration was 10–20 mg/l higher (Table 4, Fig. 2). Concentration of DOC in effluent had less deviation. Concentration of TN in influent and effluent had similar variation trends as DOC, but there was a slight increase of TN in influent and effluent in 6.7., and a decrease in 14.9 (Fig. 3). In addition, the concentration of TN in influent increased between 4.9.-7.9., but concentration in effluent remained stable. Removal of DOC was lower than removal of BOD or COD in monitoring data.

Table 4. Concentrations and removal percentages of dissolved organic carbon and total nitrogen. Table represents average and standard deviation of measurements in the form of  $\bar{x} \pm SD$ , n=15.

	DOC (mg/l)	TN (mg/l)
Influent	75.8 ± 8.9	62.3 ± 5.5
Effluent	18.8 ± 1.8	55.1 ± 4.6
Removal (%)	75.0 ± 2.7	11.5 ± 5.5

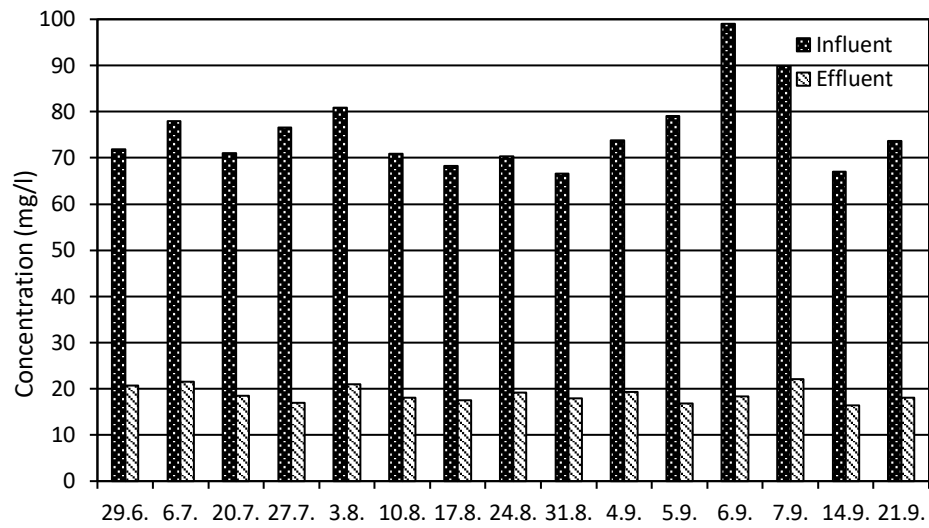


Fig. 2. Concentration of dissolved organic carbon in influent and effluent samples.

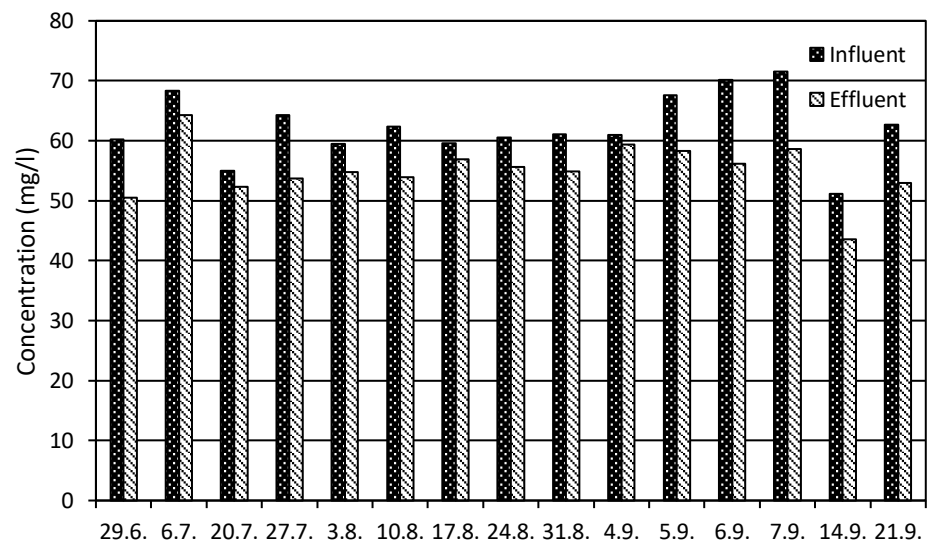
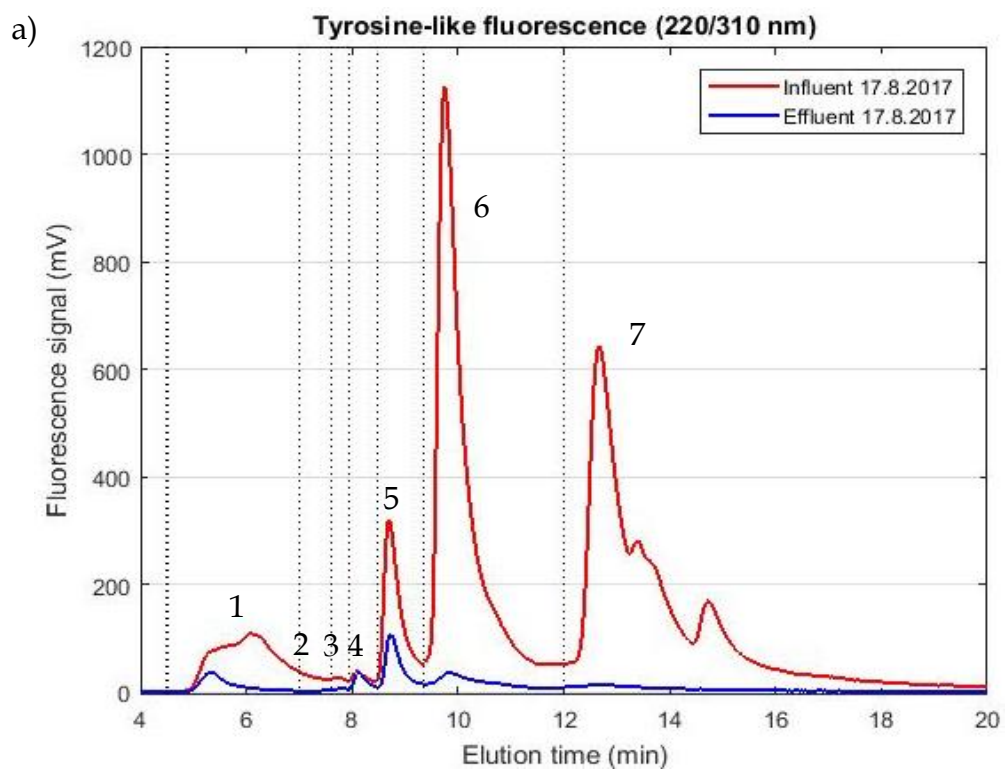


Fig. 3. Concentration of total nitrogen in influent and effluent samples.

### 4.3 Fluorescence chromatograms

Fluorescence signal of tyrosine-like compounds had four main peaks visible in the influent wastewater sample (Fig. 4a). Peaks 6 and 7 were largely disappeared in effluent, but quite large parts of peaks 4 and 5 still remained in effluent. Tryptophan-like fluorescence chromatogram had lower intensity than tyrosine-like (Fig. 4b). Peak 7 had reasonably low signal in influent, but still quite large peak in effluent compared with tyrosine-like fluorescence. Peaks 3, 4 and 5 were also largely remained in effluent.



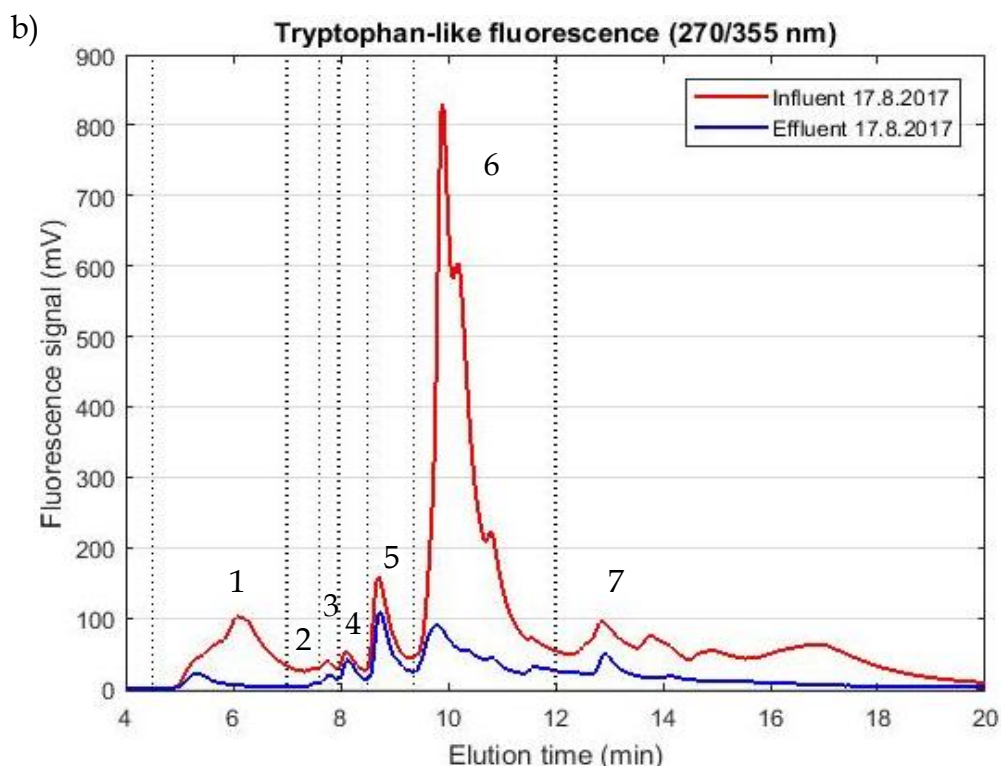


Fig. 4. Fluorescence chromatogram and peaks of a) tyrosine-like compounds (Ex./Em. 220/310 nm) and b) tryptophan-like compounds (Ex./Em. 270/355 nm) in influent and effluent samples as an example in 17.8.

Fulvic-like fluorescence chromatogram had quite different trends compared with protein-like fluorescence chromatograms (Fig. 5a). In both influent and effluent, five individual peaks were visible in chromatograms. Peak 6 was the largest in influent and decreased the most in effluent. Decreases of other peaks were rather small. Intensities of peaks 4 and 5 were increased in effluent. Peak 1 was not visible in either influent or effluent. Humic-like fluorescence chromatogram had quite similar trends as fulvic-like (Fig. 5b). Peaks 4, 5 and 6 were on similar level in influent but increased in effluent. Peak 1 did not represent much of the fluorescence intensity in influent or effluent.

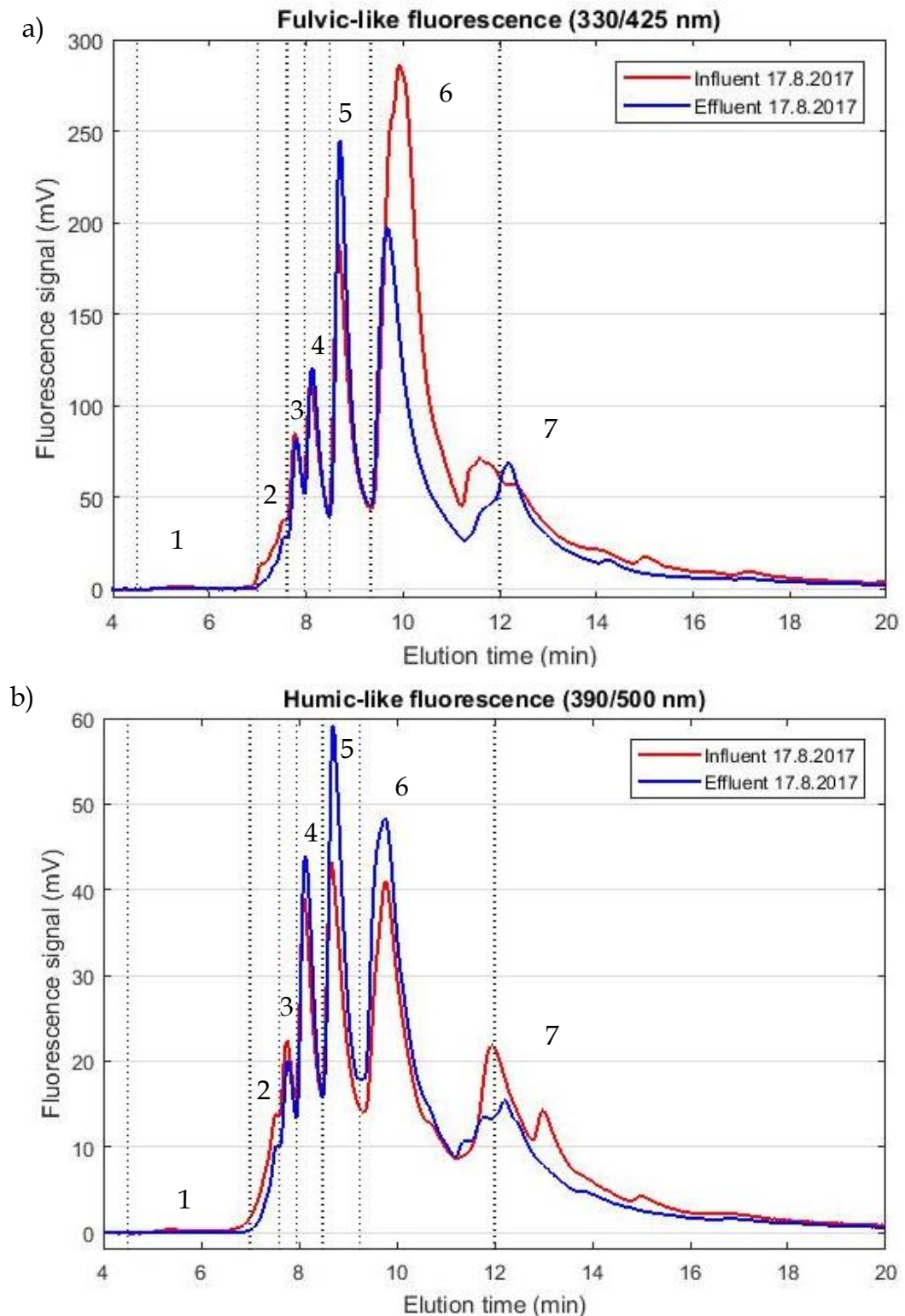


Fig. 5. Fluorescence chromatogram and peaks of a) fulvic-like compounds (ex./em. 330/425 nm) and b) humic-like compounds (ex./em. 390/500 nm) in influent and effluent samples as an example in 17.8.

#### 4.4 Total areas of fluorescence chromatograms

Protein-like compounds tyrosine and tryptophan accounted for most of total area of fluorescence chromatograms in influent water samples, on average  $2050 \pm 266$  and  $1290 \pm 143$  mV min, respectively, whereas fulvic-like and humic-like compounds had the lowest fluorescence areas, with respective averages of  $658 \pm 34$  and  $143 \pm 8$  mV min (Fig. 6). In addition, total fluorescence area of protein-like compounds had more variation than that of fulvic-like and humic-like compounds. There was noticeable increase in total fluorescence area of tyrosine-like compounds in 3.8., and between 4.9.-7.9.2017. Total fluorescence areas of tyrosine-like and tryptophan-like compounds decreased in 14.9.

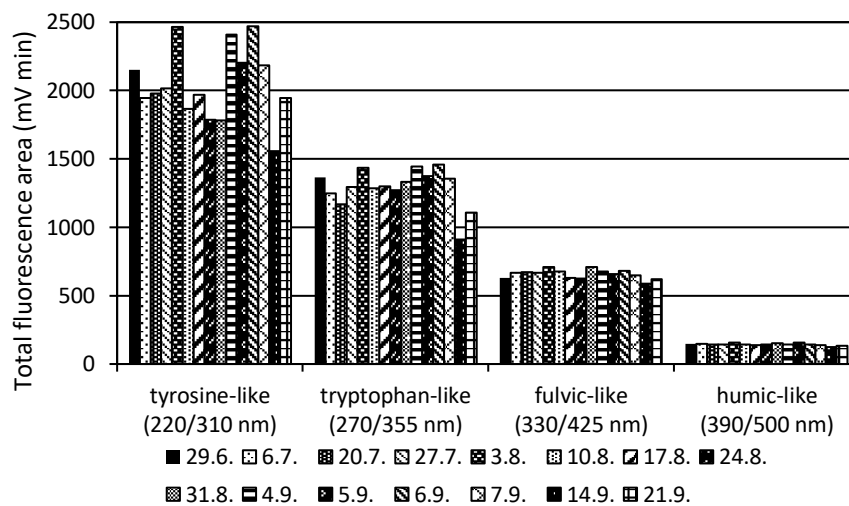


Fig. 6. Total fluorescence chromatogram areas of tyrosine-like, tryptophan-like, fulvic-like, and humic-like compounds in wastewater influent.

In wastewater effluent, total area of fluorescence was the largest for fulvic-like compounds, being on average  $454 \pm 28$  mV min (Fig. 7). Total fluorescence areas for tyrosine-like, tryptophan-like, and humic-like compounds were on average  $183 \pm 15$ ,  $299 \pm 29$ , and  $133 \pm 9$  mV min, respectively. The total fluorescence areas had mostly decreasing trend for all the compounds during the monitoring period. The area of tryptophan-like compounds was slightly higher in 6.7. In a similar way



as protein-like compounds in influent, all the compounds in effluent had slightly lower fluorescence area in 14.9.

