Wastewater treatment plants as pathways of microlitter to the aquatic environment

Julia Talvitie
Wastewater treatment plants as pathways of microlitter to the aquatic environment

Julia Talvitie

A doctoral dissertation completed for the degree of Doctor of Science (Technology) to be defended, with the permission of the Aalto University School of Engineering, at a public examination held at the lecture hall TU 1 of the school on 18 May 2018 at 12 pm.

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**Abstract**
Microlitter and its synthetic sub-type microplastics, defined as anthropogenic particles less than 5 mm in size, are an ever-increasing form of marine litter that has received considerable attention over the past decades. Among the numerous possible sources and pathways that allow this microlitter to enter aquatic environments, wastewater treatment plants (WWTPs) have been suggested as one of the more significant pathways. This thesis examines the step-wise removal of microlitter during the purification process within conventional WWTPs utilizing conventional activated sludge processes. In addition, the removal of microplastics from wastewater effluents using five different advanced final-stage treatment technologies was investigated and the microplastic discharge from existing Finnish WWTPs into the aquatic environments was assessed.

To further evaluate the environmental risk that microlitter may pose to aquatic food webs, their ingestion by Baltic blue mussels (*Mytilus trossulus*) was studied at a wastewater receiving area in the Gulf of Finland in the Northern Baltic Sea.

These results show that conventional wastewater treatment using primary and secondary treatments can efficiently remove (> 99 %) microlitter arriving at the WWTP in influent. Most (98%) of the microlitter was removed during primary treatment. The activated sludge process further decreased (~ 88%) the microlitter concentration. During the wastewater treatment, most of the microlitter (> 99.5 %) was retained in the raw and excess sludge. However, part (~ 20 %) of the retained microlitter was recycled back to the treatment process along with the reject water.

The removal of microplastics can be further enhanced by advanced final-stage wastewater treatment technologies. Membrane bioreactor removed an additional 99.9% of microplastics during treatment. Sand filtration removed 97%, dissolved air flotation removed 95 %, and disc filtration removed 40 – 98.5% of the MPs. Biologically active filtration did not have any impact on the microplastic concentration.

According to our estimations, Finnish WWTPs annually discharge ~ 480 billion microplastic particles into aquatic environments. As vast volumes of wastewaters are constantly discharged into the aquatic environments, the role of WWTPs as pathways may be significant. However, to further evaluate the relative importance of the role of WWTPs as pathway for microplastics, information on other pathways are also needed.

The results from the wastewater receiving environment indicate that the WWTPs may influence the microlitter and microplastics abundance and composition detected in biota. Blue mussels collected from the wastewater receiving area had higher microlitter content than those from the reference site.

**Keywords** microlitter, microplastics, municipal wastewaters, WWTPs, aquatic environment, blue mussels

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Väitöskirjan nimi
Jätevedenpuhdistamot mikroroskan kultureittinä vesistöihin

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Tiivistelmä
Mikroroskaksi kutsutaan yleisesti alle 5 mm:n kokoista roskaa. Mikroroskien ja erityisesti mikromuovien ekologiseen merkitykseen on alettu kiinnittää erityistä huomiota viimeisen vuosikymmenen aikana. Mikroroskia päätyy ympäristöön useista eri lähteistä kuten liikenteestä, vaatteiden pesusta ja kosmetiikasta ja erityisesti jätevedenpuhdistamoina on epäilty yhdensä merkittävimpiä mikroroskien kauttakultureitistä vesistöihin.

Tässä työssä tutkittiin mikroroskien kulkeutumista ja poistumista perinteisen aktiivilitemenetelmän perustuvan jätevedenpuhdistusprosessin sekä erilaisten jälkkäsäilytyiden aikana. Lisäksi tutkittiin jätevedenpuhdistamoiden vesistöihin aiheuttamaa mikroroskakkuormitusta Itsmeren sinisimpukoiden avulla. Tutkimuksessa verrattiin sinisimpukoista löydettyjen mikroroskien määrää jätevesiä vastaanottavan ja verrokkialueen välillä.

Tutkimuksissa havaittiin, että perinteinen jätevedenpuhdistus esikäsittelyineen ja jälkiselkeytyksen poistaa tehokkaasti (> 99%) puhdistamolle tulevan mikroroskan. Suurin osa mikroroskista (~ 98%) poistui jo esikäsittelyn aikana ja aktiiviliteprosessi vähensi edelleen (~ 88%) mikroroskien määrää esikäsittelystä jätevedestä. Suurin osa mikroroskista pidättyy jätevedenpuhdistusprosessin aikana lietteeseen. Noin 20% lietteeseen pidättävästä mikroroskasta päätyi kuitenkin takaisin prosessiin lietteen kuivauksessa syntyvän reaktiiveden mukana.

Perinteisesti käsitelystä jätevedestä voitiin edelleen poistaa mikroroskia erilaisten jälkkäsäilytyiden avulla. Membranaisuudatustekniikkaan perustuva membraanibioreaktori (engl. membrane bioreactor, MBR) poisti lähes kaikki mikroroskan (99.9 %) esikäsittelystä jätevedestä. Myös jälkiselkeytyksen jälkeistä jätevettä käsittelevät tertiäärikäsitteltyt kuten hiekasuo datin, flotaatio ja kiekkosuo datin poistivat tehokkaasti mikroroskan. Ainoastaan biologisella denitrikaatioisoudattimella ei havaittu olevan vaikutusta mikroroskien määrään jätevedessä.

Jätevedenpuhdistamot voivat olla merkittäviä mikroska- ja mikromuovikumittajia, kun ottaa huomioon, että käsitellytä jätevetta johdetaan jatkuvasti suuria määriä vesistöihin. Arviolta noin 480 miljardia mikromuovia päätyy Suomessa jätevedenpuhdistamoilta vesistöön. Toisaalta jätevedenpuhdistamot voivat tarjota ratkaisun mikromuovikuormituksen vähentämiseen.

Itsmeren sinisimpukoidissa havaittiin korkeampia mikroskaamäärää jätevesiä vastaanottavalla merialueella kuin verrokkialueella. Havaitut korkeammat mikroskaamäärät saattovat johtua jätevedenpuhdistamoiden kuormittamasta vaikutuksesta. Muiden mahdollisten mikroroskan lähteiden ja reittien vaikutusta tuloksiin ei kuitenkaan pystytty sulkemaan tutkimuksessa pois.

Avainsanat
mikroroskat, mikromuovit, yhdyskuntajätevedet, jätevedenpuhdistamot, vesivyöhykkeet, sinisimpukat

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Cover photo: microplastics from personal care products found in Finnish wastewaters.
Preface

This thesis work was carried out during the years 2014 – 2018 in the Research group of Water and Environmental Engineering, Department of Built Environment, Aalto University School of Engineering. The work was mainly funded by Maj and Tor Nessling Foundation (2014 – 2017). Maa- ja vesitekniikan tuki ry funded the thesis costs.

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Espoo, May 2018

Julia Talvitie

“Nobody said it was easy
It's such a shame for us to part
Nobody said it was easy
No one ever said it would be this hard
Oh take me back to the start”

Coldplay “Scientist”
List of original publications

This thesis is based on the following original publications and manuscripts. They are referred to in the text by the following noted Roman numerals:


Author’s contribution

Publication I: “Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea.”

The author designed the study with E. Vahtera and M. Heinonen. She performed all field sampling, laboratory analyses, and data analyses. She also wrote the article and received comments from co-authors.

Publication II: “How well is microlitter purified from wastewater? - A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant.”

The author designed the study with A. Mikola. She performed all field sampling, laboratory analyses, and data analyses. She also wrote the article and received comments from co-authors.

Publication III: “Solutions to microplastic pollution - removal of microplastics from wastewater effluent with advanced wastewater treatment technologies.”

The author designed the study with A. Mikola. She performed all field sampling, laboratory analyses, and data analyses. She also wrote the article and received comments from co-authors.

Publication IV: “Application of an enzyme digestion method reveals microlitter in Mytilus trossulus at a wastewater discharge area”