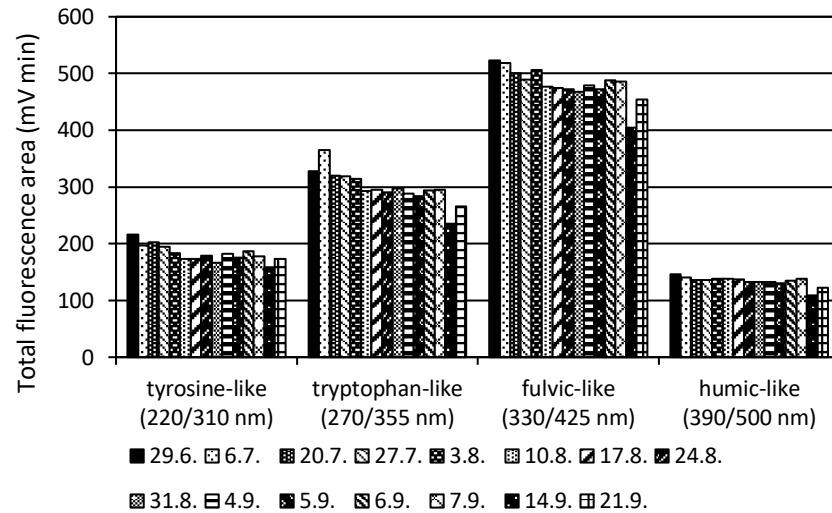


Fig. 7. Total fluorescence chromatogram areas of tyrosine-like, tryptophan-like, fulvic-like, and humic-like compounds in effluent.

#### 4.5 Total areas of fluorescence chromatograms normalized by flow

Total fluorescence areas of influent and effluent normalized by flow had quite similar variation trends as non-normalized results (Fig. 8 and 9). The fluorescence area of tyrosine-like and tryptophan-like compounds in influent fluctuated more than in effluent. For fulvic-like and humic-like compounds in influent, the variation of fluorescence area was quite low. In the same way as in non-normalized results, the fluorescence area of tyrosine-like, tryptophan-like and fulvic-like compounds in influent and all the compounds in effluent decreased in 14.9.

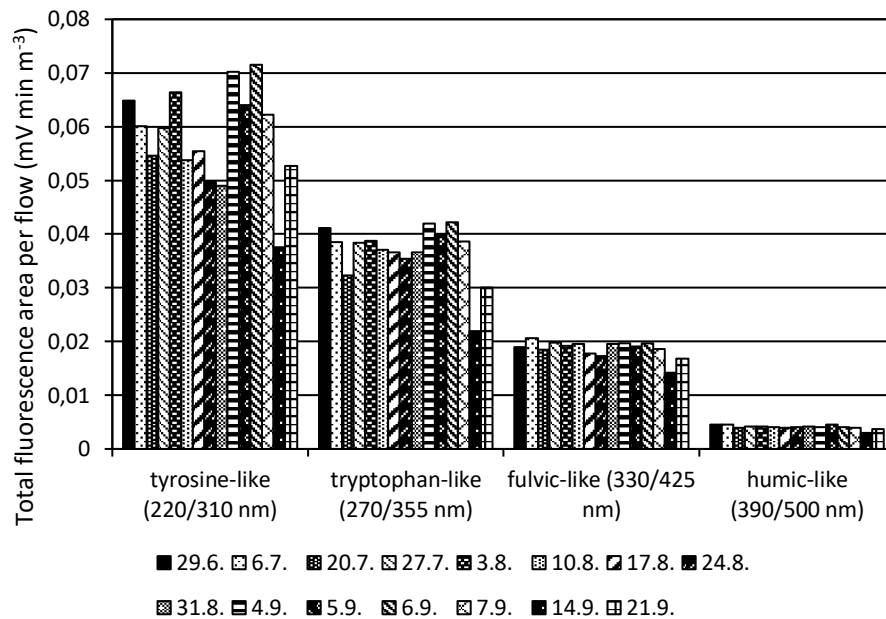


Fig. 8. Total fluorescence areas of tyrosine-like, tryptophan-like, fulvic-like, and humic-like compounds in influent normalized by flow.

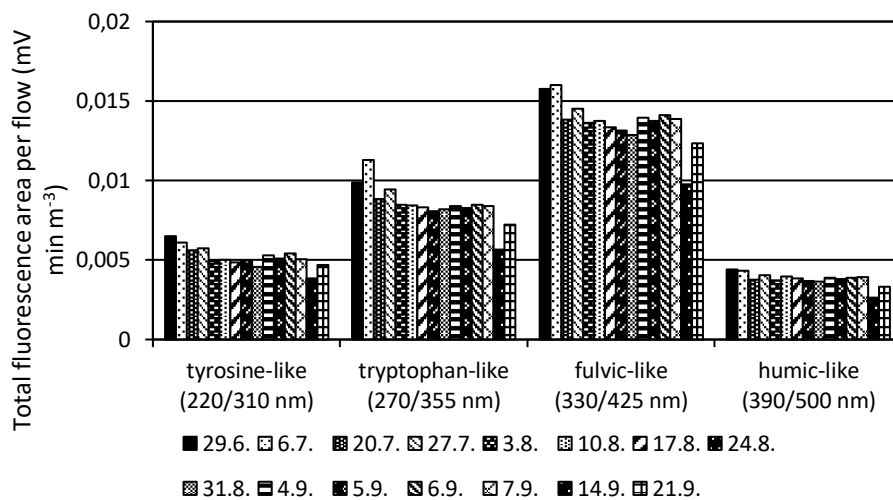


Fig. 9. Total fluorescence areas of tyrosine-like, tryptophan-like, fulvic-like, and humic-like compounds in effluent normalized by flow.

#### 4.6 Total areas of fluorescence chromatograms normalized by DOC concentration

Total fluorescence area of influent and effluent normalized by DOC concentration had different variation trends compared with non-normalized results (Fig. 10 and

11). Results of effluent had relatively more variation between sampling days. Especially fractions of fulvic-like compounds in effluent varied considerably. Fractions of tryptophan-like compounds were nearly the same in influent and effluent. On the contrary, fractions of fulvic-like and humic-like compounds of DOC concentration in effluent were twice as large as in influent.

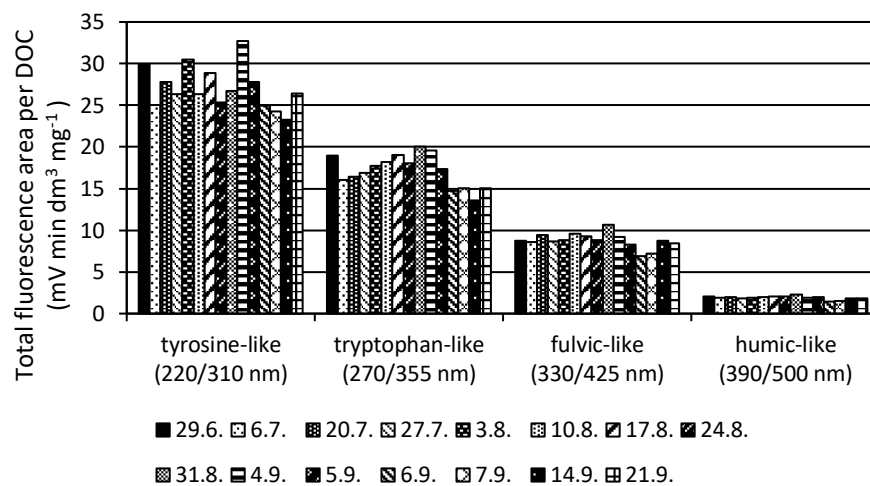


Fig. 10. Total fluorescence area of tyrosine-, tryptophan-, fulvic- and humic-like compounds in influent normalized by DOC concentration.

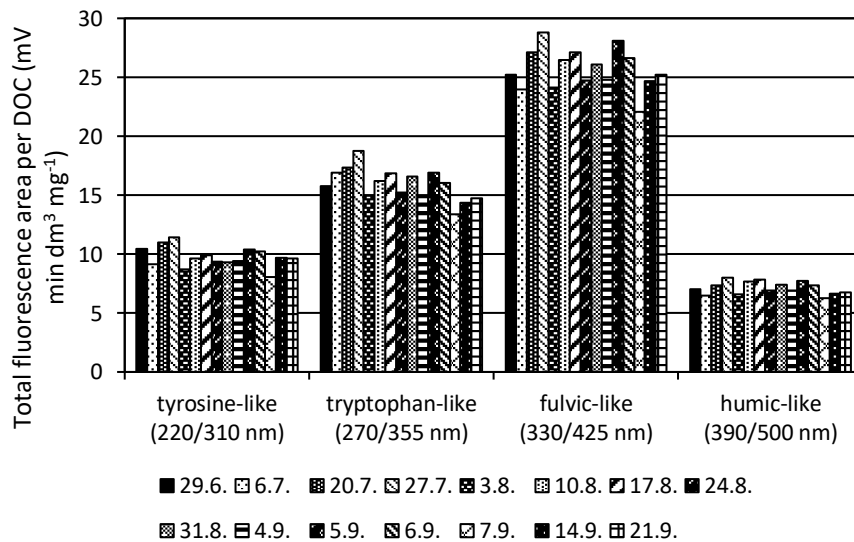


Fig. 11. Total fluorescence area of tyrosine-, tryptophan-, fulvic- and humic-like compounds in effluent normalized by DOC concentration.

#### 4.7 Removal percentages of compounds

Percentage removal of total fluorescence areas was quite steady for tyrosine-like compounds ( $90 \pm 1$  %), whereas slightly more variation could be seen for tryptophan-like compounds, with average removal of  $77 \pm 3$  % (Fig. 12). The removals of fulvic-like and humic-like compounds were low,  $27 \pm 4$  and  $7 \pm 5$  %, respectively, and variation between days was quite large. Removal percentages of compounds normalized by DOC concentration had totally different results than non-normalized results (Fig. 13). The removal percentages of fulvic-like and humic-like compounds were negative. The removal of tyrosine-like compounds was lower than in non-normalized results, and for tryptophan-like compounds the removal percentage per DOC concentration varied quite much and was in some parts negative.

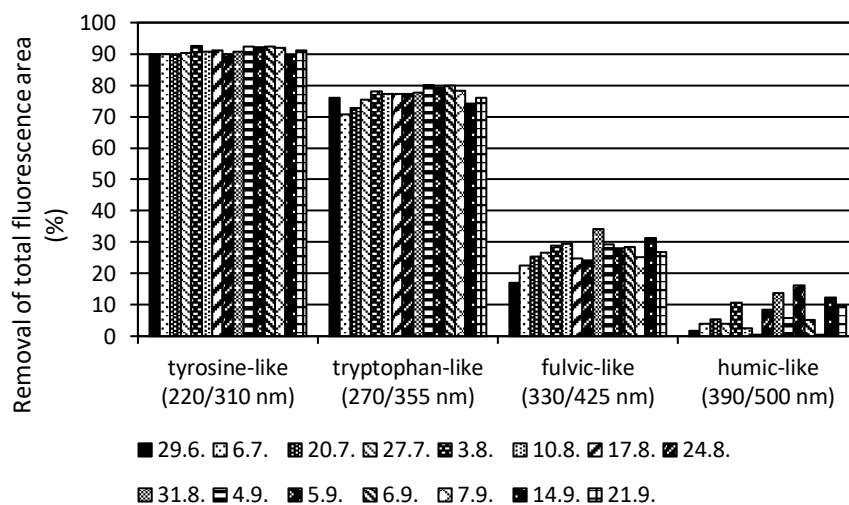


Fig. 12. Percentage removal of total fluorescence chromatogram areas of tyrosine-like, tryptophan-like, fulvic-like and humic-like compounds.

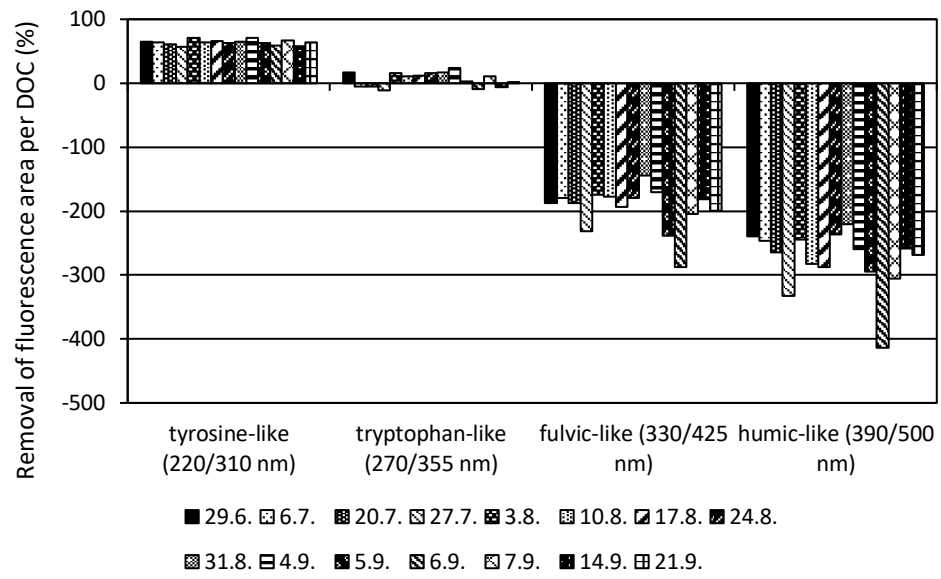


Fig. 13. Percentage removal of total fluorescence areas of tyrosine-like, tryptophan-like, fulvic-like and humic-like compounds normalized by DOC concentration.

#### 4.8 Fluorescence peak areas and removal of peaks

For tyrosine-like compounds in influent, peaks 6 and 7 covered the highest fractions of fluorescence peak areas (Fig. 14). Removal percentages of these peaks were also the highest ( $94 \pm 1\%$  and  $95 \pm 1\%$ ). Peak areas of peaks 1 and 5 were the second largest in influent, and, in addition to peaks 6 and 7, the major fractions in effluent. Removal percentage of peak 4 was the lowest ( $31\%$ ), but this peak was also one of the smallest in peak area.

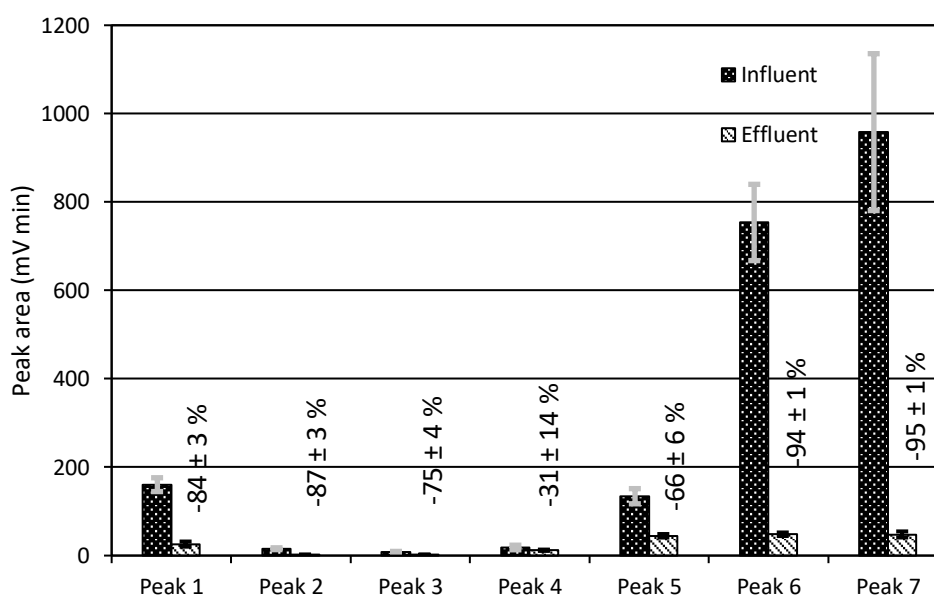


Fig. 14. Fluorescence peak areas of tyrosine-like compounds in influent and effluent and removal percentages of peaks. Graph represents average and standard deviation of results in form of  $\bar{x} \pm SD$ ,  $n=15$ .

In the same way as with tyrosine-like compounds, peaks 6 and 7 were the most abundant in tryptophan-like compounds in influent and effluent, and peaks 1 and 5 accounted for the second largest fractions (Fig. 15). Fluorescence peak area of peaks 6 and 7 had quite high variation range in influent. The average removal percentage of peaks 6 and 7 was lower than in tyrosine-like compounds,  $82 \pm 2\%$  and  $73 \pm 6\%$ , respectively, and their fraction in effluent was also larger. The lowest removal percentages were in peaks 4 ( $37 \pm 6\%$ ) and 5 ( $40 \pm 5\%$ ). As the removal percentage of peak 5 was quite low, it accounted for the third largest fraction of tryptophan-like compounds in effluent.

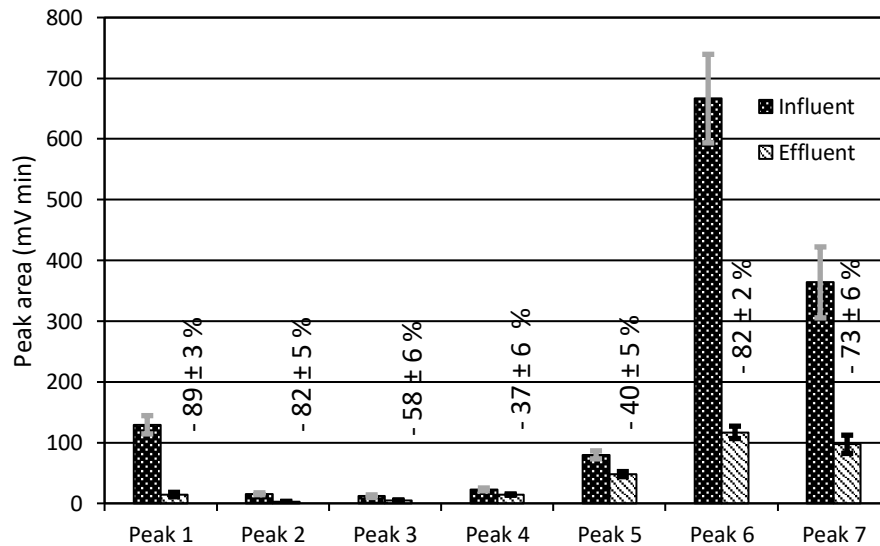


Fig. 15. Fluorescence peak areas of tryptophan-like compounds in influent and effluent and removal percentages of peaks. Graph represents average and standard deviation of results in form of  $\bar{x} \pm SD$ ,  $n=15$ .

The highest fractions of fulvic-like compounds in influent and effluent were in peaks 6 and 7 (Fig. 16). On the other hand, the removal percentages of these peaks were quite low,  $41 \pm 4 \%$  and  $21 \pm 3 \%$ , respectively. The highest removal percentage of fulvic-like compounds was in peak 1 ( $93 \pm 9 \%$ ), but this peak was the smallest in peak area. Peak 5 was the third largest in peak area, and it was increased in effluent on average.

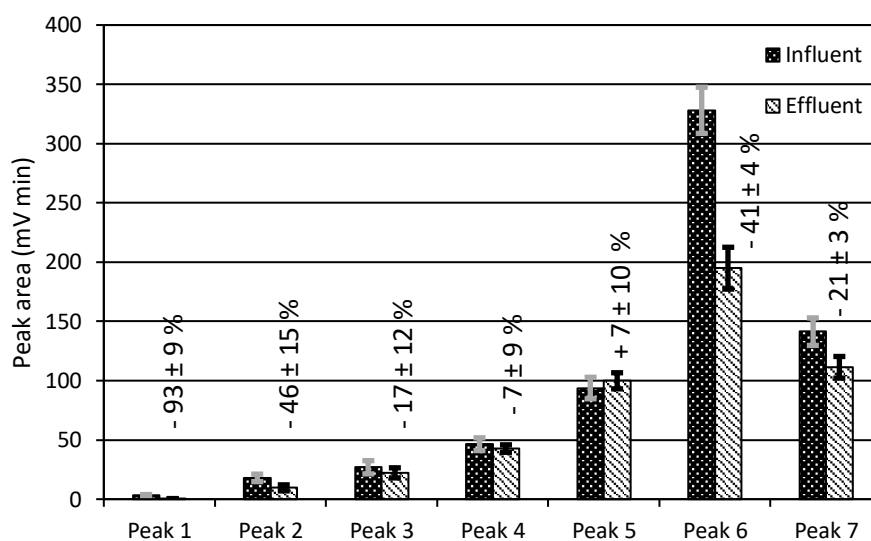


Fig. 16. Fluorescence peak areas of fulvic-like compounds in influent and effluent and removal percentages of peaks. Graph represents average and standard deviation of results in form of  $\bar{x} \pm SD$ ,  $n=15$ .



Fluorescence peak areas of humic-like compounds had similar trends as in fulvic-like compounds (Fig. 17). Peak 1 had the highest removal percentage ( $94 \pm 5\%$ ), but in the same way as in fulvic-like compounds, its peak area was also the smallest. Peaks 5, 6 and 7 accounted for the highest peak areas of humic-like compounds both in influent and in effluent. The average fractions of peaks 5 and 6 were larger in effluent than in influent. The average fractions of peaks 5 and 6 were larger in effluent than in influent.

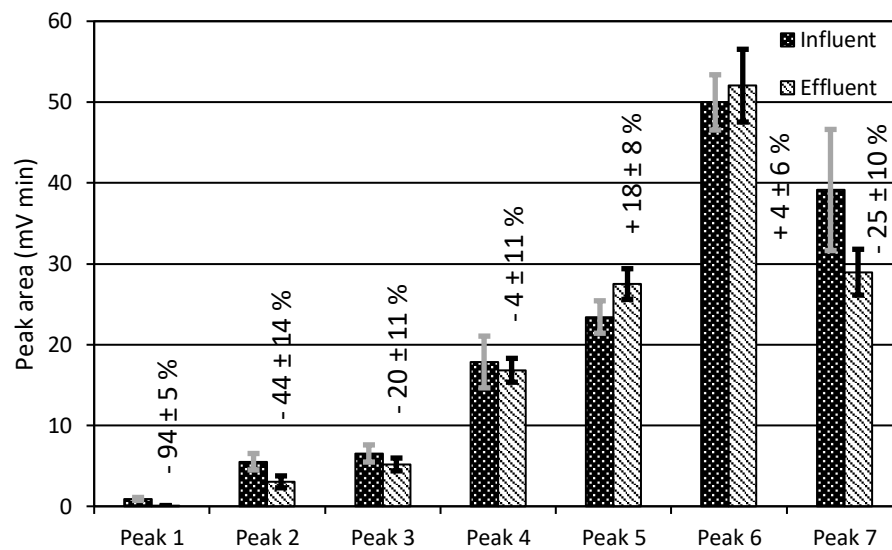


Fig. 17. Peak areas of humic-like compounds (270/355 nm) in influent and effluent and removal percentages of peaks. Graph represents average and standard deviation of results in form of  $\bar{x} \pm SD$ ,  $n=15$ .

#### 4.9 Removal percentages of fluorescence peak areas

The highest variation range in the removal percentage of tyrosine-like compounds was in peak 4, whereas the removal percentages of peaks 6 and 7 varied the least (Fig. 18). Peaks 1 and 3 had lower removal percentages than the normal range in 29.6., 77 % and 67 %, respectively. In 31.8., peaks 3 and 4 had higher removal percentages that were outliers, 83 % and 73 %, respectively. Peak 3 had higher removal percentage also in 3.8., and 24.8.

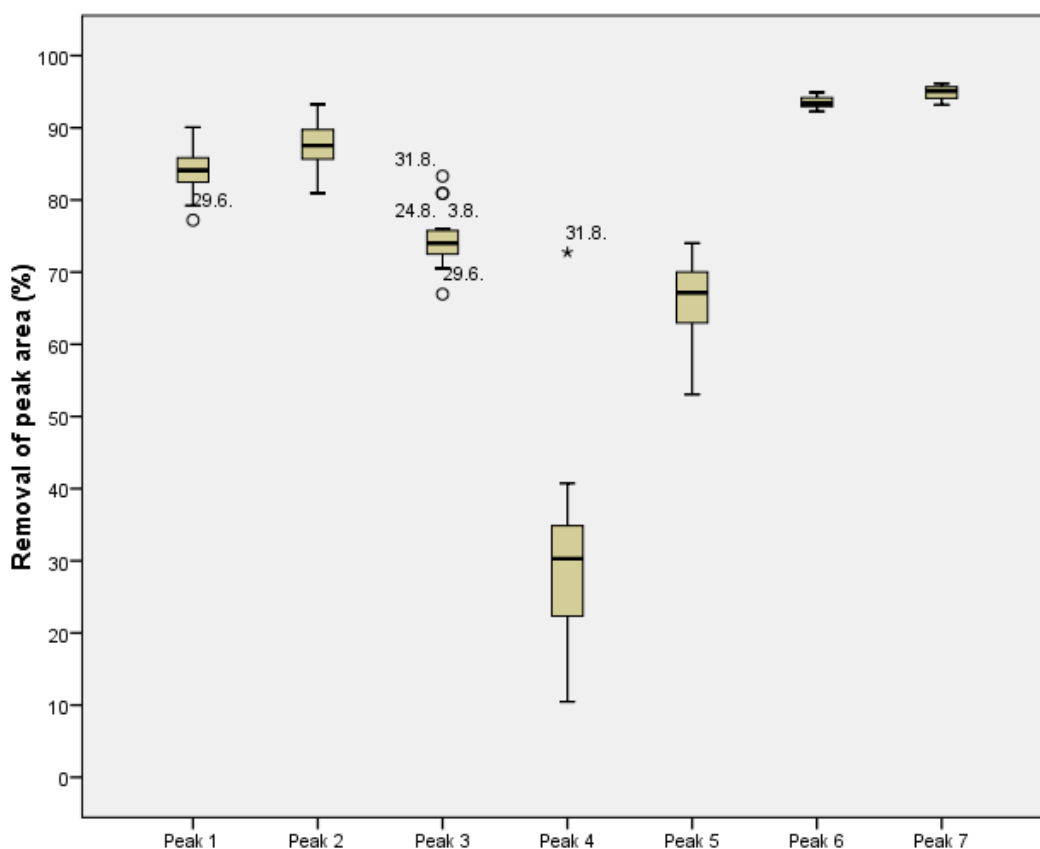


Fig. 18. Percentage removal of fluorescence chromatogram peak areas of tyrosine-like compounds.

Tryptophan-like compounds had higher variation range in the removal percentage of fluorescence peak area than tyrosine-like compounds (Fig. 19). The same way as with tyrosine, removal percentages of peaks 3 and 4 were higher than normally in 31.8, 73 % and 54 %, respectively. In addition, peak 7 had lower removal percentage in 6.7. (56 %).

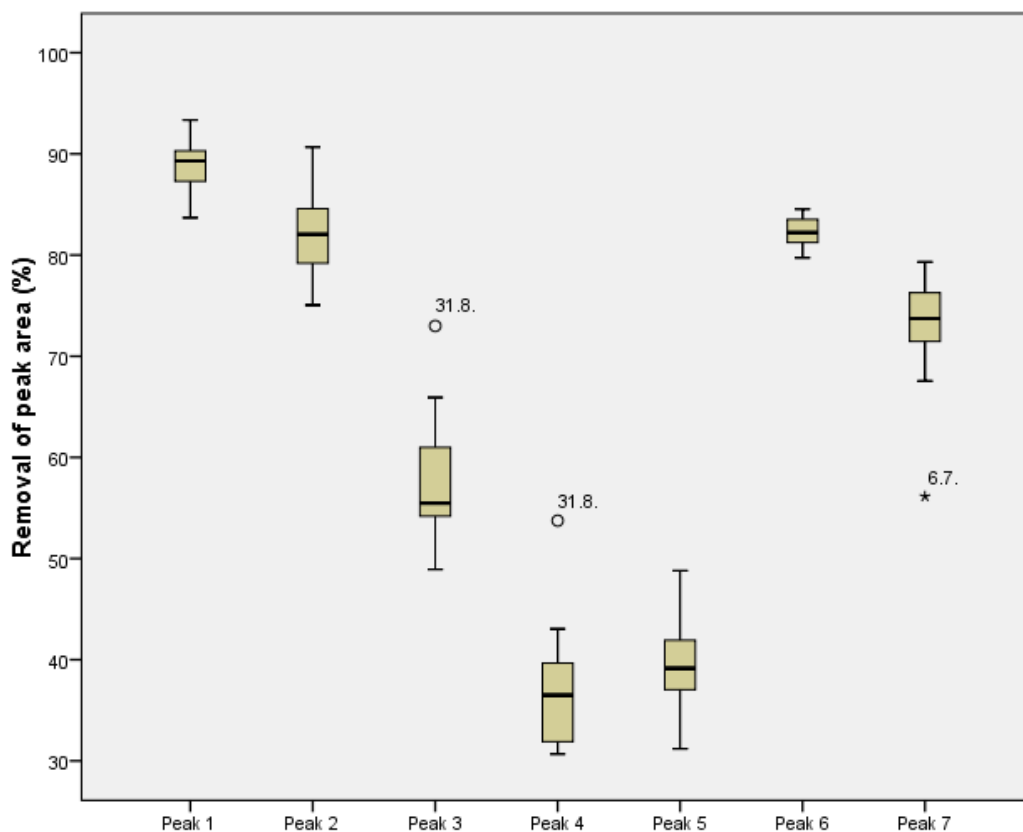


Fig. 19. Percentage removal of fluorescence chromatogram peak areas of tryptophan-like compounds.

Removal percentages of fulvic-like compounds had a large variation level, and, in comparison to protein-like compounds, removal percentages were quite low on average (Fig. 20). In 29.6., peaks 2 and 5 had lower removal percentages that were outliers. In 7.9., peak 2 had also lower removal. Peaks 2, 4 and 5 had higher removal percentages in 31.8. Peak 7 had higher and lower removal in 14.9., and in 20.7., respectively.

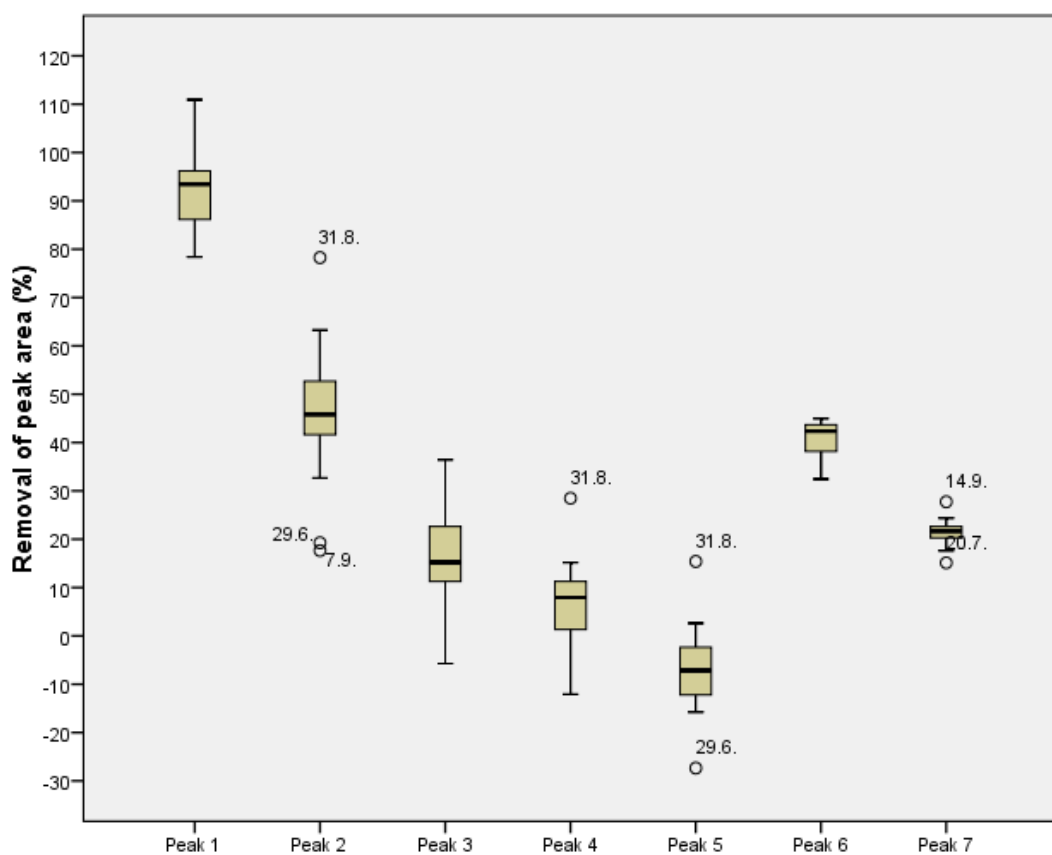


Fig. 20. Percentage removal of fluorescence chromatogram peak areas of fulvic-like compounds.

In the same way as with fulvic-like compounds, removal percentages of humic-like compounds are quite low (Fig. 21). Peaks 2 and 3 had lower removal percentages in 29.6, whereas in 31.8. peaks 2, 3 and 4 had higher removal percentages than normally. In addition, peak 7 had higher removal in 5.9.

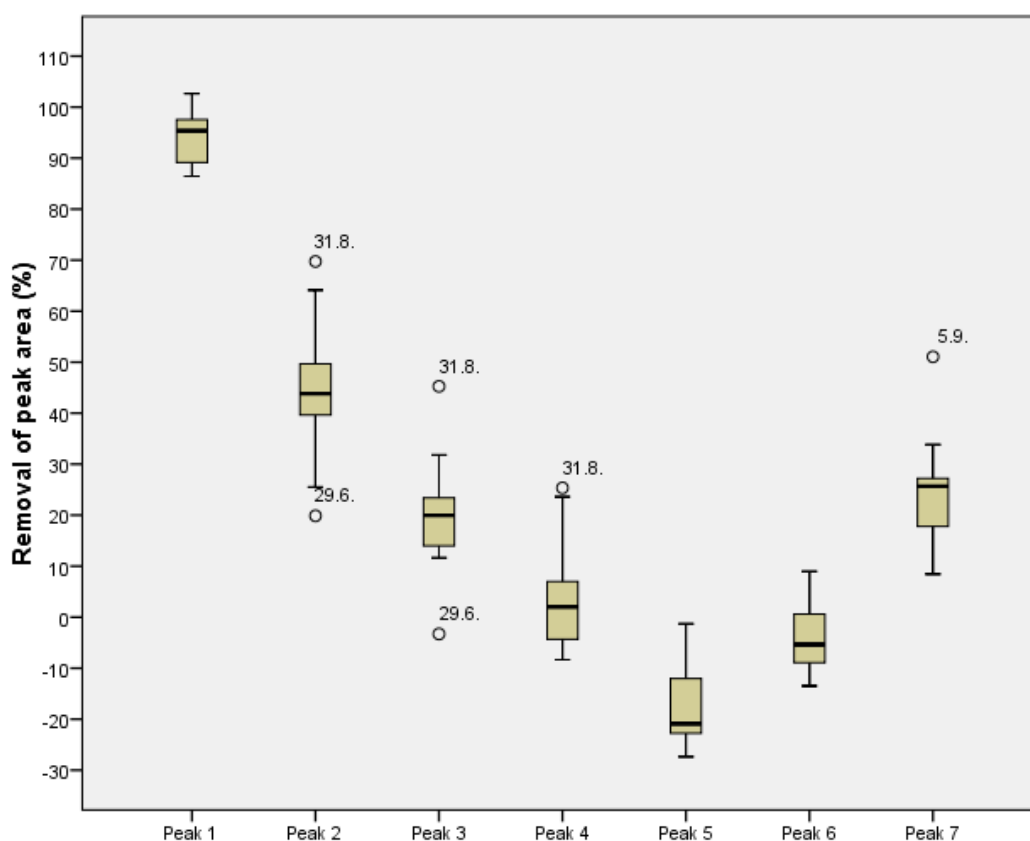


Fig. 21. Percentage removal of fluorescence peak area of humic-like compounds.