The author designed the study with co-authors. She participated in field sampling, laboratory analyses and data analyses with S. Railo. She also wrote the manuscript together with co-authors.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>activated sludge process</td>
</tr>
<tr>
<td>AD</td>
<td>anaerobic digestion</td>
</tr>
<tr>
<td>BAF</td>
<td>biologically active filter</td>
</tr>
<tr>
<td>BOD</td>
<td>biological oxygen demand</td>
</tr>
<tr>
<td>CAS</td>
<td>conventional activated sludge process</td>
</tr>
<tr>
<td>DAF</td>
<td>dissolved air flotation</td>
</tr>
<tr>
<td>DF</td>
<td>discfilter</td>
</tr>
<tr>
<td>DW</td>
<td>dry weight</td>
</tr>
<tr>
<td>EDS</td>
<td>energy-dispersive x-ray spectroscopy</td>
</tr>
<tr>
<td>FPA</td>
<td>focal plane array</td>
</tr>
<tr>
<td>FTIR</td>
<td>fourier-transform infrared spectroscopy spectrometry</td>
</tr>
<tr>
<td>FTIRI</td>
<td>imaging fourier-transform infrared spectroscopy spectrometry</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>GES</td>
<td>good environmental status</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloride</td>
</tr>
<tr>
<td>HClO₄</td>
<td>perchloric acid</td>
</tr>
<tr>
<td>HNO₃</td>
<td>nitric acid</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>KOH</td>
<td>potassium hydroxide</td>
</tr>
<tr>
<td>LS</td>
<td>lime stabilization</td>
</tr>
<tr>
<td>ML</td>
<td>microlitter</td>
</tr>
<tr>
<td>MP</td>
<td>microplastic</td>
</tr>
<tr>
<td>NA</td>
<td>not available</td>
</tr>
<tr>
<td>NaCl</td>
<td>sodium chloride</td>
</tr>
<tr>
<td>NaI</td>
<td>sodium iodide</td>
</tr>
<tr>
<td>NaOH</td>
<td>sodium hydroxide</td>
</tr>
<tr>
<td>N-tot</td>
<td>total nitrogen</td>
</tr>
<tr>
<td>PA</td>
<td>polyamide</td>
</tr>
<tr>
<td>PAX</td>
<td>polyaluminium chloride</td>
</tr>
<tr>
<td>PCPs</td>
<td>personal care products</td>
</tr>
<tr>
<td>pe</td>
<td>population equivalent</td>
</tr>
<tr>
<td>PE</td>
<td>polyethylene</td>
</tr>
<tr>
<td>PP</td>
<td>polypropylene</td>
</tr>
<tr>
<td>P-tot</td>
<td>total phosphorus</td>
</tr>
<tr>
<td>PVAL</td>
<td>poly(vinyl alcohol)</td>
</tr>
<tr>
<td>PVC</td>
<td>polyvinylchloride</td>
</tr>
<tr>
<td>PES</td>
<td>polyester</td>
</tr>
<tr>
<td>PET</td>
<td>polyethylene terephthalate</td>
</tr>
<tr>
<td>PS</td>
<td>polystyrene</td>
</tr>
<tr>
<td>PUR</td>
<td>polyurethane</td>
</tr>
<tr>
<td>RAS</td>
<td>return activated sludge</td>
</tr>
<tr>
<td>RO</td>
<td>reverse osmoses</td>
</tr>
<tr>
<td>RSF</td>
<td>rapid sand filter</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
<tr>
<td>SS</td>
<td>suspended solids</td>
</tr>
<tr>
<td>TD</td>
<td>thermal drying</td>
</tr>
<tr>
<td>WW</td>
<td>wet weight</td>
</tr>
<tr>
<td>WWTP</td>
<td>wastewater treatment plant</td>
</tr>
<tr>
<td>ZnCl</td>
<td>zinc chloride</td>
</tr>
</tbody>
</table>
1. Introduction

1.1. Background

Every year millions of tonnes of litter, i.e., man-made solid waste, end up in aquatic environments worldwide, causing environmental, economic, health and aesthetic problems. Littering affects the aquatic ecosystem through the entanglement in and ingestion of litter by aquatic species, habitat loss and transportation of alien species. In addition, littering causes serious economic damage to tourism, the fishing industry, and maritime activities (UNEP 2016). Litter is composed of variety of materials used in society, e.g., plastics, glass, paper/cardboard, metal, processed wood, and textiles (Aniansson et al. 2007, Derraik 2002, Cheshire et al. 2009, Galgani et al. 2010). Plastics are of particular concern as they are persistent, may contain harmful compounds (e.g. plasticizers and flame retardants), and may adsorb organic pollutants and heavy metals from surrounding environment (Derraik 2002).

This litter is not only those visible items found in the environment; it also includes small non-visible particles called microlitter (ML). In recent years, there has been an increased focus on the environmental problems that can arise from microlitter, particularly its synthetic subtype microplastics (MP) (GESAMP, 2016). Due to small size, microlitter is readily ingested by a variety of aquatic organisms, ranging from zooplankton to mammals (Miranda and de Carvalho-Souza 2016) and may hence enter the food chain at lower trophic levels that lead to the bioaccumulation of microlitter and its associated contaminants (Setälä et al. 2014, Rochman et al. 2013, Besseling et al. 2014).

During the time this thesis work was planned and started several reports on the potential sources and pathways for microlitter to enter the aquatic environment have been published, and wastewater treatment plants (WWTPs) have been suggested as one of those pathways (Magnusson and Norén 2014, Sundt et al. 2014, Essel et al. 2015, Lassen et al. 2015 Ziajahromi et al. 2016). The WWTPs receive microlitter from industries, households, commercial establishments, institutions and storm water run-off. Depending on the treatment level of the plant, different proportions of the incoming microlitter can be expected to pass through the wastewater treatment and become widely distributed in the receiving water environment. Several studies have reported on the microlitter in wastewater effluents (e.g. Michielssen et al. 2016, Magnusson and Norén 2014, Dris et al. 2015, Ziajahromi et al. 2017a). These data indicate that WWTPs can act as pathways for microlitter to enter the aquatic environment. As vast volumes of effluents are discharged continuously into aquatic environments and these amounts are only expected to grow due to future population growth and urbanization, the role of WWTPs as pathways for microlitter may indeed be significant. At the same time, WWTPs can offer new/better solutions to reduce the input. Despite this potential, however, very little attention has as yet been focused on the removal of microlitter during the different wastewater treatment processes.
1.2. Aims and thesis outline

The general aim of this thesis was to examine wastewater treatment plants as pathways for microlitter and microplastics into aquatic environments. WWTPs can offer solutions to reduce the microlitter input to the aquatic environment, and thus, the main focus of this thesis was to investigate microlitter removal during different wastewater treatment processes. Before this thesis was started, there were no established methods for detecting microlitter in wastewaters. Thus, in this work, sampling and analyses methods for detecting wastewater-derived microlitter was developed. The specific aims were, therefore, fourfold: (1) to develop the sampling and analyses methods for microlitter in wastewaters (I, II). (2) To assess the removal of microlitter in conventional wastewater treatment processes. In this context, the effect of microlitter size and shape on their removal in different treatments and material composition of microlitter in effluent was examined. In addition, the variation in microlitter concentration during the day and between days throughout the treatment processes was studied (II). (3). The efficiency of different advanced wastewater treatment technologies to remove microplastics from wastewater effluents was examined (II, III). Further, the annual microplastic load discharged into the aquatic environment with the existing Finnish WWTPs effluents was assessed. (4). Finally, the microlitter content in the wastewater effluent, recipient water, and local filter-feeding bivalves (blue mussels) was investigated to assess the potential effects of microlitter discharge via effluents on the receiving water body and its biota (IV).

This thesis is divided into eight chapters. Following this introduction, the concept of marine litter and its smaller forms of microlitter and microplastics are described in Chapter 2, with a specific focus on their sources and pathways into the aquatic environment. Chapter 3 focuses specifically on municipal wastewater treatment plants as pathways for microlitter. It summarizes the studies on microlitter in wastewater and wastewater-derived microlitter in aquatic environments. The general wastewater treatment level in Finland is briefly described as well as the actual wastewater treatment plants selected for this study and their applied treatment technologies. Chapter 4 describes the challenges of detecting microlitter in different environments and then discusses the different research methods applied to address this challenge. Chapter 5 offers a brief description of the particular methodology developed and used in this thesis. In Chapter 6 the main results and findings from publications are presented and discussed in. In addition, the chapter includes annual microplastic emission estimations from Finnish WWTPs. Finally, the conclusions of the study are set forth in Chapter 7 and future research needs in Chapter 8.
2. Marine litter – a global concern

Marine litter, i.e., man-made solid waste, is recognized as a major part of marine pollution that damages ecological, economic, cultural, recreational and aesthetic values of marine ecosystem and its components. According to an internationally agreed definition, marine litter is any “persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment” (UNEP 2016). Marine litter further consists of items “that have been deliberately discarded, unintentionally lost, or transported by wind and rivers into the sea and on beaches” (EU Commission 2010). In addition to marine environments, fresh water systems, like lakes and rivers, also suffer from littering (Eriksen et al. 2013, Klein et al. 2015). Monitoring studies and beach surveys show that most of the marine litter consists of plastics (Aniansson et al. 2007, Derraik 2002) and to a lesser degree, other materials, such as glass, paper/cardboard, metal, processed wood, and textiles (Aniansson et al. 2007, Cheshire et al. 2009, Galgani et al. 2010).

Plastics are one of the most widely used materials in today's modern world. Since the introduction of plastic materials more than 60 years ago, global plastic production has increased rapidly, today reaching 322 million tons produced per year and predicted to become over billion tons by 2050 (World Economic Forum 2016). Plastics are durable, malleable, lightweight and low-cost materials (Derraik 2002, Sivan 2011), and therefore, they are used in a wide variety of applications, e.g., packaging (39.5%), building and construction (20.1%), transportation (8.6%), electronics (5.7%), agriculture (3.4%) and other sectors (22.7%), including the consumer and household appliances, health, and sports and leisure activities (Plastic Europe 2016).

Plastics are produced for different applications, but the market is generally dominated by six polymers of (low-density and high-density) polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate (PET) and polystyrene (PS). In the European Union (+ Switzerland and Norway), almost 70% of the produced plastics are recycled or used for energy production (Plastic Europe 2016). Globally, the percentage is however only 28% (World Economic Forum 2016). Hence, considerable quantities of plastics are still accumulating in landfills and the environment (Thompson 2015).

It is estimated that, 4.6-12.7 million tons of plastic litter are entering the worlds’ oceans annually (Jambeck et al. 2015), accounting for 1 – 4% of total plastic production. According to another estimation based on a sampling survey and oceanographic modelling, more than 5 trillion plastic particles, weighing over 250 000 tons, float on the surface of the ocean. Most (~ 90%) of the world's ocean plastics by weight are large pieces ( > 5 mm) of litter, often referred to as meso (5mm – 5cm) and macroplastics (> 5cm). However, the dominant type of litter quantity is a smaller fraction (< 5mm), called microplastics (Eriksen et al. 2014).
Floating plastic litter, including microplastics, has accumulated in five garbage patches in subtropical gyres, as well as in coastal areas (Moore et al. 2001, Law et al. 2010, Doyle et al. 2011, Goldstein and Goodwin 2013). The coastal hot spots often appear in areas with a high population density and close proximity to a coast (Fig. 1.) (IOC/UNESCO and UNEP 2016).

Figure 1. Global spatial distribution of the abundance of floating macro- and microplastics, based on model estimates (Figure modified from IOC/UNESCO and UNEP 2016).