#### 4.10 Total areas of UV<sub>254</sub> absorbance chromatograms and removal percentages of peak areas

Total area of UV<sub>254</sub> absorbance chromatograms in influent was about twice as much as in effluent (Fig. 22a). Peak areas had seemingly similar trends as peak areas of fulvic-like fluorescence (Fig. 22b). Peaks 6 and 7 accounted for the largest fractions of UV absorbance in influent. The largest removal was in peak 1 ( $86 \pm 8\%$ ) and lowest in peak 4 ( $23 \pm 7\%$ ).

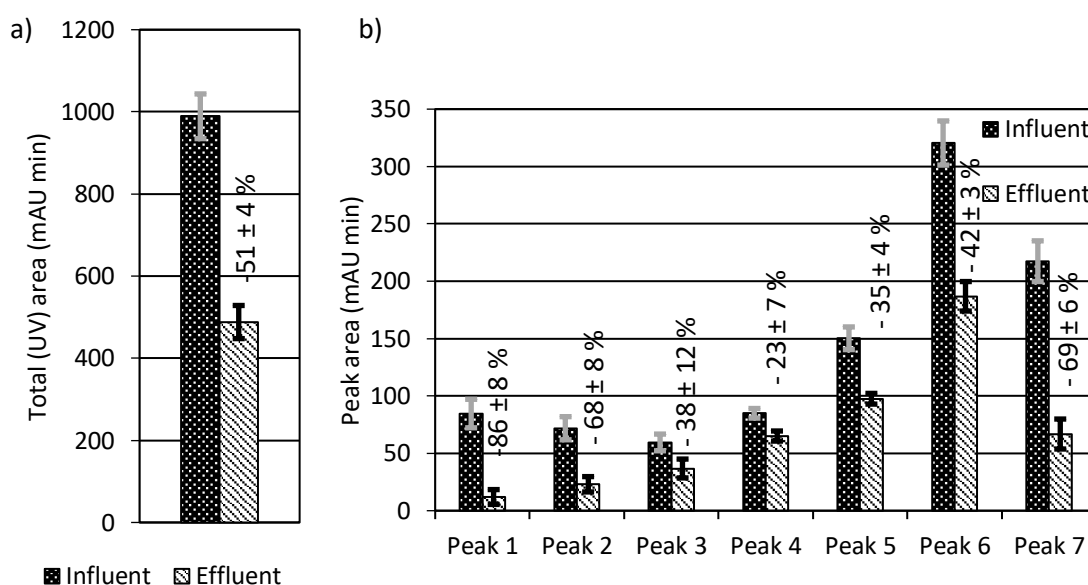
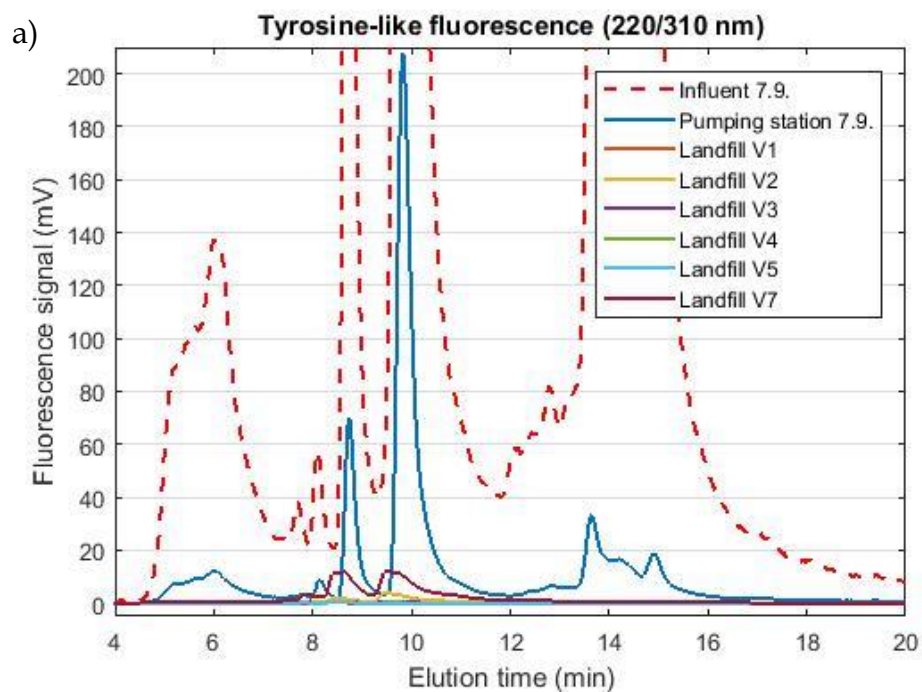


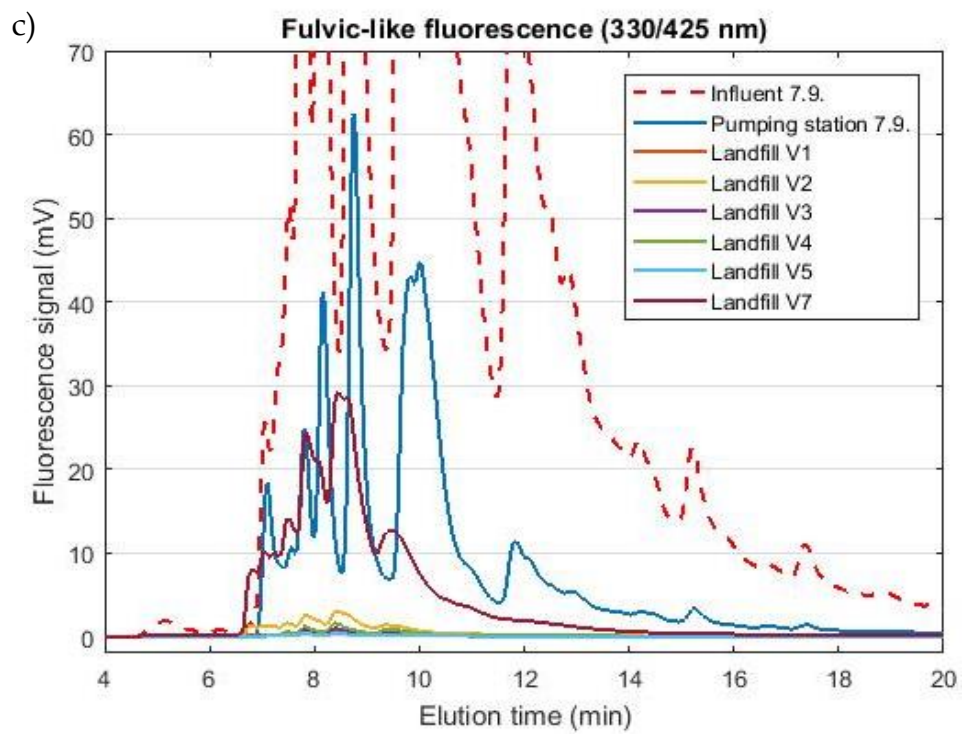
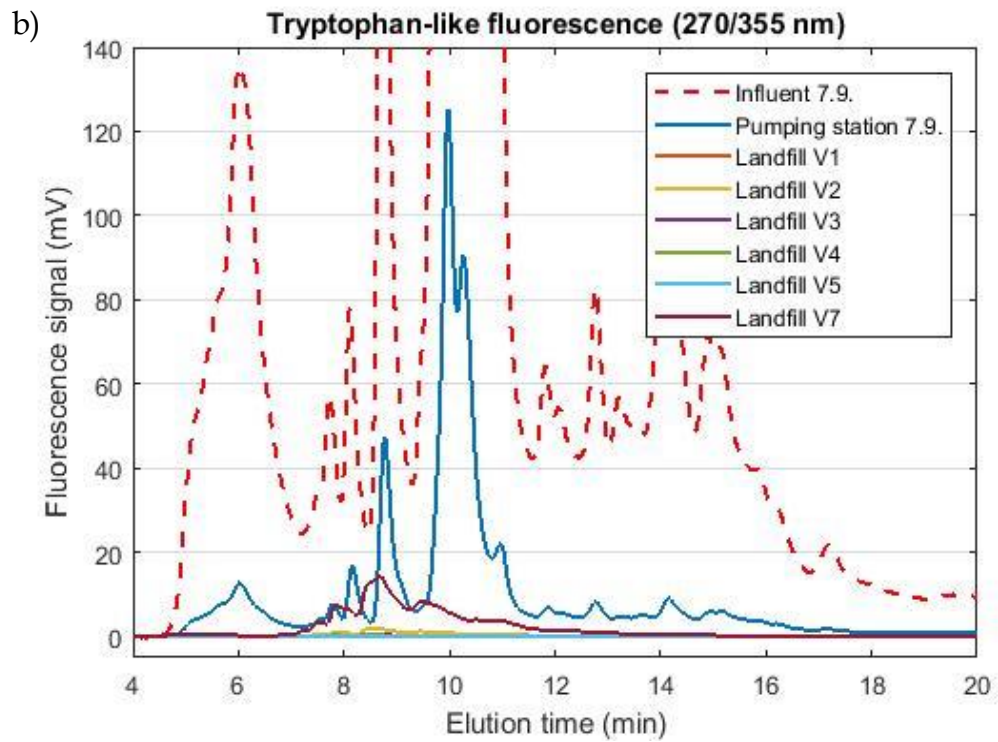
Fig. 22. a) Total area and b) peak areas of UV<sub>254</sub> absorbance chromatograms in influent and effluent and removal percentages of peaks. Graph represents average and standard deviation of results in form of  $\bar{x} \pm SD$ ,  $n=15$ .

#### 4.11 Landfill leachate fluorescence chromatograms

Tyrosine-like fluorescence in landfill leachate samples was low, compared with that in influent wastewater and pumping station sample (Fig. 23a). Landfill leachate sample V7 gave the highest fluorescence intensity of the leachate samples. Two main peaks are visible in tyrosine-like fluorescence of leachate. Tryptophan-like fluorescence of leachate samples was on a similar level as tyrosine-like fluorescence,

but three main peaks were visible (Fig. 23b). The profile was different from chromatogram profile from pumping station. Fulvic-like fluorescence of landfill leachate samples was quite large compared with pumping station sample (Fig. 23c). Peaks of larger sized compounds (in leachate) accounted for large part of those in pumping station sample. Similar trends were visible in humic-like fluorescence of leachate (Fig. 23d). Peaks of all types of fluorescence in landfill leachate samples were different in shape and in different location in elution time axis relative to the peaks in fluorescence chromatogram of pumping station samples.







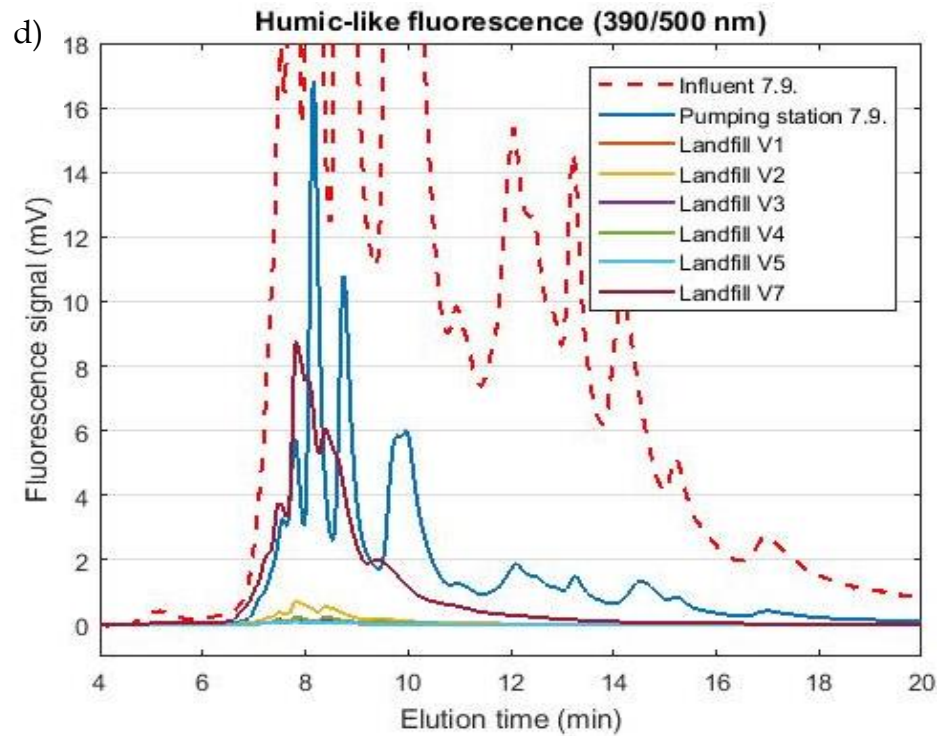


Fig. 23. Fluorescence signal of a) tyrosine-like (Ex./Em. 220/310 nm), b) tryptophan-like (Ex./Em. 270/355 nm), c) fulvic-like (Ex./Em. 330/425 nm) and d) humic-like (Ex./Em. 390/500 nm) compounds in landfill leachate samples. Pumping station and influent wastewater samples from 7.9. are represented as reference.

## 5 DISCUSSION

### 5.1 Wastewater quality parameters

In Nenäinniemi WWTP, the removal efficiencies of monitored parameters were quite similar in different years. In 2015 and 2016, removal of solids was lower, and had more variation than during both timescales in 2017. On the other hand, timescales in 2017 did not include the rest of the year, which might decrease the variation. In 2015, the disturbance was not observed, but the removal of total phosphorous was relatively low and the removal of organic matter and solids were slightly lower than usually at the end of September (Appendix 1). High variation in monitored parameters in 2016 likely resulted from the disturbance in the treatment plant in autumn, observable in removal efficiencies at that time (Appendix 2). Average removal of BOD in 29.6.-21.9.2017 was lower than in other timescales, and the variation was also higher (Appendix 3). On the contrary to previous years, sludge settleability problems in the treatment plant were not observed in 2017. Nevertheless, problems with phosphorous removal were observed at the beginning of September, and concentrations of solids, COD, BOD and total phosphorous in wastewater effluent were higher in 12.9.

DOC removal was much lower than the removals of BOD or COD, which was also most likely affected by filtration of samples, as the soluble fraction contains most organic compounds that are hard to remove (Shon *et al.* 2006). Removal of total nitrogen was lower in results from analysis made in this study compared with that of monitoring data. This was likely affected by different method used for analysis. In addition, samples were filtered before TN analysis, which also affects the results, as only the soluble fraction of compounds is included. Nitrogen removal has not been included in treatment processes in Nenäinniemi WWTP, and therefore the capacity of aeration basins is insufficient for nitrification, which causes low nitrogen

removal values. Nitrogen removal and nitrification level were higher on average during the summer, whereas in yearly average the removals were slightly lower. According to this, warm temperature increases nitrification, which is consistent with literature (Metcalf & Eddy 1991).

## 5.2 Tyrosine-like compounds

According to tyrosine-like fluorescence chromatograms in influent and effluent, only small fraction of these compounds remained in the wastewater after treatment. As a result, the removal percentage of tyrosine-like compounds was large (about 90 %). This is consistent with literature that proteins are easily degraded during wastewater treatment (Shon *et al.* 2006). In addition, good degradability of tyrosine-like compounds during wastewater treatment has been observed using fluorescence EEMS (Yu *et al.* 2013). Goffin *et al.* (2018) observed correlation between the fluorescence intensity of tyrosine-like component and BOD concentration in various WWTPs. On the other hand, Dignac *et al.* (2000) observed lower removal of tyrosine compared with removal of other proteins, indicating that tyrosine can be produced from other biodegradable compounds or they are not completely degradable. Consistent with observations in this study, tyrosine-like fluorescence has been concluded to be major part of organic matter in wastewater influent (Yu *et al.* 2013, Goffin *et al.* 2018). Furthermore, higher amount of tyrosine-like compounds in wastewater influent occurred the same time as DOC concentrations were also higher (in 3.8. and 4.9.-7.9.), demonstrating the dominance of tyrosine-like compounds in organic matter composition. On the contrary, most often the amount of tryptophan-like compounds in wastewater influent is larger than that of tyrosine-like detected by fluorescence (Yu *et al.* 2013, Goffin *et al.* 2018), and in some cases tyrosine-like fluorescence is minimal or not observed (Hudson *et al.* 2008, Yang *et al.* 2014).

A number of researchers have concluded that tyrosine-like compounds in wastewater effluent originate from SMPs formed during biological treatment (Wang and Zhang 2010, Yu *et al.* 2015). In a study of Shen *et al.* (2012), proteins accounted for quite small fraction of SMPs in wastewater from various WWTPs, whereas polysaccharides and humic substances dominated. On the other hand, industrial wastewater source seemed to increase the fluorescence intensity of protein-like compounds in SMPs (Shen *et al.* 2012). Based on this observation, industrial wastewaters might have increased tyrosine-like compounds in wastewater effluent in this study. Yu *et al.* (2015) studied organic matter composition in SMP and effluent organic matter samples using EEM with parallel factor analysis (PARAFAC) and observed that tyrosine-like fluorescence in both samples was on the same level, indicating that tyrosine-like compounds are mostly originated from SMPs. They concluded that these compounds in wastewater effluent were mostly from utilization-associated products of SMPs, whose concentration decreases within treatment, indicating good degradability of the compounds. On the other hand, as the fluorescence intensity of tyrosine-like compounds in their study was quite small in both samples from the beginning of the treatment, it could indicate that quite small amounts of tyrosine-like compounds are present in domestic wastewater used in the study (Yu *et al.* 2015). Therefore, as tyrosine-like compounds accounted for the largest fraction of organic matter in wastewater influent in this study, these compounds could originate from industrial sources.

When considering peak areas of fluorescent compounds, peaks 6 and 7 accounted for the largest fractions of tyrosine-like compounds. Therefore, high removal percentage of these peaks affected the removal of tyrosine-like compounds the most. These peaks were composed of quite small-sized compounds, as their apparent MWs were <170–1200 Da (Appendix 4). According to Jarusutthirak and Amy (2007), compounds of small size (<500 Da) can be amino acids produced

during biological processes. Despite the large removal efficiencies of peaks 6 and 7, these peaks also accounted for most of fluorescence area in effluent samples. This could indicate that these compounds contained high amount of easily degradable compounds and lower amounts of recalcitrant compounds. Peak area of peak 5 (~1200-2000 Da) was also on quite the same level in effluent as peaks 6 and 7. On the other hand, peak area of this peak was quite low in influent, and removal of this peak is much lower than those of peaks 6 and 7, which could then mean that this size fraction was mainly composed of recalcitrant compounds.

Peak 1 in wastewater influent was quite wide and seemed to contain sub-peaks at retention times of 5 min and >6 min, whereas in effluent only the peak at 5 min was visible, indicating poorer removal of these compounds. On the other hand, Jarusutthirak and Amy (2007) observed formation of SMPs with MW of > 10 000 Da, and these compounds were concluded to be proteins or polysaccharides. In their study, HPSEC with UV and DOC detection was used, and therefore, the results are not directly comparable as the DOC detector cannot distinguish between proteins and polysaccharides (Jarusutthirak and Amy 2007). One possible source for compounds in peak 1 in effluent might then be SMPs, as the peak accounted for compounds with MW approximately 5 300–21 700 Da. Removal of small-sized tyrosine-like compounds was higher, which could indicate that they were more easily degraded, whereas compounds of large size could be composed of complex structures with low degradability (Dignac *et al.* 2000).

### **5.3 Tryptophan-like compounds**

Tryptophan-like compounds comprised the second largest fraction of organic matter in wastewater influent, but their removal was lower than for tyrosine-like compounds. This observation was somewhat different with other studies, as tryptophan-like compounds are most frequently detected as the major component

of wastewater organic matter rather than tyrosine-like compounds, and to be removed for the largest part in WWTP (Yang *et al.* 2014, Cohen *et al.* 2014, Yu *et al.* 2015). On the other hand, tyrosine-like compounds were not observed as EEM PARAFAC component in the previously mentioned studies. Difference in the observations might result from variations in wastewater sources caused by industrial wastewaters. For example, wastewaters from a slaughter house have been observed to have high fluorescence intensity of tryptophan-like compounds (Louvet *et al.* 2013). Similarly as with tyrosine-like compounds, good degradability of tryptophan-like compounds has been observed based on correlations between the fluorescence intensity of tryptophan-like compounds and concentrations of organic matter parameters, such as COD or BOD (Hudson *et al.* 2008, Cohen *et al.* 2014, Yang *et al.* 2014). Overall, tryptophan-like compounds in this study showed high level of biodegradability and removal of these compounds was larger than fulvic-like and humic-like compounds as in other studies (Yu *et al.* 2013, Cohen *et al.* 2014, Yang *et al.* 2014).

In a study of Guo *et al.* (2011), tryptophan-like fluorescence was used for the detection of protein-like compounds with HPSEC. They studied behavior of wastewater organic matter during different kinds of WWTPs operating with the same wastewater source. The MWs of the largest peaks were different from those in this study, as the highest fluorescence response was observed from compounds with MW of 100-300 Da (Guo *et al.* 2011), whereas in this study the highest tryptophan-like fluorescence response was in compounds with higher MW of peak 6. This peak is composed of compounds with MW approximately of 170-2000 Da and therefore compounds eluting T the beginning of this peak had larger MW than 100-300 Da.

Peaks 6 and 7 of tryptophan-like fluorescence chromatograms accounted for most tryptophan-like compounds in wastewater influent, and the removal of these peaks

was smaller than with tyrosine-like compounds. On the other hand, peak 7 accounted for the largest fraction of tyrosine-like compounds, and lower fraction of tryptophan-like compounds. These observations could indicate that tyrosine-like compounds contained high amount of easily degradable small-sized compounds, whereas tryptophan-like compounds were mostly slightly larger in size. When further comparing the fluorescence chromatograms of tyrosine-like and tryptophan-like compounds, similar shift in the location of peak 1 (MW ~5300 – 21 700 Da) in influent and effluent was visible in both compound types. Similar kind of shift has been observed by study of Guo *et al.* (2011) in fluorescence chromatogram representing protein-like compounds with MW>10 000 Da. Compounds with MW > 10 000 Da have been considered to be composed of SMPs (Jarusutthirak and Amy 2007, Guo *et al.* 2011).

Yu *et al.* 2015 studied the composition of organic matter in SMP samples and wastewater effluent and concluded that some part of tryptophan-like compounds in wastewater effluent could originate from SMP-UAPs. As the fluorescence intensity of tryptophan-like compounds in effluent organic matter samples was observed to be higher than that of SMP, these compounds also originate from other sources, possibly synthetic organic compounds (Yu *et al.* 2015). Based on these findings, peak 1 in both tyrosine-like and tryptophan-like compounds in wastewater effluent could be composed of SMPs. On the other hand, the formation of SMPs does not explain the source of tryptophan-like compounds in wastewater influent.

#### **5.4 Fulvic-like compounds**

As can be seen from the fluorescence chromatogram, not much of fulvic-like compounds was removed in the WWTP. Consistent with the results of this study, most often fulvic-like fluorescent compounds have been observed to be less

removed than protein-like compounds detected with fluorescence EEMS (Yu *et al.* 2013, Yang *et al.* 2014, Yang *et al.* 2015a). Correlation between fluorescence intensity of fulvic-like compounds and BOD in wastewater has been observed (Yang *et al.* 2014), which indicates that at least part of fulvic-like compounds are biodegradable. Furthermore, amount of fulvic-like compounds in wastewater influent is usually lower than that of protein-like compounds, and relatively higher in effluent, similarly as in this study (Yu *et al.* 2013, Yang *et al.* 2014, Goffin *et al.* 2018). However, fulvic-like and humic-like compounds are usually more dominant in natural waters compared with protein-like compounds, and therefore NOM is likely source for part of fulvic-like compounds in wastewater observed in this study (Peiris *et al.* 2011, Peleato and Andrews 2015).

Peaks 1 and 2 covered only a small fraction of fulvic-like compounds, and removal percentages of these peaks were the largest. Peaks 5, 6 and 7 accounted for the largest fractions of fulvic-like compounds in influent and effluent, and therefore low removal percentages and increase in amount of these peaks affected the removal of total fluorescence area the most. Furthermore, peak 5 was increased 7 % on average, indicating possible formation of these compounds during treatment in most sampling days. Peaks 6 contained sub-peak that is partially overlapping with peak 7 and according to chromatograms, it seems that compounds of larger size were removed and amount of slightly smaller compounds was increased. This might result from degradation of other compounds and production of others. Increase of some part of fulvic-like compounds in effluent might be caused by formation of SMP-BAP, as was concluded by Yu *et al.* (2015). On the other hand, the wavelengths at which this component was observed are different than those used for detection of fulvic-like compounds in this study, representing mostly microbially derived compounds (Yu *et al.* 2015). Other possible source for fulvic-like compounds are synthetic organic compounds, which are commonly not easily degraded (Yu *et al.* 2015).



## 5.5 Humic-like compounds

Fluorescence signal of humic-like compounds in wastewater effluent is partially higher than in influent, which could mean that these compounds were formed in WWTP. This seemed to decrease the average removal of humic-like compounds the most. Peaks 5 and 6 of humic-like compounds were mostly increased on average after wastewater treatment, which indicates that compounds with MWs of ~170 – 2000 Da are likely among compounds that are produced during biological treatment. Minor removal or even an increase of fluorescent component similar to humic-like compounds during wastewater treatment have been observed using fluorescence EEMS (Cohen *et al.* 2014, Yang *et al.* 2014). Yu *et al.* (2015) observed decrease in the fluorescence intensity of humic-like compounds in sequencing batch reactors at the beginning of cycle and slight increase at the end. In addition, they observed increase of humic-like compounds in SMP samples, indicating that increase in biological treatment could result in production of non-degradable compounds, possibly originating from biomass-associated products (Yu *et al.* 2015). According to Dignac *et al.* (2000), organic matter in wastewater effluent that was not characterized using various chromatographic methods might contain humic substances that are likely formed from degraded proteins and sugars in wastewater. Relative amount of non-characterized fraction was increased in wastewater effluent, indicating increase in compounds of this fraction (Dignac *et al.* 2000). Therefore, part of humic-like compound that were increased in this study could be produced from protein-like compounds and the increase can be affected by enhanced degradation of proteins.

Humic-like compounds with MWs of 500-2000 Da that has been observed to contain recalcitrant humic-compounds partly derived from biological process, were contained in peaks 5 and 6 in this study (Jarusutthirak and Amy 2007, Guo *et al.* 2011). In addition, in a study of Guo *et al.* (2011) increase in compounds of this size

after biological treatment was observed and decrease after denitrification and phosphate removal. In the results of this study, peaks 5 and 6 in fulvic-like and humic-like compounds were mostly retained in wastewater effluent and partly increased, possibly caused by compounds formed in the biological process.

Major removal of natural organic matter or humic-like compounds during coagulation in drinking water treatment have commonly been observed (Matilainen *et al.* 2005, Sanchez *et al.* 2013), and therefore phosphorous removal by coagulation in the WWTP might also remove humic-like compounds. Fulvic-like and humic-like compounds were commonly of smaller size than protein-like compounds, as peak 1 accounted for the smallest fraction of these compounds in both wastewater influent and effluent. Other similarities between fulvic-like and humic-like compounds can be observed, as a shift in a peak between peaks 6 and 7 seemed to be quite similar in both compound types.

## 5.6 UV absorbance

Removal of total UV absorbance chromatogram area was about 50 %, which was more than the removals of fulvic-like and humic-like compounds. The trends in peak areas were quite similar as with fulvic-like and humic like compounds, commonly known to be detectable with UV absorbance because of their high aromaticity (Brezonik and Arnold 2011). On the other hand, no increases in average peak areas after wastewater treatment were visible. In addition, peak area of peak 1 was relatively higher with UV absorbance than with fluorescence peak areas of fulvic-like and humic-like compounds, which could indicate that not only humic compounds are detectable with UV absorbance, but also other compounds. In most studies, UV detection has been used with HPSEC to indicate the presence of humic-like compounds (Her *et al.* 2003, Jarusutthirak and Amy 2007, Guo *et al.* 2011). Based on results in this study, other types of humic compounds or tyrosine-like and

tryptophan-like compounds can also be observed with UV detection (Lambert *et al.* 1998).

Guo *et al.* (2011) observed a decrease in small organic compounds with MW < 500 Da during wastewater treatment. They used HPSEC with fluorescence and UV detection, of which UV detection was indicative of humic compounds. This is not so consistent with results of this study, as the removal of UV chromatogram area of peak 6 including these small-sized compounds was not very high. On the contrary, the removal of humic-like compounds of smaller size was much lower in this study, indicating presence of other types of UV absorbing compounds, such as amino acids (Jarusutthirak and Amy 2007). Tap water has been observed to contain peak with MW of 650 Da, representing humic-like compounds remaining from natural water source (Guo *et al.* 2011), which could account for some part of compounds in peak 6 in wastewater influent and effluent. Guo *et al.* (2011) concluded that SMPs with MW of >10 000 Da are not humic compounds, as no UV response was observed. In this study, UV peak area in peak 1 was low in effluent, possibly indicating the same, as no fulvic-like and humic-like compounds were detected either. The UV signal in this fraction might originate from complex compounds, which possibly also have protein-like fluorescing structure.

Peaks in UV absorbance chromatograms were slightly different than for fluorescence chromatograms, as only the area within retention time of 4.3-14.2 min was integrated, being smaller than for fluorescence chromatograms. This was because the baseline of chromatograms was in some samples under the zero-line, which would have resulted in negative values in areas of peak 7 and reduced the total chromatogram area. On the other hand, no peaks were usually visible after retention time longer than 15 min, and the peaks after 12.5 minutes were outside the range of calibration. In addition, UV chromatograms were integrated manually, which might have slight effects on the results.

## 5.7 Normalized fluorescence chromatogram areas

Areas of fluorescence chromatograms normalized by flow indicate the fluctuation of organic matter concentration in different sampling days and that the areas were not dependent of the wastewater flow. These areas of tyrosine-like and tryptophan-like compounds had more variation than fulvic-like and humic-like compounds, which was also seen in non-normalized results. This could indicate higher variation in the amount of protein-like compounds in wastewater influent, possibly caused by industrial wastewaters. As part of humic-like and fulvic-like compounds likely originated from NOM, the low variation in the amounts of these compounds might have resulted from quite stable concentration of NOM in the wastewater source.

Areas of fluorescence chromatograms normalized by DOC concentration showed the variation in the share of a specific compound of dissolved organic carbon in different samples. Variation in the normalized areas indicated variation in the relative amount of specific compound in the sample. Especially tyrosine-like and tryptophan-like compounds had quite large variation in fluorescence area relative to DOC concentration and the variation was different from non-normalized results. Larger variation in normalized areas of tyrosine-like, tryptophan-like and fulvic-like compounds was seen in effluent samples compared with non-normalized samples. This resulted from higher variation in DOC concentration in wastewater effluent, as non-normalized fluorescence chromatogram areas were more stable.

Large removal of tyrosine-like compounds in removal percentages normalized by DOC indicates good degradability of tyrosine-like compounds, as has already been concluded. On the contrary, tryptophan-like compounds had weaker removal relative to DOC concentration and in some cases, their removal percentage was negative. Negative removal of compounds indicate that normalized fluorescence area divided by DOC concentration was smaller in influent than in effluent, which might have resulted from relatively higher DOC concentration in influent or low

concentration in effluent. As the removal of tryptophan-like compounds was quite stable in non-normalized results, negative removal caused by higher DOC concentration could have resulted from higher amount of other types of organic compounds, which increased the DOC concentration. In the case of fulvic-like and humic-like compounds, the removal percentage normalized by DOC concentration was constantly negative, which indicate that these compounds were not removed to the same level as DOC concentration. As their amount in the effluent relative to DOC concentration was higher than the corresponding amount in influent, these compounds could be concluded to be recalcitrant compounds or formed during the treatment.

### **5.8 Variation of results between days**

The highest variation in removal efficiency of tyrosine-like compounds was observed in peaks 4 and 5 and lowest in peaks 6 and 7. On the other hand, peaks 4 and 5 accounted for only minor parts of tyrosine-like compounds in influent, and therefore they did not have large effect on total removal of these compounds. High variation in removal efficiencies might have resulted from varying amount of these compounds in wastewater. Removal efficiencies of peaks 6 and 7 in tyrosine-like compounds had quite small variation between days, indicating that possible fluctuations in wastewater composition or WWTP conditions did not have much effect on the removal of these compounds. Peak 4 in tyrosine-like compounds was quite steady in influent, but had more variation in effluent, indicating that treatment conditions can affect the removal of this peak. In addition, the amount of peak 4 was large in 31.8. in influent and quite small in effluent (Appendix 5), which could indicate that this fraction was composed of some compounds that were not so well removed, and others that are well removed. Increase in the amount of this peak in influent could have occurred as a result of increase of well-degradable compounds,

and therefore they were easily removed during WWTP, which lead to higher removal percentage than usually.

In tryptophan-like compounds, higher variation in peak removals was visible, and therefore this can be affected more easily by variations in wastewater composition or treatment conditions. Especially peak areas of peaks 6 and 7 of tryptophan-like compounds in wastewater influent had large variation, but this did not seem to affect the removal of peak 6 considerably. High variation in removals of peaks 2-5 was likely affected by variation in wastewater composition. Lower removal of peak 7 was observed in 6.7., which seemed to result from considerably lower area of this peak in influent and higher in effluent.

In 31.8., removal percentages of a couple of peaks in all compound types were higher than usually, and, on the contrary, in 29.6. lower than usually. High removal percentages seemed to be caused by increases in amounts of these compounds in influent, which could indicate that there was higher amount of easily degradable compounds in the influent. For fulvic-like and humic-like compounds, removal of total fluorescence chromatogram area was lower in 31.8, and areas of certain peaks were higher in influent. On the other hand, higher removal percentages in 31.8. was observed in peaks 3 and 4 that account for quite small fractions of protein-like compounds in wastewater influent, and therefore enhanced removal does not affect the total removal significantly.

As it was mentioned before, there was decrease in removal of phosphorous from wastewater in the beginning of September 2017. Observed increase in the amounts of tyrosine-like compounds in wastewater influent between 4.9.-7.9.2017 might be related to the disturbance observed. No outliers in removal percentages of tyrosine-like and tryptophan-like peaks was observed at that time, but areas of peaks 6 and 7 of tyrosine-like compounds in wastewater influent were higher.

## 5.9 Landfill leachate

According to fluorescence chromatograms from landfill leachate samples, fulvic-like and humic-like compounds dominated in these samples. Quite similar observations have been made with studies using UV for detection of humic-like compounds (Guo *et al.* 2011, Keen 2017). The amount of fulvic-like and humic-like compounds in leachate sample relative to the amount of these compounds in influent and pumping station samples was higher than that of protein-like compounds. In a study using fluorescence EEMS, high fluorescence intensity of fulvic-like compounds, and also fluorescence of tryptophan-like compounds was observed in landfill leachate (Baker and Curry 2004). In this study, compounds ranging from medium MW to quite low MW seemed to originate from landfill leachate, which has also been observed in other studies (Calace *et al.* 2001, Kang *et al.* 2002, Guo *et al.* 2011). In a study of Calace *et al.* (2001), young landfill leachate was observed to contain mostly compounds with lower MW, whereas larger compounds were present in older leachate. Furthermore, humification of leachate and increase in MW of organic compounds in older landfills has also been observed elsewhere (Kang *et al.* 2002). As most peaks in fulvic-like and humic-like compounds were quite poorly removed, these compounds from landfill leachate might be recalcitrant compounds. For example, Keen (2017) observed removal of high-MW compounds after biological treatment of landfill leachate, whereas compounds with MW <7000 Da were still present in effluent. However, as the degradability of organic compounds depends on the treatment process used, not considerable conclusions can be made of the degradability of organic matter in landfill leachate based on the results of this study.