One of the key factors that influences the behavior of plastics in water environments is their relative density to water. The densities of common plastics range from 0.90 - 1.39 kg m\(^{-3}\) while water density varies from ~ 1.00 to 1.029 kg m\(^{-3}\), depending on its salinity and temperature. This means that only low-density plastics, e.g., polyethylene (0.88 – 0.96 g/cm\(^{3}\)) and polypropylene (0.86 – 0.95 g/cm\(^{3}\)) are expected to float. However, other factors like entrapped air, wind, water movement, biofouling and ingestion by organisms can impact the fate and transportation of common plastics in aquatic environments (Cole et al. 2011).

### 2.1. Microlitter and microplastics – size matters

Litter particles smaller than 5 mm in size are called microlitter. There is no definitely determined lower size limit for microlitter, but it is commonly separated from nano-size particles (< 1 μm). In practice, the analytical instruments (e.g. FTIR, Raman spectroscopy, electron microscopy etc.) typically determine the smallest size-fraction that can be analyzed and hence included in the examinations. Microlitter also consists of particles of different shapes (e.g., fibers, fragments, flakes and films) of synthetic and non-synthetic (natural) origin. Plastic subtype of microlitter is commonly called microplastics. Based on its formation, microlitter is also divided into primary and secondary particles. Primary particles are initially manufactured as a small size while secondary particles are the result of the breakdown of larger items. Both types – primary and secondary microlitter are found in the aquatic environments (Barnes et al. 2009).
2.2. Sources of microlitter and their pathways to the aquatic environment

Most of all the litter entering marine environments (80-90%) is land-based, meaning that their sources, means of release, transport mechanisms and pathways are geographically originally situated on land (Andrady 2011, UNEP 2016, OSPAR 2017). For microlitter, such estimations actually include assessments of the potential sources of microplastics only, with less focus on other materials. While fragmentation of plastic materials already present in the environment forms microscopic plastic fragments (Cole et al. 2011, Barnes et al. 2009, Andrady 2011), other potential sources have also been recognized.

Although the empirical data record on the sources and pathways of microlitter/microplastics is currently still very limited, few recent national reports have provided useful microplastic emission estimations. According to the reports from Sweden (Magnusson et al. 2016b), Norway (Sundt et al. 2014), Denmark (Lassen et al. 2015) and Germany (Essel et al. 2015), important potential sources of microplastics emissions include traffic through road wear and abrasion of tires, and the loss of plastic pellets that are used as raw material in plastic manufacturing (Table 1.).

Table 1. Microplastic emissions from significant land-based sources in Sweden (Magnusson et al. 2016b), Norway (Sundt et al. 2014), Denmark (Lassen et al. 2015) and Germany (Essel et al. 2015). The emissions are given as tons per year. Tire abrasion, synthetic microfiber and microbead emissions are also given as grams per individual per year.

<table>
<thead>
<tr>
<th>Microplastic sources</th>
<th>Sweden (t/year)</th>
<th>Norway (t/year)</th>
<th>Denmark (t/year)</th>
<th>Germany (t/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tire abrasion</td>
<td>7670</td>
<td>4500</td>
<td>4200 – 6600</td>
<td>60 – 111 000</td>
</tr>
<tr>
<td></td>
<td>800 g/ind/y</td>
<td>900 g/ind/y</td>
<td>750 – 1200 g/ind/y</td>
<td>1 – 1400 g/ind/y</td>
</tr>
<tr>
<td>Synthetic fibers</td>
<td>8 – 960</td>
<td>600</td>
<td>200 – 1000</td>
<td>80 – 400</td>
</tr>
<tr>
<td></td>
<td>1 – 100 g/ind/y</td>
<td>120 g/ind/y</td>
<td>36 – 179 g/ind/y</td>
<td>1 – 5 g/ind/y</td>
</tr>
<tr>
<td>Personal care products</td>
<td>66</td>
<td>40</td>
<td>9 – 29</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>6.9 g/ind/y</td>
<td>8.0 g/ind/y</td>
<td>1.6 – 5.2 g/ind/y</td>
<td>6.25 g/ind/y</td>
</tr>
<tr>
<td>Pellet loss</td>
<td>310 – 530</td>
<td>450</td>
<td>3 – 56</td>
<td>21000 – 210000</td>
</tr>
</tbody>
</table>
Other emission sources to be considered are e.g., 3D printing powders, personal care products (PCPs) (van Wezel et al. 2016, Galloway and Lewis 2017), and textiles (Browne et al. 2011). The major part of microbeads and textile fibers are estimated to be transported to wastewater treatment plants, and depending on the treatment level of WWTP, part of these microplastics are expected to end up in the aquatic environment.

Marine- or freshwater-based microplastics are mostly formed from the fragmentation of materials used in e.g., fishing and the maritime sector (including ship paint) and recreational activities (Magnusson et al. 2016b). Land-based litter enters the aquatic environments via different waterways e.g., streams, brooks, rivers, wastewaters and storm waters (Fig. 2.).

Figure 2. The main land-based sources and pathways of microplastics to the aquatic environment (Setälä et al. 2017. Original by P. J. Kershaw).
2.3. Fate and impacts of microlitter in aquatic environments

Microlitter and microplastics are ubiquitously present in aquatic systems, including surface water (Eriksen et al. 2013, Setälä et al. 2016a, Dubaish and Liebezeit 2013) the pelagic zone (Reisser et al. 2015), the seafloor (Claessens et al. 2011, Van Cauwenberghe et al. 2013, Graca et al. 2017) and in biota, ingested by aquatic organisms (Avio et al. 2015, Li et al. 2016, Taylor et al. 2016). The distribution and fate of microlitter in the aquatic environment is influenced by multiple environmental factors, including water currents, vertical transport, and retention in sediment and biota. Depending on their relative density compared to the surrounding water, microlitter can float on the water surface or become submerged in the water column. In addition, degradation, aggregation, and biofouling can change the density of any material, thereby altering its fate in a water environment (Van Cauwenberghe et al. 2013, Karlsson et al. 2017). The seafloor is suggested as the ultimate sink for microlitter (Barnes et al. 2009).

The harmful effects of larger marine litter on aquatic ecosystems have been well documented, including e.g., entanglement and ingestion of wildlife, habitat damage, and alien species transport (Derraik 2002, Andrady 2011). Most of the concerns regarding microlitter have been linked to microplastics; however, other microlitter types, such as combustion particles (black carbon) (Dubaish and Liebezeit 2013) and natural textile fibers (Ladewig et al. 2015) also have the potential to cause adverse effects on biota. The primary environmental risk associated with microlitter and microplastics is their suspected bioavailability for marine organisms. Due to their small size, microlitter and microplastics are available for ingestion by a wide range of aquatic organisms, and hence they can enter the food chain at lower trophic levels (Setälä et al. 2014, Li et al. 2016, Taylor et al. 2016).

Due to the hydrophobic nature and large surface to volume ratio, these particles can efficiently adsorb a variety of micropollutants, especially toxic metals (Rochman et al. 2014) and persistent organic pollutants which may cause harmful effects on exposed organisms (Rios et al. 2010, Chua et al. 2014, Besseling et al. 2014). The adverse effects of microlitter on exposed aquatic organisms is not solely due to ingestion; it can also produce external physical damage that can affect survival, growth, and reproduction (Ogonowski et al. 2016, Ziajahromi et al. 2017b). Microplastics can also provide substrates for microorganisms that last much longer than natural ones and hence transport non-indigenous and harmful species (Zettler et al. 2013).

However, the knowledge we have today about the effects of microlitter on aquatic organisms derives mainly from laboratory experiments where higher concentrations of microplastics are used compared to environmentally relevant concentrations. These studies also use only a few test organisms of a single species compared to the variety of species actually exposed to microplastic pollution in the environment (Ogonowski et al. 2016, Jemec et al. 2016). Consequently, the degree of the risks that microplastics and their associated contaminants may pose to aquatic ecosystems and human health is not yet fully understood.
3. Wastewater treatment plants as pathways for microlitter to the aquatic environment

3.1. Microlitter in wastewater

Wastewater treatment plants have been suggested as one of the pathways microlitter takes to enter the aquatic environment (Eriksen et al. 2013, Gallagher et al. 2016, Dris et al. 2015, McCormick et al. 2014). Large amounts of microlitter, such as the microbeads from PCPs and textile fibers from washing machine effluent, end up in the WWTPs via the sewage system (Ziajahromi et al. 2017a). Due to their small size, microlitter have been suspected to pass the treatment processes. To examine the WWTPs as possible pathways for microlitter, several studies on final wastewater effluent have been conducted (Table 2.). These studies confirm that wastewater effluent can indeed contain microlitter and microplastics.

Due the differences in methods applied in different studies (e.g. sample volume, mesh-sizes of the filters, sample processing and material characterization) it is difficult to directly compare the results. However, if the results from the same study are compared, the impact of the purification level of a plant (effluent type) on the microplastic concentration in the final effluent can be detected. Effluent after primary treatment contains clearly more microplastics when compared to the effluent after secondary or tertiary treatment (Ziajahromi et al. 2017a).
Table 2. Microlitter and microplastic abundances reported in municipal wastewater effluent.