The highest fluorescence intensity was observed from leachate sample V7, which had also the highest DOC concentration, 262 mg/l (Appendix 4). Sample V2 had the second highest DOC concentration, 250 mg/l, and also the second highest

fluorescence intensity. Quite low fluorescence intensities of other leachate samples were observed, which is quite consistent with the low DOC concentrations of these samples. On the other hand, the fluorescence intensities of leachate samples were affected by the normalization of the chromatograms with the flow, which might not be very accurate, if the flow of the leachate was not the same for all wells.

The shapes of landfill leachate fluorescence chromatograms were quite different than those of pumping station sample and wastewater influent samples. Difference can be due to higher concentration of leachate samples, which can affect the resolution of peaks, if too large injection volumes are used (Szabo *et al.* 2016). Also, landfill leachate samples were taken at different time than the sample from the pumping station, which weakens the comparability between samples, as the composition of organic matter in the pumping station sample can vary between days. In addition, sample from pumping station was an 8-hour aggregate sample, and samples of Nenäinniemi WWTP were 24-hour aggregate samples, whereas those from landfill leachate were taken as individual samples, being therefore susceptible to variations at sampling moment. Therefore, not very reliable comparisons can be made. Some of the samples were held in freezer prior to analysis, which caused precipitation to two samples, and therefore new samples for HPSEC were filtered from the corresponding leachate samples, which were held in refrigerator for about 24 hours. On the other hand, these results can be used to estimate the relative amounts of different types of compounds in landfill leachate.

### **5.10 Sources of error and other considerations**

HPSEC have been proved to be suitable method for determining composition of organic matter in wastewater (Her *et al.* 2003, Jarusutthirak and Amy 2007, Szabo *et al.* 2016). With regards to the results, certain considerations should be made. For example, it should be taken into account that peaks occurring in the beginning and



in the end of the retention time-scale are composed of compounds that are affected by interactions with the column material (Specht and Frimmel 2000). In addition, the retention time range of which molecular weights of compounds can be determined depends on MWs of standards used. In this study, the calibration range between elution times of polystyrene sulfonate standards and acetone was 5.44 – 12.5 min. For example, peak 7 in this study is partially outside calibration range and eluted among last compounds, and therefore no conclusions of sizes of compounds in this peak can be made. Furthermore, the equation chosen based on the calibration curve affects the MWs calculated for each peak.

Furthermore, the location of baseline of the chromatogram relative to the zero-line had variations between samples and therefore could have affected the peak areas and chromatogram areas obtained. This is because the chromatograms were integrated only above zero-line. For example, peak area of peak 1 in fulvic-like compounds was negative in some samples, resulting from the baseline being under zero-line. On the other hand, this mainly affects the areas of small peaks, but no considerable impacts on the total area of chromatogram should occur, if variation is small. As a result, this need to be taken into consideration when processing the results and choosing the chromatogram area to be integrated.

The comparability of these results to previous literature is weakened by the fact that the method has not been so widely used for monitoring of organic matter quality in wastewater using the same detectors. Results obtained with fluorescence EEM or other methods provide information about relative amount of detected compounds in wastewater (Yang *et al.* 2015b). Fluorescence intensities can be compared with those obtained with fluorescence detection with HPSEC, as the areas of fluorescence chromatograms represents the relative amount of certain compounds. On the other hand, as only specific wavelength combination is used in fluorescence detection with HPSEC, it can be different than that of the same fluorescent compound

detected with fluorescence EEM (Hudson *et al.* 2007). This need to be taken into consideration when comparing the results, as the wavelength at which the specific compound fluoresces might vary in different samples (Yang *et al.* 2015b).

## 6 CONCLUSIONS

In this study, tyrosine-like and tryptophan-like compounds were observed to be mostly removed in the WWTP, whereas fulvic-like and humic-like compounds were less removed. For all compound-types, medium-sized compounds were usually removed the lowest. Furthermore, part of fulvic-like and humic-like compounds were increased in wastewater effluent, indicating formation of recalcitrant compounds. These compounds could possibly originate from degraded protein-like compounds, as has been observed in literature. Based on landfill leachate chromatograms, part of fulvic-like and humic-like compounds in wastewater can originate from landfill leachate. According to literature, possible sources for all compound types could be synthetic organic compounds originating from various industries. Presence of observed compounds in wastewater effluent can be explained by formation of soluble microbial products during biological treatment, or presence of non-degradable compounds. Certain conclusions of origins of non-degradable compounds in each compound type would require analysis of samples from different points in wastewater network.

This study provides information about which size fractions of compounds are easily degraded during wastewater treatment and which compounds are likely formed. Organic matter analysis with HPSEC combined with UV and fluorescence detectors gives more information about important compound-types in wastewater than conventional methods. Monitoring with HPSEC-UV-fluorescence would provide

information about variation in characteristics of compounds during different times of the year and their source in wastewater effluent. It could be essential to study correlations between traditionally measured parameters and amounts of different compounds types to distinguish which compound types account for each parameter the most. Characterization of organic matter would be essential to conduct during different processes of WWTP, to enhance knowledge about which compound fractions are removed or formed in each treatment process. As the new treatment processes are introduced in Nenäinniemi WWTP, the effect of the processes on organic matter composition would be interesting area of study. Additionally, monitoring during possible disturbances in the treatment plant would help track possible changes in organic matter composition.

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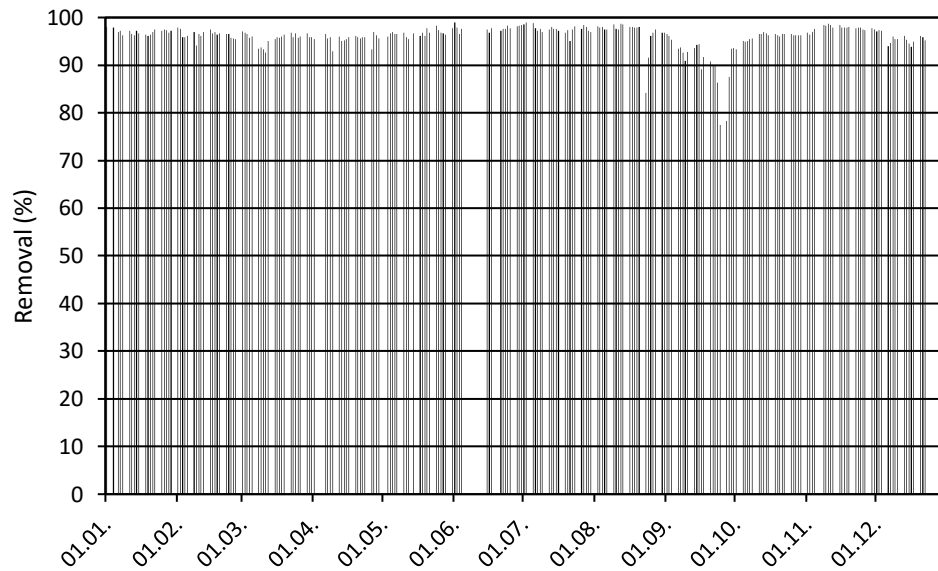
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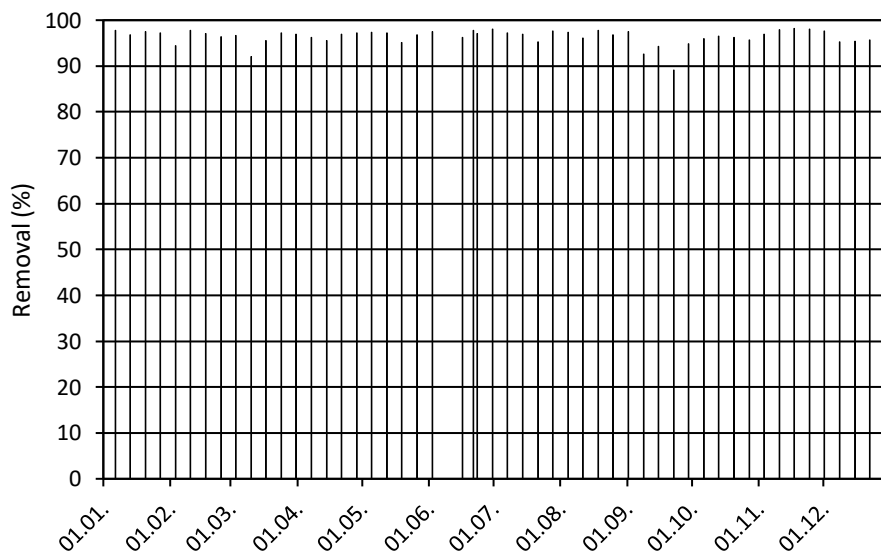
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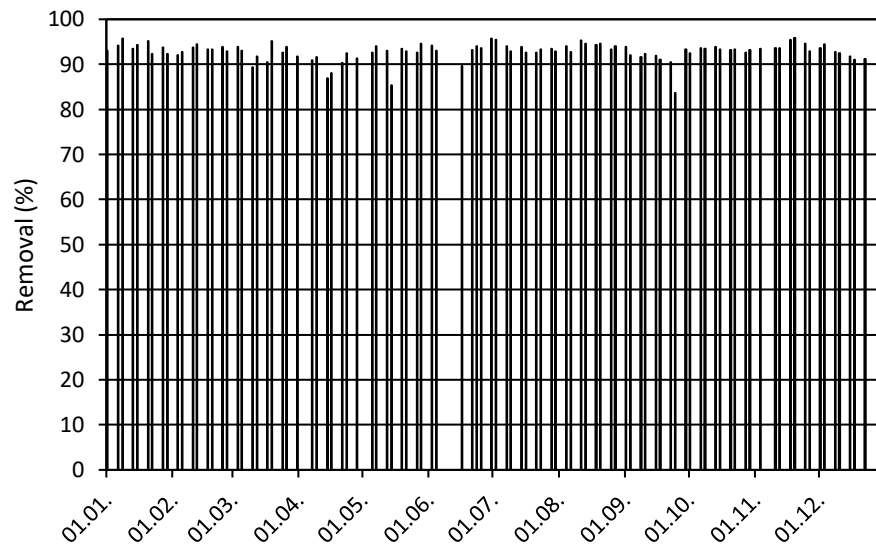
## APPENDIX 1: Removal efficiencies of monitored parameters in Nenäinniemi WWTP 2015



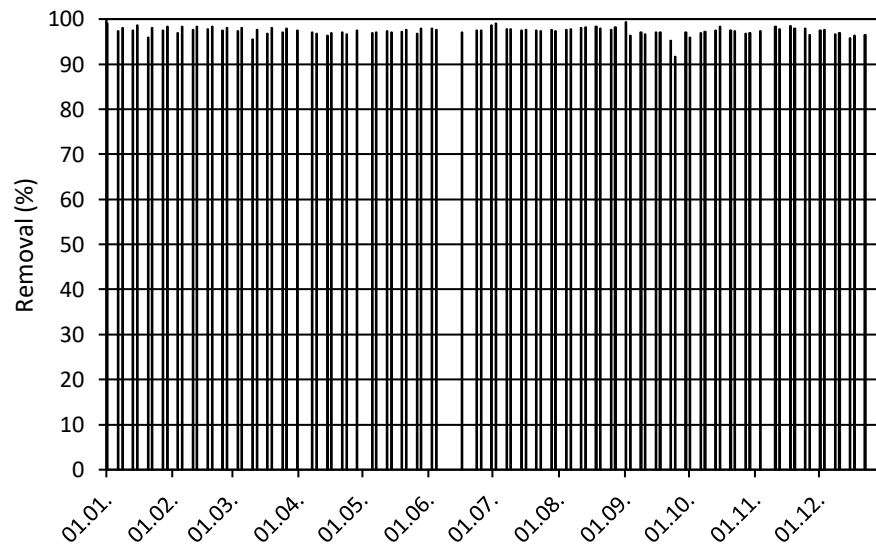
Removal efficiency of phosphorous in 2015.



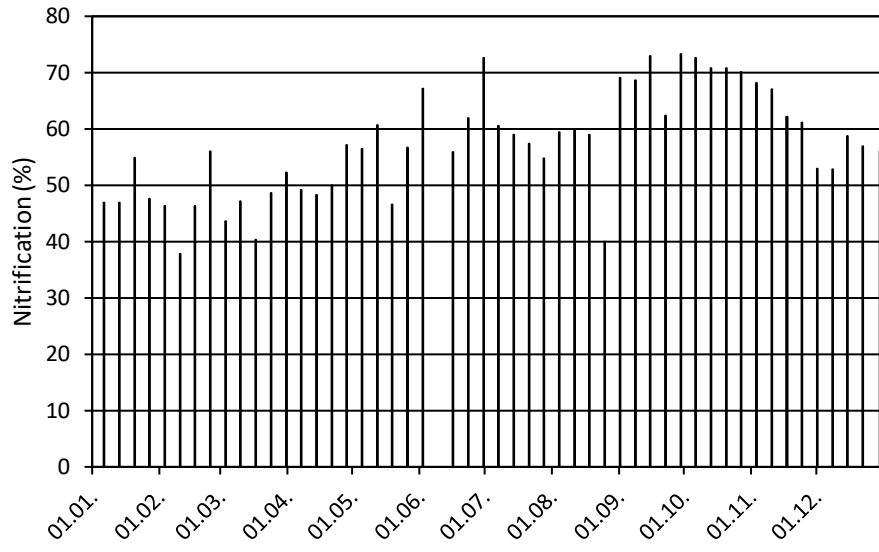
Removal efficiency of solids in 2015.



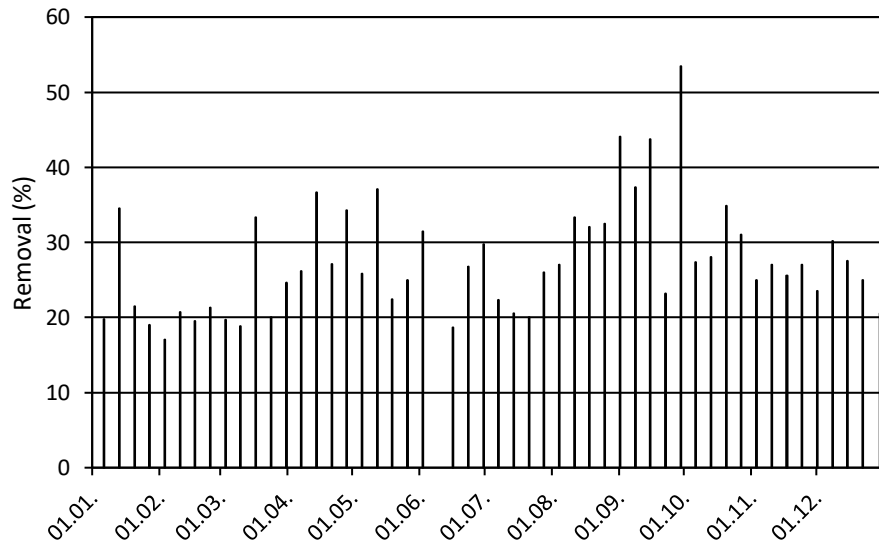
Removal efficiency of chemical oxygen demand in 2015.



Removal efficiency of biochemical oxygen demand in 2015.

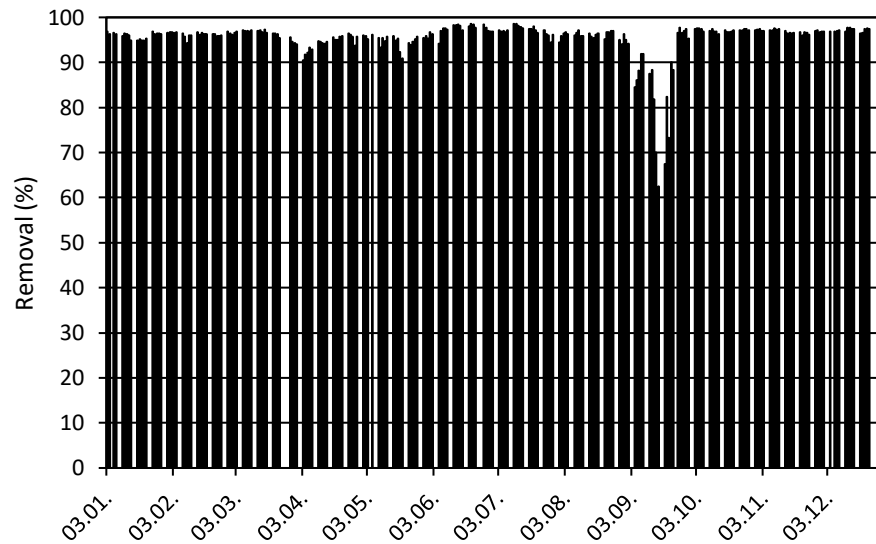


Nitrification level in 2015.

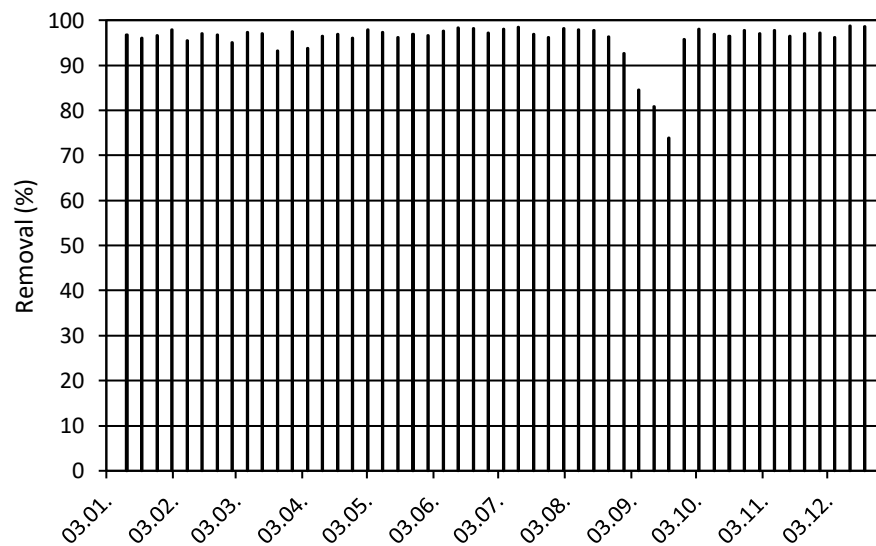


Removal efficiency of total nitrogen in 2015.

## APPENDIX 2: Removal efficiencies of monitored parameters in Nenäinniemi WWTP 2016

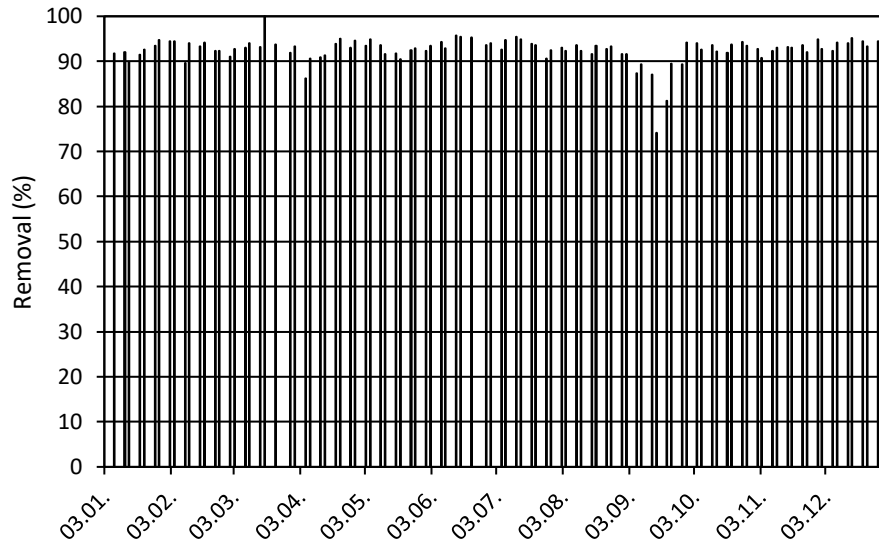


Removal efficiency of total phosphorous in 2016.

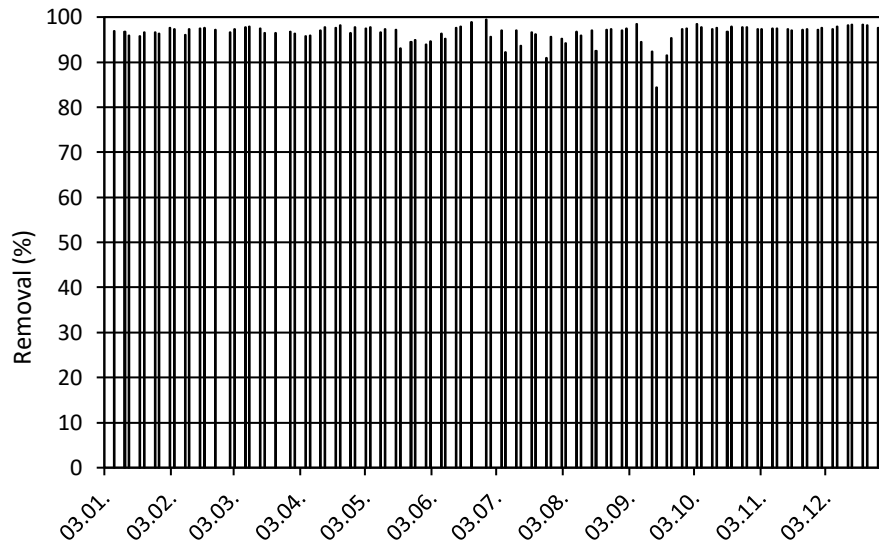


Removal efficiency of solids in 2016.

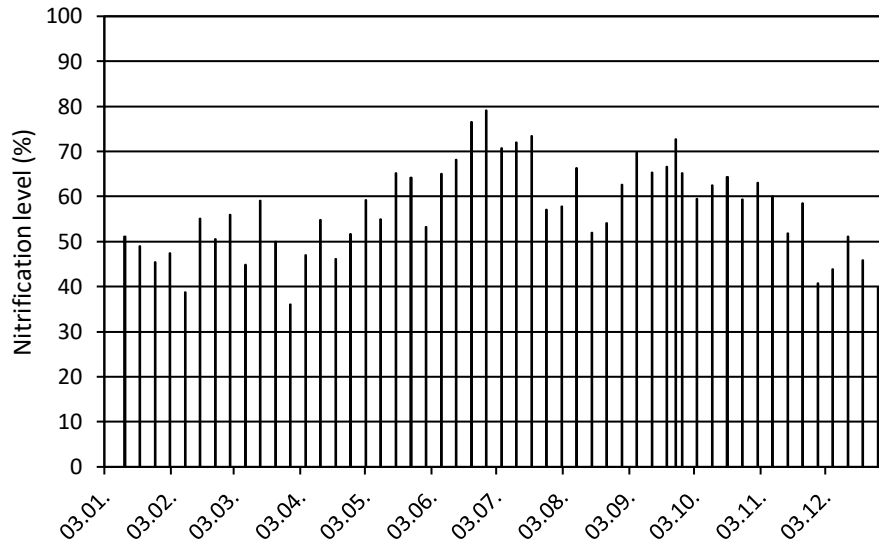




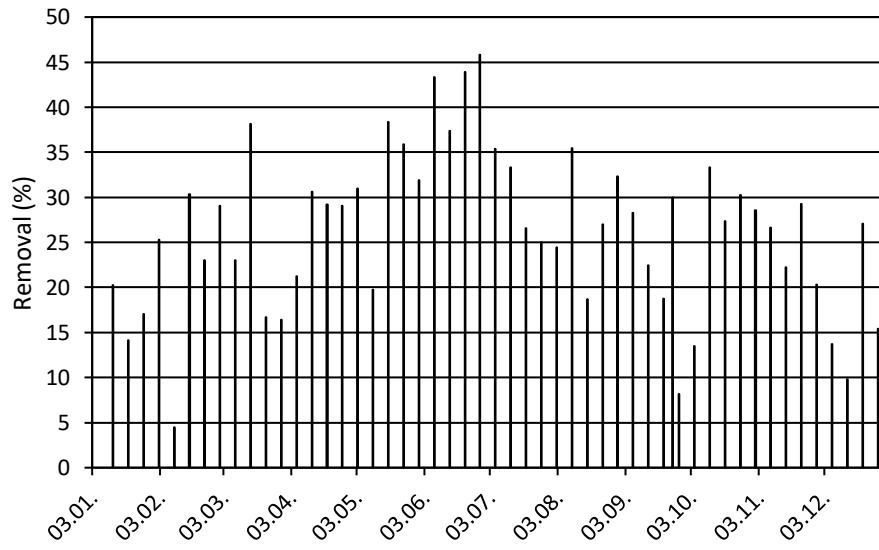
Removal efficiency of chemical oxygen demand in 2016.



Removal efficiency of biochemical oxygen demand in 2016.

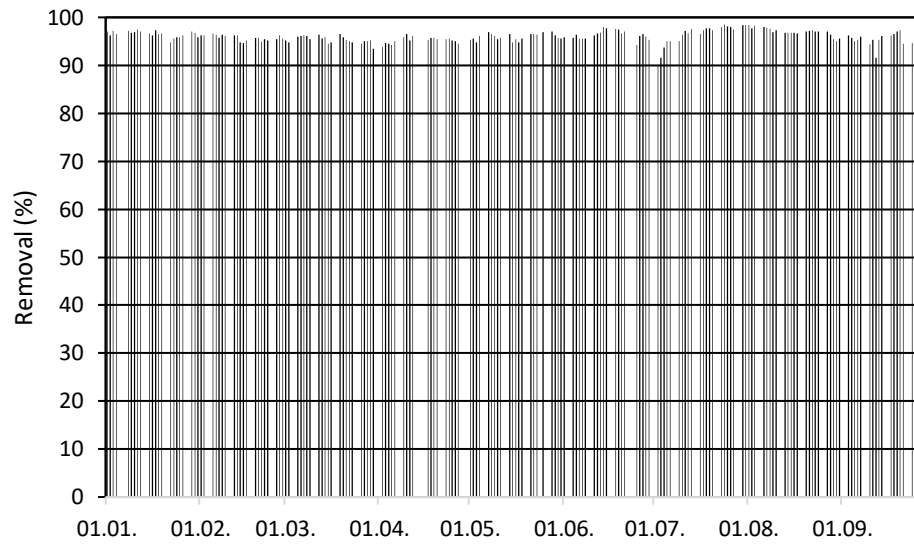


Nitrification level in 2016.

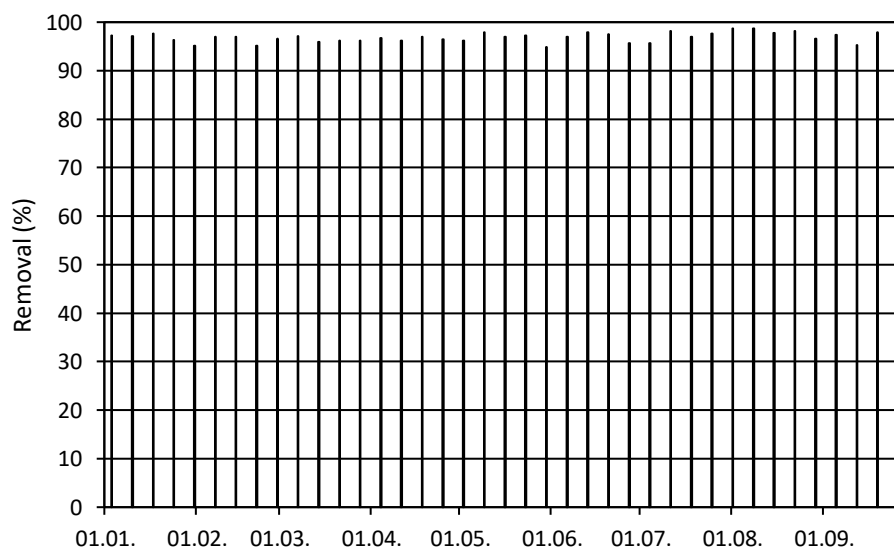


Removal efficiency of total nitrogen in 2016.

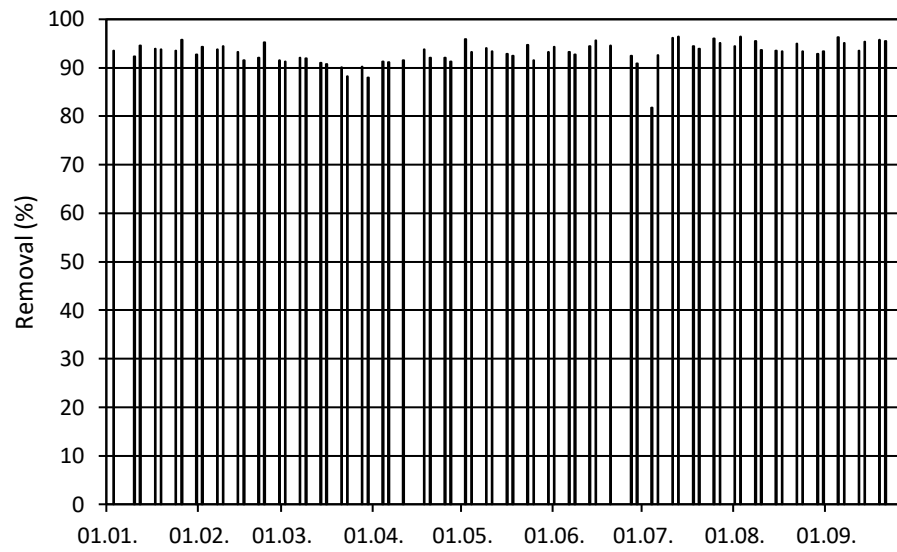
### APPENDIX 3: Removal efficiencies of monitored parameters in Nenäinniemi WWTP 2017



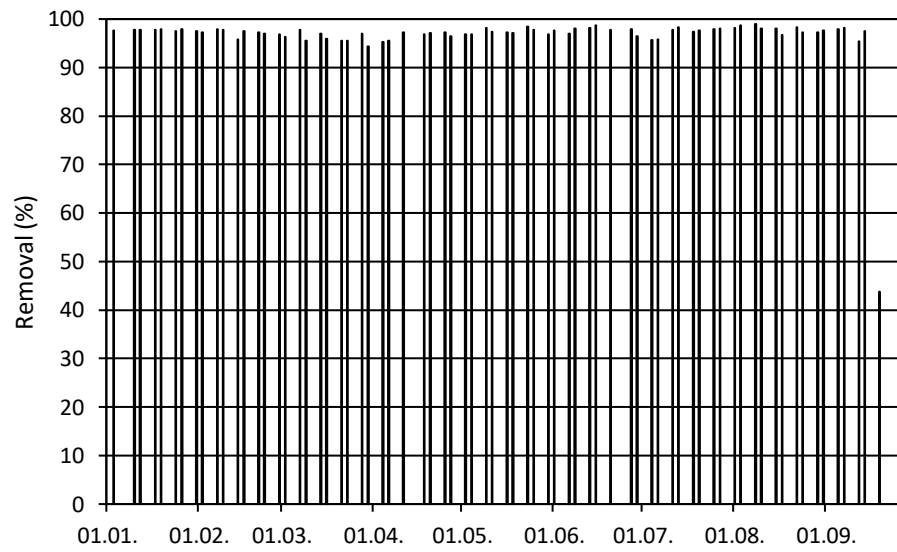
Removal efficiency of total phosphorous in 2017.



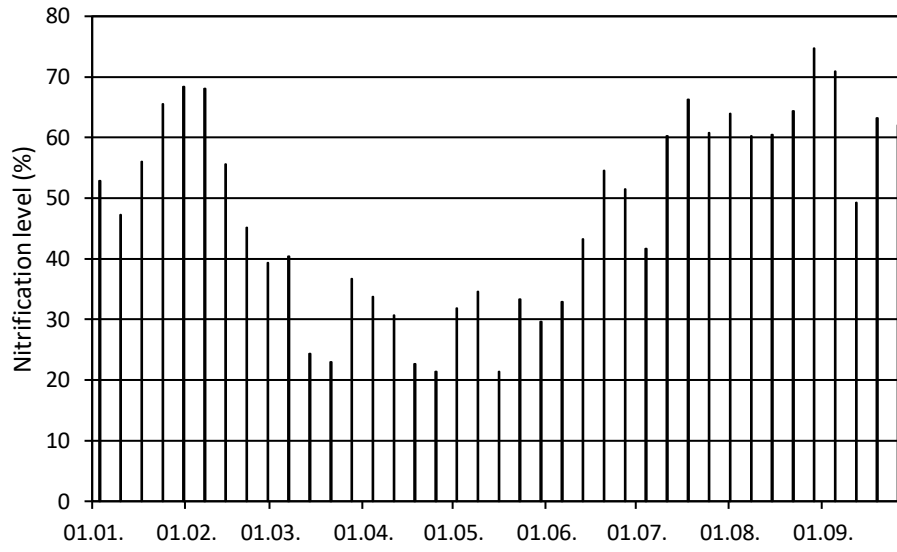
Removal efficiency of solids in 2017.



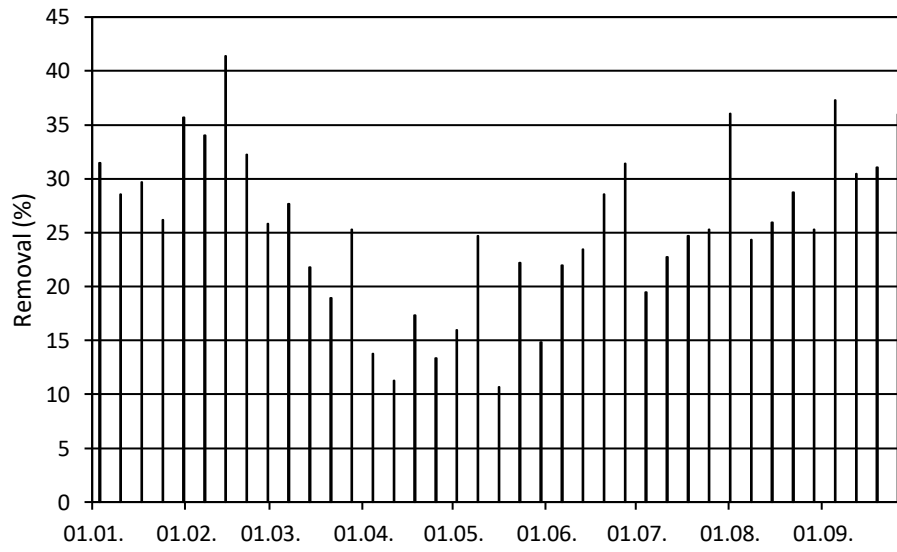
Removal efficiency of chemical oxygen demand in 2017.



Removal efficiency of biochemical oxygen demand in 2017.