<table>
<thead>
<tr>
<th>Effluent type</th>
<th>MP concentration (L⁻¹)</th>
<th>Size (μm)</th>
<th>Sample volume (L)</th>
<th>Material analyses</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>1.54</td>
<td>&gt; 25</td>
<td>16.5 – 100</td>
<td>Visual + FTIR</td>
<td>Ziajahromi et al. 2017a</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.008</td>
<td>&gt; 300</td>
<td>1000</td>
<td>Visual + FTIR</td>
<td>Magnusson and Norén 2014</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.0009</td>
<td>&gt; 180</td>
<td>5680</td>
<td>Visual + FTIR</td>
<td>Carr et al. 2016</td>
</tr>
<tr>
<td>Secondary</td>
<td>35</td>
<td>&gt; 100</td>
<td>0.05</td>
<td>Visual</td>
<td>Dris et al. 2015</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.25</td>
<td>&gt; 65</td>
<td>50 L</td>
<td>Visual + FTIR</td>
<td>Murphy et al. 2016</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.48</td>
<td>&gt; 25</td>
<td>150</td>
<td>Visual + FTIR</td>
<td>Ziajahromi et al. 2017a</td>
</tr>
<tr>
<td>Tertiary</td>
<td>0.05</td>
<td>&gt; 125</td>
<td>500 - 41000</td>
<td>Visual</td>
<td>Mason et al. 2016</td>
</tr>
<tr>
<td>Tertiary</td>
<td>0.28</td>
<td>&gt; 25</td>
<td>200</td>
<td>Visual + FTIR</td>
<td>Ziajahromi et al. 2017a</td>
</tr>
<tr>
<td>Tertiary</td>
<td>0</td>
<td>&gt; 45</td>
<td>189000</td>
<td>Visual + FTIR</td>
<td>Carr et al. 2016</td>
</tr>
<tr>
<td>Tertiary</td>
<td>0.28</td>
<td>&gt; 25</td>
<td>200</td>
<td>Visual + FTIR</td>
<td>Ziajahromi et al. 2017a</td>
</tr>
<tr>
<td>Tertiary</td>
<td>0 – 9</td>
<td>&gt; 20</td>
<td>390 – 1000</td>
<td>FTIR</td>
<td>Mintenig et al. 2017</td>
</tr>
</tbody>
</table>

The studies also show, that microlitter can be efficiently removed from the influent during primary and secondary treatment processes and that only a small part (~ 1%) of the incoming microlitter will be discharged into the environment with final effluent (Magnusson and Norén 2014, Carr et al. 2016, Murphy et al. 2016). Nevertheless, effluents may still act as a significant point source of microlitter, depending on the treatment level of the WWTPs and given the large volumes that are constantly discharged into the aquatic environments. For example, a typical Finnish 10 000 m³d⁻¹ WWTP with the microplastic concentration of 1 MP L⁻¹ in final effluent, would discharge 10 million microplastic particles every day.

A few studies also indicate that during the wastewater treatment, microlitter is retained mainly in the sewage sludge (Magnusson and Norén 2014, Dris et al. 2015, Carr et al. 2016). As sewage sludge is used as e.g. fertilizer in agriculture and for green construction, the use of sludge-based biosolids may be a pathway for microlitter to enter the environment (Mahon et al. 2017, Mintenig et al. 2017). However, the actual fate and transportation of microlitter during the different sludge treatments is practically unknown at present.
Wastewater treatment plants can act as a source, but also as a sink, for microlitter by offering solutions to help reducing the input of microlitter into the environment. Despite this positive potential, very little research has been focused on the actual removal of microlitter during the different types of wastewater treatment processes.

### 3.2. Wastewater-derived microlitter in the aquatic environment

The most commonly identified wastewater-derived microlitter found in aquatic environments includes polyethylene and polypropylene microbeads from PCPs and textile fibers from washing textiles. Microbeads from PCPs are light in weight and can thus be found on the water surface. For example, Eriksen et al. (2013) found multi-colored PCP microbeads on the surface of the Laurentian Great Lakes in the U.S., most likely ending up in the fresh water system from wastewater discharges. Gallagher et al. (2016) found microbeads and the textile fibers in surface waters of the Solent estuarine system in the UK, also suspected as derived from WWTPs. Cheung and Fok (2016) found microbeads in the coastal waters of Hong Kong, which they suggested to found their way into the sea via wastewater or storm waters.

A few studies have also compared the microplastic concentration and composition between the wastewater receiving site and reference site without the direct impact of wastewaters, to try to detect possible microlitter hot spots caused by wastewater discharges. Browne et al. (2011) found 250% more microplastics, mainly polyester and acrylic microfibers, from wastewater discharge site sediment compared to the reference site. Magnusson and Norén (2014) found decreasing microfiber concentration away from the wastewater discharge pipe on the Swedish west coast. In addition, the wastewater receiving area had overall a higher microfiber concentration compared to the reference site. Dris et al. (2015) and McCormick et al. (2014) found higher microplastic concentration in the highly urbanized rivers of the Seine in France and the Chicago River in U.S., respectively, after the introduction of effluent discharge from large (population equivalent (pe) > 1 million) WWTPs. On the other hand, Klein et al. (2015) did not detect any relationship between the microplastics concentration and the proximity of WWTPs when examining the sediment in the Rhine.

### 3.3. Wastewater treatment in Finland

Finland has more than 500 municipal WWTPs, 90 of which are designed for more than 10 000 pe. These 90 largest WWTPs treat almost 90% of the total wastewater volume generated in Finland (VAHTI, data system of the Finnish Environmental Institute, 2017). According to Finnish national legislation (Government Decree on Urban Wastewater Treatment 888/2006, Finlex) as directed by the European Union’s Urban Wastewater Treatment Directive (91/271/EEC), these wastewaters are required to be treated with secondary (biological) or an equivalent treatment. On average, the Finnish WWTPs remove 97% of BOD$_7$, 96% of total
phosphorus and 56 % of total nitrogen (FIWA, 2017). However, the exact requirements are defined individually for WWTPs in the WWTPs environmental permit. At the moment, there are no standards for acceptable concentrations in the environment for microlitter removal or emissions. The most commonly used treatment technique for the Finnish WWTPs is activated sludge (AS). WWTPs based on this AS process are usually preceded by pre-treatment. The pre-treatment includes screening, sand, and grit removal, and primary sedimentation (Fig. 3.). The main purpose of the screening and grit removal is to remove larger particles from the influent that could harm the later treatment processes. In primary sedimentation, particulate organic matter and organically bound nutrients are removed from the wastewater through settling. The removal efficiency can be enhanced with coagulants (e.g., iron and aluminum salts). The coagulants precipitate phosphorus and enhance the aggregation of small suspended solids into larger particles, called flocs that settle faster. Wastewater that has undergone such pre-treatment is called primary effluent.

Figure 3. A schematic of the wastewater treatment based on the AS process.

The purpose of the activated sludge process is to degrade organics and remove nutrients from the primary effluent. There is a large variety of design; however, in principle, AS consists of three main components: an aeration tank, which serves as a bioreactor; a sedimentation tank for separation of solids and treated wastewater, and return activated sludge equipment to transfer settled AS from the sedimentation tank to the aeration tank (Fig. 3.). The coagulants and flocculants are used for precipitation and enhancing the aggregation of the flocs. Part of the flocs are recycled from the secondary sedimentation process back to the AS tank.

The secondary effluent can then be discharged into aquatic environment or treated in a tertiary treatment facility. The tertiary treatments most typically applied in the Finnish WWTPs include sand filtration, biologically active filtering, and flotation techniques. Figure 4 shows the geographical distribution of plants with their different treatments in Finland. Altogether ~ 500 000 000 m³ of Finnish effluents are released annually into the Baltic Sea or into the fresh water environment, such as rivers or lakes, which then eventually flow into the Baltic Sea (Säylä, 2015).
About 150 000 tons of sewage sludge (dry weight = dw) is produced annually in Finland (Vilpanen and Toivikko 2017). The sludge formed at the wastewater treatment plants is thickened and dried mechanically. The most frequently used sludge handling method applied in the Finnish WWTPs is anaerobic digestion. The dried sludge is usually composted. Other processing methods include lime stabilization, and thermal drying. The end product, referred to as biosolids, is most commonly used for green construction (48%) and in agriculture (41%) (Vilpanen and Toivikko 2017).
3.4. Description of the selected WWTPs and the applied treatment technologies

All WWTPs included in this thesis, Viikinmäki (II, III), Kenkäveronniemi, Paroinen, Kakolanmäki (III) and Suursuo (IV) are based on a conventional activated sludge process (CAS) including pre-treatment. As a final treatment step, WWTPs (excluding Suursuo) have either a tertiary treatment, or membrane bioreactor (MBR) (Table 3.). The tertiary treatments include a biologically active filter (BAF), a disc filter (DF), a rapid sand filter (RSF) and dissolved air flotation (DAF). These treatment technologies are selected as they are either commonly applied in Finnish WWTPs or considered to be applied in near future.

Table 3. Removal of BOD₇, SS, P-tot, and N-tot with the applied treatment methods in the WWTPs. *The pilot scale treatments are not included in the removal values. Pe = population equivalent of the plant and efflux = annual effluent discharge (III, IV).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Viikinmäki</th>
<th>Kakolanmäki</th>
<th>Paroinen</th>
<th>Kenkäveronniemi</th>
<th>Suursuo</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD₇</td>
<td>95</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>96</td>
</tr>
<tr>
<td>SS</td>
<td>98</td>
<td>95</td>
<td>98</td>
<td>98</td>
<td>86</td>
</tr>
<tr>
<td>P-tot</td>
<td>95</td>
<td>95</td>
<td>99</td>
<td>97</td>
<td>90</td>
</tr>
<tr>
<td>N-tot</td>
<td>90</td>
<td>79</td>
<td>61</td>
<td>NA</td>
<td>65</td>
</tr>
<tr>
<td>Pe</td>
<td>800 000</td>
<td>300 000</td>
<td>117 000</td>
<td>50 000</td>
<td>10 000</td>
</tr>
<tr>
<td>Efflux (m³·10⁶)</td>
<td>100</td>
<td>32</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Treatments</td>
<td>CAS + BAF + DF*</td>
<td>CAS + RSF</td>
<td>CAS + DAF</td>
<td>CAS or MBR*</td>
<td>CAS</td>
</tr>
</tbody>
</table>
4. Methods for microlitter detection - how to find a needle in a haystack

A variety of detection methods for microlitter within environmental samples have been developed and employed. The selection of equipment used for sampling and the sample processing depends on the matrices studied, the size of the particles, and the level of identification. Natural samples usually contain high amounts of organic material like detritus, plankton, plants, cellulose, or even sand and clay, while the number of microlitters at the same time may be relatively low. Sample processing thus includes both the removal of natural particles and the extraction of microlitter. Although no established, widely accepted and implemented methods for sampling and analyzing microlitter have yet been set, a variety of sampling, processing and detection techniques have been developed to be able to quantify the microlitter taken from different matrices.

4.1. Sampling methods

In surface water, microlitter is commonly sampled by using surface nets like manta trawls (Eriksen et al. 2014, Setälä et al. 2016a, Kovač Viršek et al. 2016) and from a water column using plankton nets (Doyle et al. 2011). These methods do not usually sample particles < 300 μm of size, but they do allow sampling of large sample volumes (> 10 m³) that are needed specifically in low microlitter concentration environments (Löder et al. 2015a). Occasionally other techniques like submerged pumps (Setälä et al. 2016a, Magnusson and Norén 2011), low-volume bulk samples (Dubaish and Liebezeit 2013, Ng and Obbard 2006) and continuous plankton recorder (Thompson et al. 2004) have been used to assess microlitter abundances in the water environment. Wastewater sampling is performed usually with the direct in-situ filtering technique introduced the first time by Magnusson and Norén (2014). Before the analyses of microlitter, the samples often require further processing in a laboratory.

4.2. Sample processing

The main processing steps include volume reduction and the digestion (=destruction) of organic matter from the samples and the separation of litter particles from the matrix, usually by applying a method based on the specific density of the litter particles (Masura et al. 2015, GESAMP 2016). Volume reduction is an important step, in particular when large samples volumes are required and expensive chemicals are needed in later sample processing steps. Volume reduction is usually performed by using sieving, filtering, or elutriation techniques (Claessens et al. 2013, Hidalgo-Ruz et al. 2012). The elutriation method decreases the volume...
of solid substances like sand and clay from the samples and is targeted mainly for sandy sediments, although it has also been applied to sludge samples (Claessens et al. 2013, Mahon et al. 2017).

The purpose of the digestion methods is to remove organic matter from the samples. Various digestion methods, using simple and/or mixtures of strong acids (HCl, HNO₃, HClO₄) (Claessens et al. 2013, De Witte et al. 2014, Van Cauwenbergh and Janssen 2014) and bases (NaOH, KOH) (Claessens et al. 2013, Dehaut et al. 2016, Rochman et al. 2015) have been developed. Some of these methods can damage or destroy examined litter particles, leading to underestimations of microlitter content (Dehaut et al. 2016). As an example, the nitric acid (HNO₃), which is used in some investigations, has been proven to destroy some of the polyamides, indeed among the most commonly produced plastic polymers (Avio et al. 2015, Catarino et al. 2017, Cole et al. 2014).

Oxidizing chemicals (H₂O₂) (Li et al. 2016, Dehaut et al. 2016, Ziajahromi et al. 2017a) and enzymes (Catarino et al. 2017, Cole et al. 2014) either separately or in combination (Cole et al. 2014, Mintenig et al. 2017) have produced good results for digesting organic material, without damaging plastics, including sensitive microplastics like synthetic fibers.

Density separation is the most commonly used method for extracting microplastics from sediment (Hidalgo-Ruz et al. 2012) and other high organic matter matrices like wastewater and sludge (Mahon et al. 2017, Ziajahromi et al. 2017a, Mintenig et al. 2017). This density separation is commonly performed by mixing the sediment sample with concentrated sodium chloride (NaCl) solution, as described by Thompson et al. (2004). The NaCl solution, with a density of 1.2 g cm⁻³ does not, however, extract higher density plastics like PET, and PES. To increase the extraction efficiency, higher density salt solutions, such as ZnCl with a density 1.5 – 1.7 g cm⁻³ (Mahon et al. 2017) and NaI with a density 1.6 – 1.8 g cm⁻³ (Karami et al. 2017, Ziajahromi et al. 2017a) have been applied.

4.3. Microlitter characterization

Most of the studies have relied on the visual identification of microlitter to separate the plastics and other examined materials from organic matter. Visual identification is often performed with the help of a dissecting microscope or in the case of the larger litter items (> 1 mm), by directly examining the samples with the naked eye (Hidalgo-Ruz et al. 2012). During the microscopic analysis, particles are measured, categorized (e.g., fibers/lines, fragments, flakes/films, spherical, and sometimes foams) and their coloration documented. Easily disintegrating particles with cellular structures are excluded from further examination as organic matter (Masura et al. 2015, Kovač Viršek et al. 2016). While numerous studies have utilized visual-only identification, the instrumental analysis methods are required for precise polymer identification.
Polymer identification is most commonly performed with FTIR or Raman spectroscopy. As plastic polymers possess specific IR and Raman spectra, these techniques are ideal for microplastic identification (Löder et al. 2015b). Other analytical techniques, such as pyrolysis gas-chromatography-mass spectrometry (pyr-GC-MS) (Claessens et al. 2013, Fries et al. 2013) and electron microscopy (e.g. SEM, SEM-EDS) have also been used successfully to identify microlitter materials (Li et al. 2016, Magnusson and Norén 2011). If this chemical analysis includes a visual identification (pre-selection) step, then these results may contain the risk of underestimation due to missed particles. Particularly, small and transparent particles can go unnoticed. More recently, chemical mapping with Focal Plane Array (FPA)-based micro-FTIR (Tagg et al. 2015, Löder et al. 2015b, Mintenig et al. 2017) has been suggested as an unbiased method for microplastic identification. In this analysis technique, samples are analyzed automatically, and hence, the technique does not include visual pre-selection with that risk of a misdetection of particles.

4.4. Contamination of samples

When working with environmental samples there is a continuous risk for sample contamination. Contamination can take place during both sampling and sample processing. Contamination may include e.g. fragments from sampling device, labware and clothing, but most often contamination in microlitter samples is airborne and consists of textile fibers (Nuelle et al. 2014, Woodall et al. 2015). The evaluation of the microlitter abundances in the environment can therefore easily be affected by contamination of samples.

Several procedures have been suggested to control the contamination. All of the equipment should be rinsed with microlitter-free water (e.g. filtered tap water, or distilled water) and all fluids and chemicals filtered prior the use. Plastic materials should be avoided during sampling and sample processing when possible (Woodall et al. 2015). In addition, sample processing should take place in clean labs and sample exposure to the air should be minimized (e.g., sealing of filters in Petri dishes) (Hidalgo-Ruz et al. 2012, Woodall et al. 2015).

Despite these efforts, avoiding the microlitter contamination of the samples is challenging and therefore different control sampling (including procedural blanks) should be performed (Hidalgo-Ruz et al. 2012). Furthermore, laboratory contamination levels during sample processing can be evaluated by using dampened filter paper to collect air-borne particles (e.g. Thompson et al. 2004, Browne et al. 2011, Woodall et al. 2015).

As the airborne fibers are most commonly contaminating the samples, some studies have excluded them from the examination (Dekiff et al. 2014; Goldstein and Goodwin 2013, Van Cauwenberghe et al. 2013). However, as textile fibers are often dominating microlitter type in environment (Browne et al. 2011, Mathalon and Hill 2014, Woodall et al. 2014, Li et al. 2016), excluding them may bias the interpretation of the microlitter contamination levels in the environment.
5. Methods used in this thesis for microlitter detection

5.1. Developing sampling method for wastewater-derived microlitter

Due to the lack of a standardized method, a simple filtering device was developed to sample microlitter from wastewater and the receiving environment (I). Sampling was carried out by either pouring or pumping water into a filtering tower that holds a series of different mesh-size filters in decreasing mesh size order (Fig 4.). The filter mesh sizes applied throughout all the studies presented in this thesis were 300, 100, and 20 μm, producing particle size fractions of > 300μm, 100 – 300 μm and 20 – 100 μm, respectively.

Figure 4. The filtration device. On left: filter placed on the filter holder between connectors. On right: The filtration device assembled with three different filter holders.
5.2. Sampling wastewater and sludge

5.2.1. Removal of microlitter in conventional wastewater treatment

The removal of microlitter during conventional wastewater treatment process was examined in Viikinmäki WWTP (II). The wastewater treatment at Viikinmäki consists of coarse screening (10 mm), grit removal, pre-aeration, primary sedimentation, activated sludge treatment, secondary sedimentation and a tertiary biological filtration (Table 3.). Three replicates were collected from influent, primary effluent, secondary effluent, and reject water (water from sludge dewatering process) during one day. The primary effluent and secondary effluent samples were collected with the in-situ filtering while influent and reject water samples were collected with a metallic beaker, then poured into pre-cleaned plastic containers, and transported into the laboratory for filtration. The sample volumes depended mainly on the sampling site and mesh-size of the filter and they are given in table 4.

Table 4. The sample volumes with sampling site and mesh sizes of the filters.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Sample volume 300 μm filter</th>
<th>Sample volume 100 μm filter</th>
<th>Sample volume 20 μm filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>0.1 L</td>
<td>0.1 L</td>
<td>0.1 L</td>
</tr>
<tr>
<td>Primary effluent</td>
<td>50 – 150 L</td>
<td>10 L</td>
<td>0.5 L</td>
</tr>
<tr>
<td>Secondary effluent</td>
<td>200 L</td>
<td>30 L</td>
<td>1 L</td>
</tr>
<tr>
<td>Reject water</td>
<td>0.01 L</td>
<td>0.01 L</td>
<td>0.01 L</td>
</tr>
</tbody>
</table>

5.2.2. Microlitter in sludge

To examine the sewage sludge as pathway for microlitter into the environment three replicates of the raw and excess sludge as well as dried sludge samples were collected. The raw and excess sludge was collected with a metallic beaker and dried sludge by hand from the conveyor belt. These samples were placed into pre-cleaned plastic containers and transported into the laboratory for further processing. Subsamples of 1.0 g and 0.2 g from raw and excess sludge as well as from dried sludge were taken, respectively, for further examination. (II). The solid content of the raw and excess sludge in Viikinmäki WWTP was approximately 2%, and in the dried sludge 29%.
5.2.3. **Intra-day and inter-day variation for microlitter in wastewater**

To examine the variation of microlitter concentration during the day (intra-day variation), twenty four 1 L samples from the influent and effluent were taken hourly over a 24h period using an automated sequencing sampler (II).