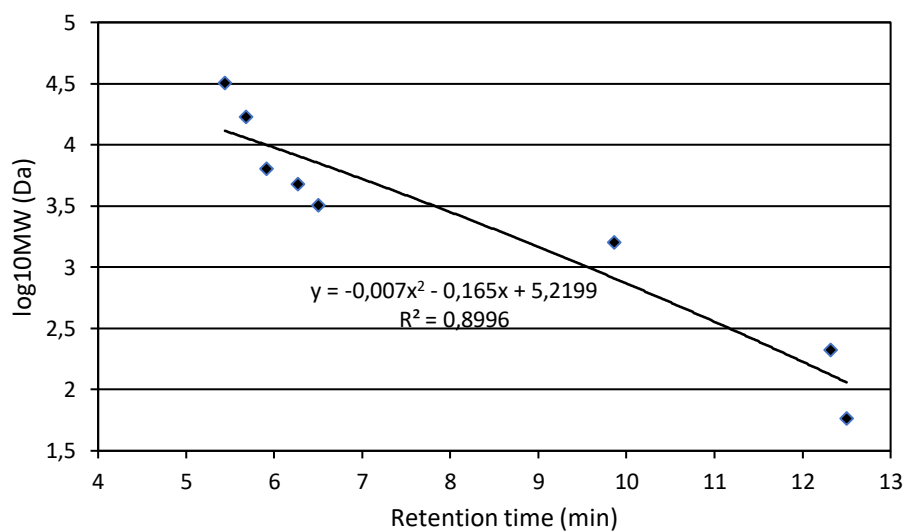


Nitrification level in 2017.



Removal efficiency of total nitrogen in 2017.

## APPENDIX 4: Calibration curve of standards and molecular weight ranges of peaks

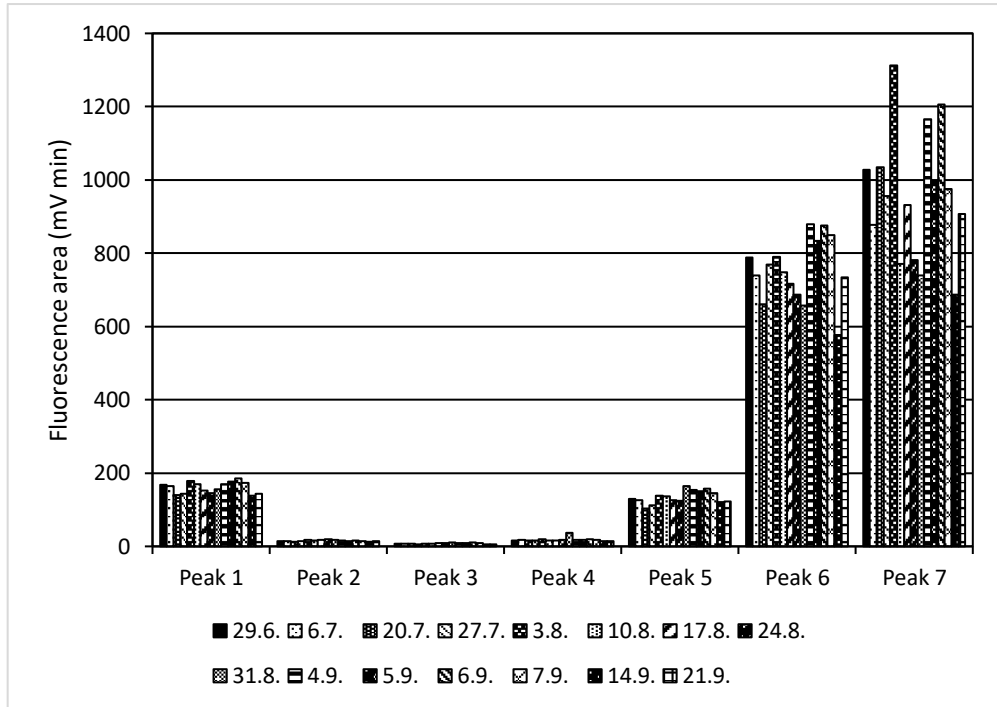


Logarithm to base 10 of molecular weights of calibration standards as a function of retention time. The trendline was set formed as second degree polynomial function,  $R^2 = 0,8996$ .

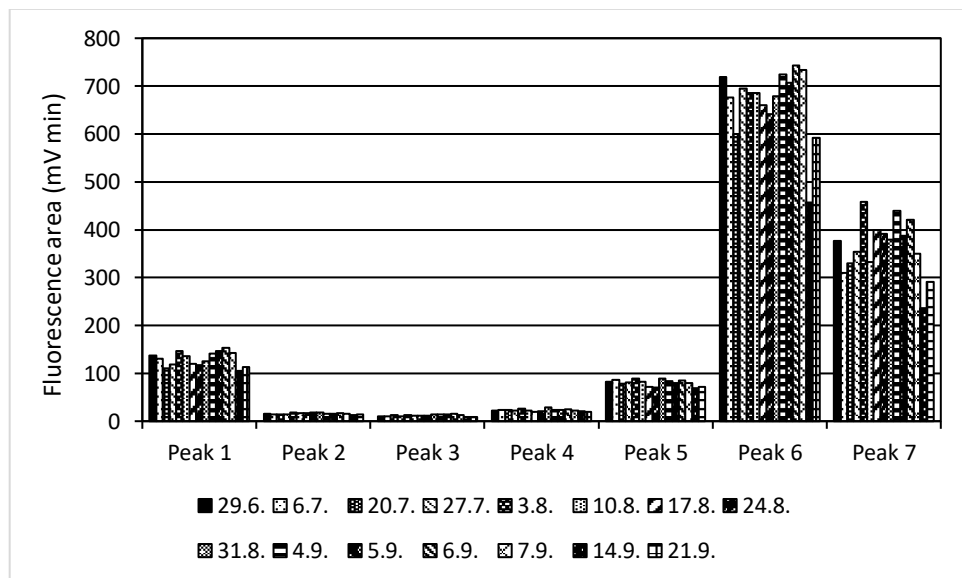
Molecular weights of each peak based on equation from calibration standards' trendline.

Peak	MW range (Da)
Peak 1	5300 - 21 700
Peak 2	3600 - 5300
Peak 3	2900 - 3600
Peak 4	2000 - 2900
Peak 5	1200 - 2000
Peak 6	170 - 1200
Peak 7	< 170

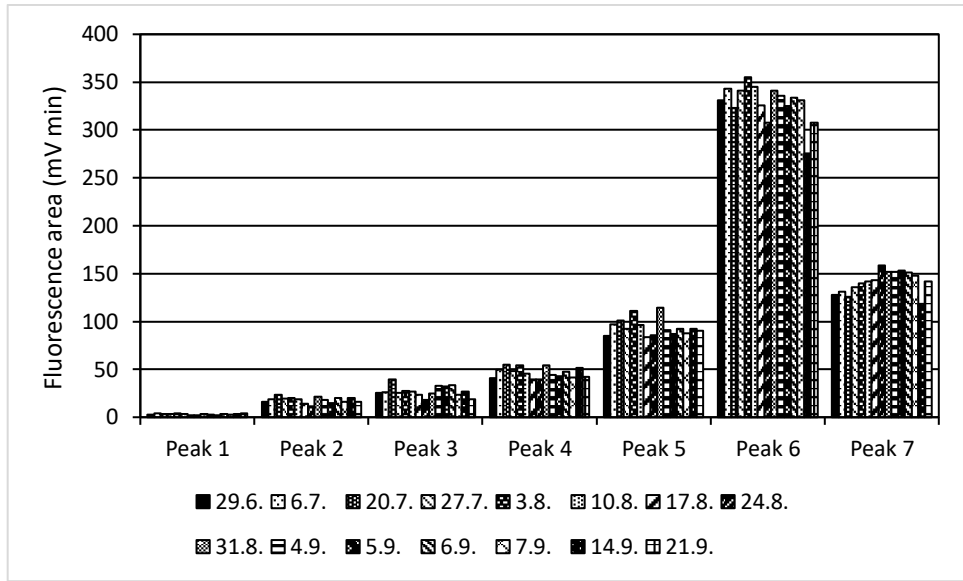
### APPENDIX 5: Peak areas of fluorescent compounds in wastewater influent



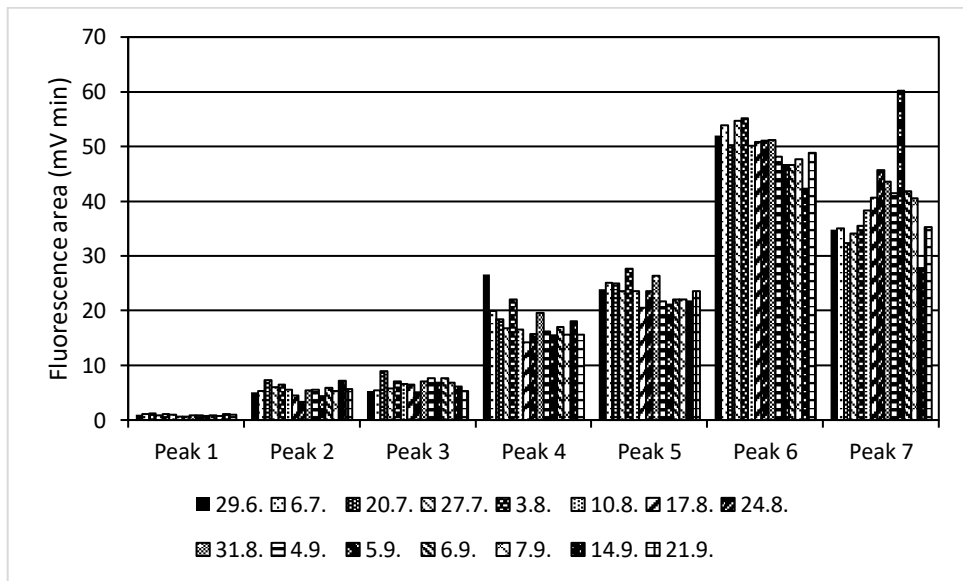
Peak areas of tyrosine-like compounds in wastewater influent.



Peak areas of tryptophan-like compounds in wastewater influent.



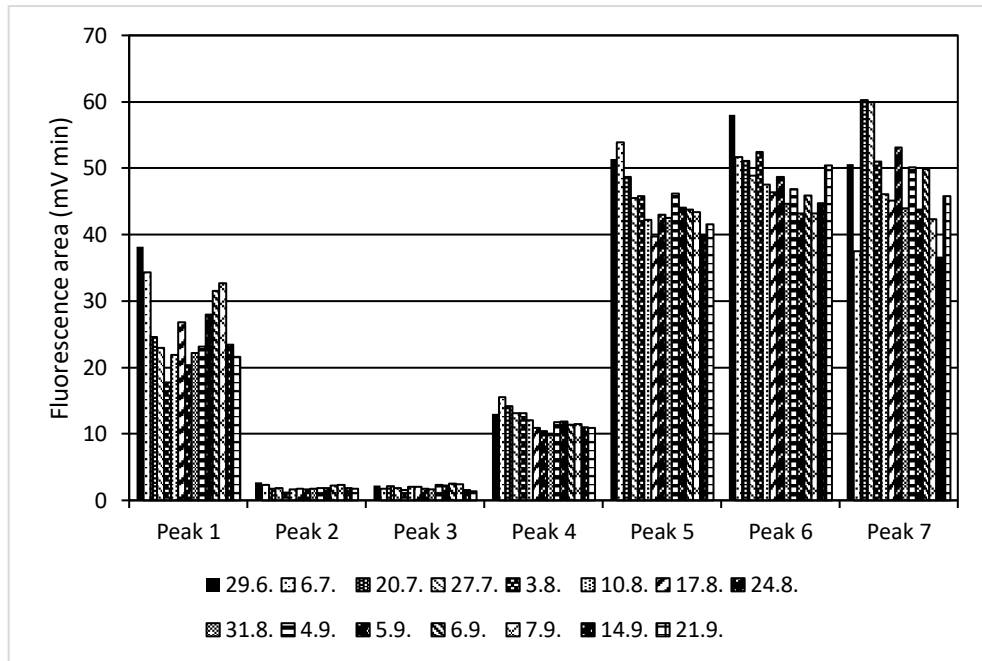
Peak areas of fulvic-like compounds in wastewater influent.



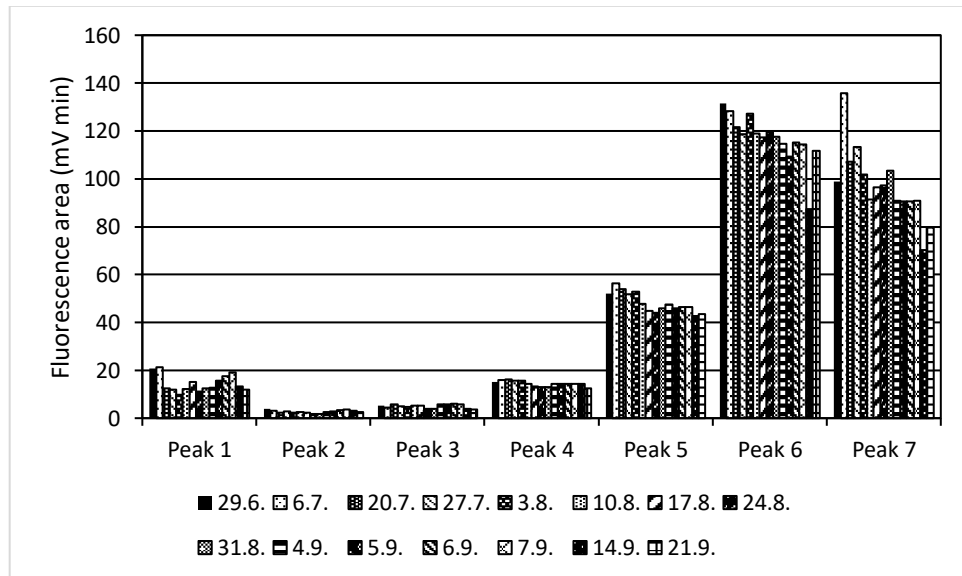
Peak areas of humic-like compounds in wastewater influent.



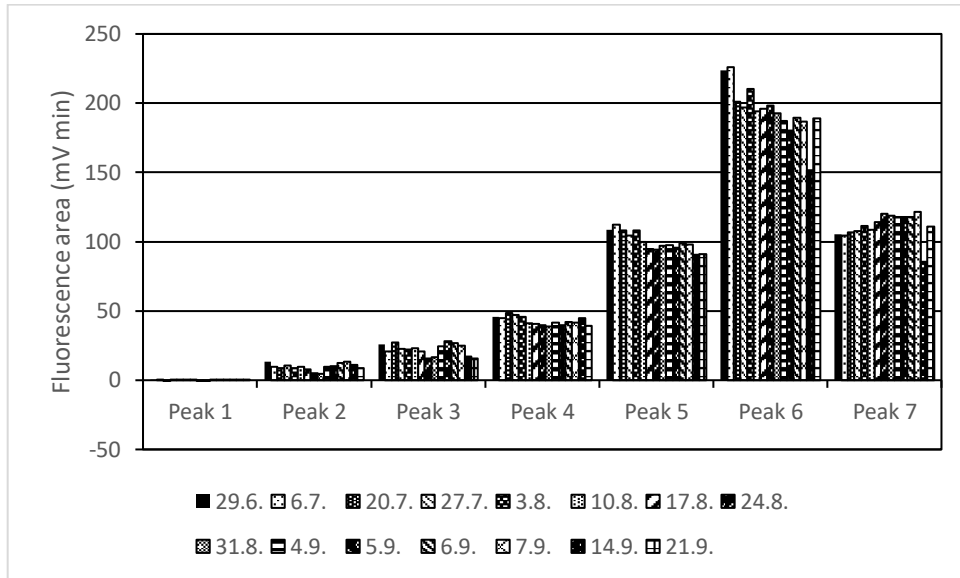
### APPENDIX 6: Peak areas of fluorescent compounds in wastewater effluent



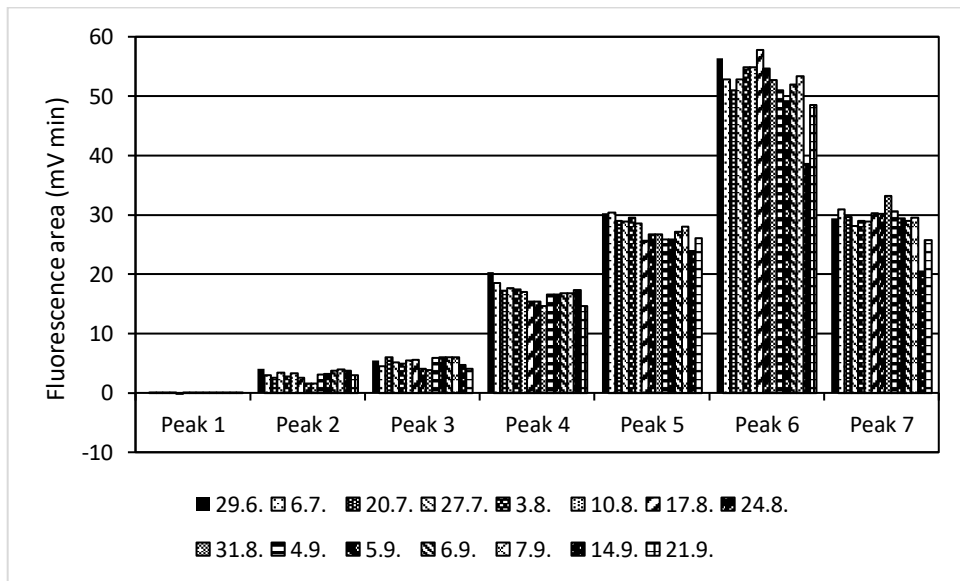
Peak areas of tyrosine-like compounds in wastewater effluent.



Peak areas of tryptophan-like compounds in wastewater effluent.



Peak areas of fulvic-like compounds in wastewater effluent.



Peak areas of humic-like compounds in wastewater effluent.

**APPENDIX 7: DOC concentrations of landfill leachate samples**

Dissolved organic carbon concentrations of landfill leachate samples.

Leachate sample	DOC (mg/l)
V1	16.5
V2	250
V3	35.9
V4	33.5
V5	10.4
V7	262