Variations in the microlitter concentration between the days (inter-day variation) were examined by sampling every three days for one week (Monday, Wednesday and Saturday). Samples from the influent, primary effluent, secondary effluent, and tertiary effluent were included in the examination.

To take the inter-day variation into account, the wastewater sampling through the week was repeated with 24-h automated composite samplers. The device took samples from Monday to Tuesday, from Wednesday to Thursday, and from Saturday to Sunday. Composite samplers at each sampling site (influent, primary effluent, secondary effluent, tertiary effluent) took flow-proportional, discrete samples at regular 15 min intervals over a 24-h period (II).

5.2.4. **Removal of microlitter using advanced final-stage treatment technologies**

The removal of microlitter from primary and secondary effluents was examined with pilot-scale MBR and DF and three other full-scale tertiary treatment processes (BAF, RSF, DAF) (II, III). Effluent samples with three replicates were collected before and after these treatments. A filtering device with a pump was used when sampling full-scale treatments (BAF, RSF, and DAF). The pump was not needed in the pilot-scale studies (DF, MBR) as they contained sampling taps designed to perform sampling before and after the treatments; hence the filtration was performed directly from the taps.
5.3. **Sampling microlitter in the wastewater receiving environment**

5.3.1. **Surface water and water column**

Background information on the microlitter concentration in the Suursuo WWTP receiving environment was collected from the water surface and the water column to collect different size-fractions (Table 5). Surface water and the water column were sampled from both sites where blue mussels were caged (IV). In connection with this environmental sampling, the wastewater effluent was sampled to investigate the microlitter discharge to the receiving environment.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Sampling method</th>
<th>Filter mesh-size</th>
<th>sample volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea water (surface)</td>
<td>Pump and filter</td>
<td>20, 100, 300 μm</td>
<td>20 – 1000 L</td>
</tr>
<tr>
<td>Sea water (column)</td>
<td>Plankton net</td>
<td>100 μm</td>
<td>470 – 930 L</td>
</tr>
<tr>
<td>Effluent</td>
<td>Pump and filter</td>
<td>20, 100, 300 μm</td>
<td>2 – 333 L</td>
</tr>
</tbody>
</table>

5.3.2. **Collection of blue mussels and a caging set-up**

An experimental approach was applied to study the ingestion of wastewater-derived microlitter in biota. In that study, blue mussels (*Mytilus trossulus*) were incubated in cages at the wastewater receiving environment, on the Southern coast of Finland, Baltic Sea (IV). Approximately 450 adult mussels with shell lengths of 2-3cm, were collected from the wastewater receiving area; 50 mussels were immediately collected for laboratory analyses, to represent the original mussel community (=source population); the remaining 400 individuals were placed in two stainless steel cages (200 individuals / cage). Cage 1 was placed at the original site where the animals were captured; cage 2 was located 700 meters away from Cage 1 and 30 meters from the wastewater discharge pipe. The cages were left in water for 4 weeks, and every week, 50 randomly selected healthy appearing mussels were collected for microlitter content examination. To compare the microlitter content of the mussels from the wastewater receiving area, reference mussels were collected from the eastern side of Lågskär, Sea of Åland, at an area not subjected to any known direct anthropogenic stress sources.
5.4. Sample processing

With these wastewater and seawater samples, the sample volume was optimized, so that sampling was stopped before the filters were filled with organic material. The clogging of the filters was observed through the transparent walls of the filtering device (II, III, IV). The sludge, influent, and reject water samples were diluted by mixing the samples with tap water and then filtering them with the filtering device (II).

To select the most suitable digestion method to use to extract microlitter from mussels, different methods were tested and compared (IV). The aim was to select an effective, but gentle, method for digesting mussel tissues, while at the same time preserving all textile fibers, including non-synthetic ones.

The digestion protocol based on enzymatic treatment, adjusted from Löder et al. (2017), was selected. The protocol included a 2:1 mixture of sodium dodecyl sulfate (SDS, 5 g L\(^{-1}\)) and enzymes (1:1 Biozym F and Biozym SE, Spinnard, Bad Segeberg, Germany). The mussels were incubated with the digestion solution in an oven at 37.5 °C for 48-72 hours. After the digestion, the samples were filtered through 20 \(\mu\)m filters using a vacuum filtration device.

5.5. Microlitter characterization

All samples were visually examined using a stereomicroscope (Wild heerbrugg, magnification \(\times50\)) (II. II, IV). During that examination, microlitter particles were identified, counted, and divided into fibers, fragments, flakes, films, and spheres and their coloration documented. The morphological properties of the particles were inspected using micro- tweezers to exclude organic matter (Masura et al. 2015). In this thesis, the term “microlitter” includes both synthetic and non-synthetic materials. The term “microplastic” includes only synthetic polymers verified with the FTIR.

The material characterization of these microlitter particles was performed using Fourier Transform infrared spectroscopic imaging (FTIRI) (Spectrum Spotlight 300, PerkinElmer, Waltham, MA, USA) (II, III, IV). The FTIR spectra were recorded in transmittance mode, in the wavelength region of 700 – 4000 cm\(^{-1}\) at 4 cm\(^{-1}\) resolution and with 15 scans. To analyze the spectra, the Thermo Scientific™ Hummel Polymer Library was used. The spectra of textile fibers were analyzed with a textile fiber library made from pure model fibers of cotton, wool, linen, silk, viscose, polyester, polyacryl, polyanide, and polypropene (II). The characterization technique allowed the inclusion of all particles size > 20 \(\mu\)m.
5.6. Sample contamination

To reduce the contamination, all equipment included in the microlitter sampling protocol was rinsed with tap water prior to use. Filters were inspected with a microscope to ensure sufficient rinsing. If any particles were found in the filters, they were removed with tweezers. All chemicals used in the sample processing were filtered with a 20 μm filter prior to use. Despite these efforts, avoiding microlitter contamination of the samples is always challenging; therefore different control samples were taken for the different sampling techniques (II, III, IV).

For the wastewater samples, control sampling was performed by filtering 100 L of tap water directly into the filtering device. For the composite and sequential sampling, three blank samples of tap water were collected and put into containers and bottles, respectively, and kept inside the sampling devices for 24-hours, like actual samples (II, III).

The sampling, filtering and analyses procedures for influent, reject water and sludge samples were almost identical to those used in 24 sequential sampling. Hence, the sequential sampling controls functions also as controls for these samples.

For the mussel samples, empty glass vials were processed according to the laboratory method applied for mussels and used as procedural blanks to verify contamination. Further, to study the impact of the shell removal for the background contamination levels, half of the mussel samples went through shell removal prior to digestion, and half were digested with their shells still on (IV).

For the seawater samples, three control samples were taken using a pump and the filtering method upstream from the Käräjäkoski River from a site used as an official reference site for water quality studies of the river recipient for several WWTPs.

5.7. Data analyses

SPSS version 24 was used to run the statistical tests. As the requirements of parametric tests were fulfilled, Independent Samples t-test was used to test for differences in the microlitter and microplastic contents between the wastewater receiving area mussels (cage 1, cage 2 and source population) and between wastewater receiving and the reference site mussels. Independent samples T-tests were also performed to learn whether there were differences in the microlitter contents of shelled and non-shelled mussels. Both the ML (and MP) ind\(^{-1}\) and ML (and MP) g ww\(^{-1}\) values were utilized.

The microlitter concentration in mussels did not differ significantly between the study sites for the wastewater receiving area (Cage 1, Cage 2, source population) nor did the concentration depend on the time of collection (Independent Samples T-test, p>0.05). Therefore, mussel
results from the wastewater receiving area (cage 1, cage 2, source population) as well as the results of mussels collected at different times during the 4-week caging period were pooled.

Independent samples T-test was also applied to test for differences in microlitter concentration in surface water and in the water column between cage 1 and cage 2 locations, as well as between the microlitter results (size fraction >100μm) yielded from the two different methods; pump and filtering and plankton net.

The microplastic emissions from the 87 largest Finnish WWTPs (> 10 000 pe) were estimated by multiplying annual efflux (m³/a) of each plant with the corresponding average microplastic concentrations of the effluent type discharged from the plant. In 2016, these plants discharged altogether 435 000 000 m³ of effluent. Secondary treated effluent (72 plants) covered 359 000 000 m³, tertiary effluent treated with sand filtration (6 plants) 51 000 000 m³ and tertiary effluent treated with flotation technique (9 plants) 25 000 000 m³ of the total discharge (VAHTI, data system of the Finnish Environmental Institute, 2017). The microplastic concentrations of the effluents included in the estimations are 1.32 MP L⁻¹ (secondary effluent, calculated as average from the secondary effluent values given in figure 5), 0.02 MP L⁻¹ (sand filtration) and 0.1 MP L⁻¹ (dissolved air flotation).

It was further estimated how upgrading 15 largest secondary WWTPs (pe >100 000) with different tertiary treatments (sand filtration, flotation, disc filter) or MBR, would reduce the total microplastic emissions from Finnish WWTPs. In 2016, the annual efflux of these 15 WWTPs was 239 000 000 m³ (VAHTI, data system of the Finnish Environmental Institute, 2017). Microplastic emissions from these WWTPs were calculated by multiplying the annual efflux with average microplastic concentration of secondary effluent. The emissions after upgrading these plants with different treatments were estimated by multiplying the annual efflux with average microplastic concentrations detected after these different treatments. The microplastics concentration after disc filter (20μm) was 0.03 MP L⁻¹, after flotation 0.1 MP L⁻¹, after sand filter 0.02 MP L⁻¹ and after MBR 0.005 MP L⁻¹ (Fig. 5.).
6. Results and discussion

6.1. Removal of microlitter in conventional wastewater treatment

The removal of microlitter from municipal wastewater influent during pre- and secondary treatment processes was examined in Viikinmäki WWTP (II). Most (> 99%) of the microlitter in wastewater influent was removed during the pre- and secondary treatments. The pre-treatment alone removed ~ 98% of the microlitter (from 686.7 to 10.9 ML L⁻¹). The activated sludge process (AS) further removed (~ 88%) microlitter from the primary effluent (from 10.9 to 1.3 ML L⁻¹). Only <0.5% of the incoming microlitter was thus left in the secondary effluent.

Most of the microlitter was retained in the sludge. However, according to the balance analyses (II), approximately 20% of the microlitter retained in the raw and excess sludge was recycled back to the treatment process with the reject water. Hence, the reject water may act as a considerable inner source of the microlitter transport back to the wastewater treatment process. Additional treatment to remove microlitter from the reject water would hence decrease the microlitter load to the influent.

It is difficult to compare the actual microlitter concentrations between the studies carried out at different WWTPs due the differences in wastewater treatment processes applied in WWTPs, different sampling methods (e.g. mesh-size of the filters, sample volumes, analyses of the microlitter) and sampling sites. Despite of these differences, the similar removal trends can be seen. For example, Carr et al. (2016) detected microplastics and other fibrous particles from the surface scum of primary sedimentation and aeration tanks, but found hardly any from the return activated sludge (RAS) or from the secondary or tertiary effluent. Based on these findings, they concluded that the majority of microplastics are most likely removed during the pre-treatment stages of skimming and settling. Due to problems with the sampling and sample processing, the study was unable to identify microplastics from the influent, and hence it could not provide any removal estimations.

Michielsens et al. (2016) found a high removal (~96%) of microlitter in a WWTP applying secondary treatment (from 133 to 5.9 ML L⁻¹). Most (90%) of the microlitter was removed during pre-treatment of screening, grit removal, and primary clarifiers (from 133 to 12.9 ML L⁻¹) with an additional removal of 54% accomplished in the secondary treatment of activated sludge process.

Murphy et al. (2016) reported >98% removal of microplastics in a WWTP with secondary treatment. The pre-treatment, including fine screening (6 mm), grit- and grease removal and primary sedimentation, removed ~80% of the incoming microplastics (from 15.7 to 3.4 MP L⁻¹). The microplastics were further removed (~90%) from the primary effluent during secondary sedimentation (from 3.4 to 0.25 MP L⁻¹). Their analysis of the solid fraction of grit, grease, and
dried sludge samples also showed high amounts of microplastic accumulating in these three matrices. Ziajahromi et al. (2017a) reported a removal rate of 66% (from 1.44 to 0.48 MP L\(^{-1}\)) during secondary treatment of AS process and UV disinfection.

According to the results presented in this thesis and other studies presented above as well, pre-treatment has the most fundamental effect on the removal of microlitter. During pre-treatment, microlitter and microplastic particles are probably attached to larger litter items and removed from the wastewater during the skimming and primary sedimentation steps (II).

In addition, these results show that secondary treatment can further remove microlitter. It is a finding also supported by parallel studies (Ziajahromi et al. 2017a, Murphy et al. 2016, Michielssen et al. 2016). During the AS process, microlitter is probably mixed with flocs and settles into the sludge during secondary sedimentation (II). Flocculants, such as ferric sulfate and polyacrylamides, are often used in these processes in order to enhance the aggregation of suspended particulate matter into larger flocs. These chemicals are not designed, however, to aggregate microplastics, so more information is needed to clarify whether the chemical dosage could have an effect on the microlitter and microplastics present in the wastewater.

### 6.2. Microlitter concentration in sludge

The average microlitter concentrations detected in raw and excess sludge and in dried sludge were 3 815 000 (±215 000) ML kg\(^{-1}\) (dw) and 644 00 (±89 700) ML kg\(^{-1}\) (dw), respectively. This indicates that part of the microlitter may be removed (e.g. due the microbial degradation) from the raw and excess sludge during the anaerobic digestion (AD) and dewatering processes. However, a systematic study on the removal of microlitter during different sludge treatment would be required to confirm this. The proportion of microplastics was not determined from the sludge samples; however, approximately 18% of the microlitter in effluent water at the same WWTP and sampled at the same time was determined as plastics (II). If it is assumed, that 18% of the microlitter would be plastics also in sludge samples, the microplastic concentration in raw and excess sludge would be 1 370 000 (±77 000) MP kg\(^{-1}\) (dw) and in dried sludge 116 000 (±16 000) MP kg\(^{-1}\) (dw). This is, however, just an assumption and does not necessary represent the reality.

In Germany, Mintenig et al. (2017) studied dried sewage sludge at six WWTPs. The samples showed strong variations between the WWTPs in their estimated microplastics concentrations, ranging from 1000 to 24 000 MP kg\(^{-1}\) (dw). In Ireland, Mahon et al. (2017) characterized microplastics in sludge samples that underwent treatments of AD, thermal drying (TD) and lime stabilization (LS). Microplastic concentrations in that study ranged from 4196 to 15 385 MP kg\(^{-1}\) (dw). That study showed the lowest microplastic concentration in the AD samples and suggested that the process may reduce microplastics via microbial degradation. In contrast, higher concentrations, but smaller sizes of microplastics in the LS samples suggested that the treatment process may also enhance the fragmentation of microplastics.
The microplastic concentration in dried sludge presented in this thesis was ten-fold higher compared to concentrations found in other studies (Mintenig et al. 2017, Mahon et al. 2017). These results are likely not comparable between studies due to the differences in sample volumes (0.2 – 40 g), mesh-sizes of the filters (0.2 – 250 μm), and the sample processing techniques. In addition, the microplastic concentrations of sludge presented in this thesis is estimated, not an absolute detected value. Further, in Mintenig et al. (2017) fibers were excluded from the examination, while in this thesis and in that by Mahon et al. (2017) textile fibers were common microlitter type. Despite these differences, the results do confirm that sewage sludge can act as a pathway for microlitter and microplastics entrance into the environment, when it is applied in land use. Further, the results of this thesis and in Mahon et al. (2017) suggest that some sludge treatments (e.g., LS, AD, and dewatering) may affect the abundance of microplastics in sewage sludge.

Over time, there may be consequences for the accumulation of microplastics in terrestrial ecosystems via land-applied sewage sludge (Nizzetto et al. 2016). These possible consequences can include e.g., interaction with the contaminants in the soil (Turner et al. 2015, Peyton et al. 2016), ingestion of terrestrial organisms (Huerta Lwanga et al. 2016, Zhao et al. 2016, Rillig et al. 2017), fragmentation into smaller particles (Barnes et al. 2009) and further transportation to the aquatic environments (Peyton et al. 2016). However, at the moment, little is known about the impacts of microplastics in terrestrial ecosystems. In addition, whether land-applied sewage sludge is a sink or a pathway for microplastics to the aquatic environment yet remains unknown.

6.3. Microlitter characteristics in wastewater

6.3.1. Size and shape distribution

The removal of different sizes and shapes of microlitter particles during wastewater treatment was also examined. Pre-treatment removed the larger size fractions (300μm – 5mm and 100 – 300 μm) particularly well (II). This outcome is in line with the purpose of pre-treatment, particularly screening and grit removal, which is to remove larger particles from the wastewater to protect further treatment steps. This finding is also supported by Mintenig et al. (2017), which reported the most common (~ 60 %) size class of microplastics being in a range of 50-100 μm in the final effluent in German WWTPs. In their study, however, only ~ 10% of the MPs were over 200 μm in size.

Of the different shapes of microlitter, fibers were the most common in all treatment processes. In the influent, fibers accounted for 68 % of the microlitter, followed by flakes (14%), fragments (10%), and films (8%). In the primary effluent, fibers accounted for 44%, followed by fragments (27%), flakes (15 %), and films (13%). In the secondary effluent, the corresponding percentages were 49%, 29%, 20 %, and 1% (II). During primary treatment,
fibers probably attached easily to grit and to other larger organic waste material and are settled into the sludge efficiently in primary sedimentation. Despite the relatively efficient removal of fibers during pre-treatment, they were still the most common shape in the primary as well as in the secondary effluent.

In addition, the proportion of fragments increased during the process (II). For example, PE fragments that originated from the PCPs were commonly detected in the primary and secondary effluent samples, but they were not found in the influent samples at all. It is likely that the low influent sample volume (0.1 L) used in this thesis was not adequate enough to detect these rare particle types. Neither was another type of microlitter, spherical PE particle (identified as microbeads from PCPs) detected in influent samples, but was found in small amounts (~ 0.1 %) in secondary effluent (II).

In this thesis as well as in other studies (Magnusson and Noren. 2014, Carr et al. 2016, Murphy et al. 2016, Mason et al. 2016, Ziajahromi et al. 2017a) fibers and fragments were reported to be the most common microlitter and microplastics types in the effluent samples. In addition, Murphy et al. (2016) reported that the wastewater contained flakes (67.3%), fibers (18.5%), film (9.9%), beads (3.0%), and foam (1.3%), when combining all the treatment processes (influent primary and secondary effluent). The study concluded that the estimation of flakes might, however, have been overestimated, as the flakes fragmented easily during identification. Hence, it is good to note that the fragmentation of particles during sampling and sample processing can actually affect the results.

6.3.2. Material characterization

The material identification was performed only on the effluent samples (II). The most common microlitter materials detected in the effluent were cotton (39%), PES (32%), and linen (14%) derived mainly from textile fibers. The most commonly detected plastics were PES, PE (4%), PP (1%), and PS (< 1%). These results highlight the importance of textile fiber, as a common microlitter type in wastewater, for affecting the detected material composition of microlitter and microplastics. Both PE and PP are among the most commonly produced and used plastics in our society; hence, it can also be expected to be commonly found as microplastic material in wastewater.

When textile fibers are included in microplastics studies, PES usually dominates the samples. According to one Australian study (Browne et al. 2011) the most common polymers in effluent are PES (30.4%), PA (21%), and acrylic (13%), derived from textile fibers. In another Australian study, the most frequently detected MPs were PES fibers and irregular PE fragments, which likely originated from the washing of clothes and the use of personal care products, respectively (Ziajahromi et al. 2017a). In their study, Murphy et al. (2016) reported the common polymers of PES (28%), PA (20%), PP (12%) and acrylics (%). However, in the study where material analyses were based on automated chemical mapping, PE dominated the effluent samples (40%), followed by polyvinyl alcohol (PVAL) (16%), and PA and PS (each
at 8%) (Mintenig et al. 2017). This is probably due the fact that only part of the textile fibers were included in the examination; hence, the results may underestimate the PES values.

6.4. Intra-Day and inter-day variation of microlitter in wastewater

Intra-day variation of the microlitter concentration was detected in both the influent and effluent samples with 24-hour sequential sampling (Fig 2, Paper II). The lower microlitter concentrations were detected during nighttime (10 pm – 7 am) (influent: 477 ML L⁻¹ and effluent 0.8 ML L⁻¹), when compared to the daytime (7 am – 10 pm) (influent: 584 ML L⁻¹ and effluent 1.7 ML L⁻¹). Additionally, the lowest microlitter concentration during daytime was detected from 1 – 4 pm with the concentration increasing toward the early evening hours.

Also, an inter-day variation was detected (Table 6, Paper II). Microlitter concentration in the influent varied from 380 (±52.2) ML L⁻¹ to 686.7 (±155.0) ML L⁻¹ and for effluent from 0.7 (±0.6) ML L⁻¹ to 3.5 (±1.3) ML L⁻¹ during the sampling week (II). The inter-day sampling was performed always at the same time of day to exclude the effect of intra-day variation on the results.

According to the results from the pump and filtering method as well as from the composite sampler, the inter-day variation was three to fivefold. In addition, the results from the sequential sampler indicate that the inter-day variation is even higher, close to tenfold in tertiary effluent (Table 6.). Therefore both the intra-day and inter-day variations should be taken into consideration when estimating the total microlitter load discharged from the WWTPs into aquatic environments. It may be more appropriate to take frequent samples throughout the day and in different days rather than snapshots.

Table 6. The microlitter concentration ranges in different sampling sites detected using different sampling methods. Data are given in the number of microliter particles per liter of wastewater (ML L⁻¹). NA = not available.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Sequential sampling</th>
<th>Pump &amp; filtering</th>
<th>Composite sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>440.0 – 670.0</td>
<td>380.0 – 686.7</td>
<td>389.4 – 899.4</td>
</tr>
<tr>
<td>Primary effluent</td>
<td>NA</td>
<td>9.9 – 14.2</td>
<td>3.5 – 23.2</td>
</tr>
<tr>
<td>Secondary effluent</td>
<td>NA</td>
<td>1.0 – 2.0</td>
<td>0.9 – 2.5</td>
</tr>
<tr>
<td>Tertiary effluent</td>
<td>0.3 – 2.6</td>
<td>0.7 – 3.5</td>
<td>0.8 – 2.2</td>
</tr>
</tbody>
</table>
6.5. Removal of microplastics with advanced wastewater treatment technologies

The removal of microplastics from municipal wastewater effluents using five different advanced final-stage treatment technologies was examined. The MBR removed 99.9% of the microplastics from primary effluent during the treatment (Fig. 5.). The RSF removed 97% DAF 95% and DF removed 40 – 98.5% of the microplastics from secondary effluent during the treatments. Only BAF did not decrease the microplastic concentration. In addition, these treatments removed all shapes and size fractions of microplastics (excl. BAF). Before and after the treatments, the smallest size fraction (20 – 100 μm) dominated the microplastic size composition while fibers were the most abundant microplastic shape.

![Image of treatment technologies]

Figure 5. The removal of microplastics from wastewater effluent using the five different final-stage treatment technologies of membrane bioreactor (MBR), rapid sand filtration (RSF), dissolved air flotation (DAF), disc filter (DF) and biologically active filter (BAF).

A small number of studies have reported the removal of microlitter and microplastics during tertiary/final-stage wastewater treatment phases (Table 7.). Mason et al. (2016) reported a minor (15%) reduction of microplastics during the tertiary sand filtration. However, they concluded that other factors besides tertiary treatments, such as population equivalent, microplastics sources, combined sewage system, and the quantity and quality of storm waters received by WWTP, could also explain the microplastic abundance detected in the final effluent (Mason et al. 2016). While this conclusion may be true, according to the results in current study, sand filtration (RSF) efficiently removed microplastics from wastewater, including all size classes and shapes.
Michielssen et al. (2016) reported that granular sand filtration removed 56% of the microlitter from secondary effluent, and the pilot scale MBR removed 92% of the microlitter from primary effluent. Mintenig et al. (2017) reported that the pile cloth media filtration technique removed 95% of the MPs (20 – 500 μm) from secondary effluent. These observations are similar to the results presented in this thesis, despite the differences in the sampling methods.

Further, Ziajahromi et al. (2017a) found ultrafiltration, together with secondary (biological) treatment to decrease 87% of the microplastics, while reverse osmoses (RO) further removed 25%. While the microplastic concentration clearly decreased during these advanced treatments, it is not fully explained how the remaining microplastics escaped. In particular, the concentration of microplastics larger than > 25 μm after RO was high (Table 7.), as the RO should be able to remove even micro-scale bacteria and protozoa and even nano-scale viruses and pharmaceuticals and pass only water molecules.

Table 7. The removal of microplastics during advanced final-stage treatments as reported in different studies. MBR = membrane bioreactor, RSF = rapid sand filtration, DAF = dissolved air flotation, DF = disc filter, BAF = biologically active filter.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Source water quality</th>
<th>Before (MP L⁻¹)</th>
<th>After (MP L⁻¹)</th>
<th>Removal (%)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSF</td>
<td>Secondary</td>
<td>0.7</td>
<td>0.02</td>
<td>97</td>
<td>III</td>
</tr>
<tr>
<td>DAF</td>
<td>Secondary</td>
<td>2.0</td>
<td>0.1</td>
<td>95</td>
<td>III</td>
</tr>
<tr>
<td>DF</td>
<td>Secondary</td>
<td>2.0</td>
<td>0.03</td>
<td>98.5</td>
<td>III</td>
</tr>
<tr>
<td>BAF</td>
<td>Secondary</td>
<td>1.4</td>
<td>2.5</td>
<td>0</td>
<td>III</td>
</tr>
<tr>
<td>MBR</td>
<td>Primary</td>
<td>6.9</td>
<td>0.005</td>
<td>99.9</td>
<td>III</td>
</tr>
<tr>
<td>Sand filtration</td>
<td>Secondary</td>
<td>5.9</td>
<td>2.6</td>
<td>65</td>
<td>Michielssen et al. 2016</td>
</tr>
<tr>
<td>MBR</td>
<td>Primary</td>
<td>5.9</td>
<td>0.5</td>
<td>92</td>
<td>Michielssen et al. 2016</td>
</tr>
<tr>
<td>Cloth filtering</td>
<td>Secondary</td>
<td>0.2</td>
<td>0.01</td>
<td>95</td>
<td>Mintenig et al. 2017</td>
</tr>
<tr>
<td>Ultra filtration</td>
<td>Secondary</td>
<td>NA</td>
<td>0.28</td>
<td>NA</td>
<td>Ziajahromi et al. 2017a</td>
</tr>
<tr>
<td>Reverse osmoses</td>
<td>Secondary</td>
<td>0.28</td>
<td>0.21</td>
<td>25</td>
<td>Ziajahromi et al. 2017a</td>
</tr>
</tbody>
</table>

The first studies showed that tertiary and other final-stage treatments like MBR can efficiently decrease the amount of microplastics released to aquatic environments via effluents (Table 7.). However, wastewater treatment technologies are not specifically designed to remove microplastics, and not all of them necessarily remove microplastics. In the future, microplastic pollution should be taken into consideration whenever designing advanced final-stage wastewater treatment technologies and applying them to WWTPs.
6.6. Microplastic emissions from WWTPs in Finland

According to my estimations ~ 480 billion microplastics are annually discharged from the 87 largest Finnish WWTPs (pe > 10 000). Out of the 87 WWTPs, 18 receive wastewater from more than 100 000 connected persons. Three of these 18 plants already have tertiary treatment in use, either sand filtration or flotation technique. The remaining plants (15) covers 66% of the total microplastics emissions via wastewater effluents of the 87 plants. By upgrading these 15 conventional WWTPs with more advanced treatment technology, the microplastics emissions could be significantly decreased. For example, the microplastic concentration in effluent after sand filtration is 98.5 % lower than after secondary treatment. Hence if sand filtration would be added to these 15 plants, total emissions of the 87 plants would decrease by 65.0 % (Table 8.).

Table 8. Potential microplastic emissions reductions from Finnish WWTPs when upgrading 15 largest conventional (secondary) WWTPs with different advanced final-stage treatments.

<table>
<thead>
<tr>
<th>wastewater treatment</th>
<th>Microplastic emissions (billion MP/a)</th>
<th>Reduction Secondary (billion MP/a)</th>
<th>vs. Secondary (%)</th>
<th>Reduction secondary (%)</th>
<th>vs. total emissions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary</td>
<td>315.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>MBR</td>
<td>1.2</td>
<td>314.1</td>
<td>99.6</td>
<td>65.7</td>
<td></td>
</tr>
<tr>
<td>Sand filtration</td>
<td>4.8</td>
<td>310.6</td>
<td>98.5</td>
<td>65.0</td>
<td></td>
</tr>
<tr>
<td>Flotation</td>
<td>23.9</td>
<td>291.4</td>
<td>92.4</td>
<td>61.0</td>
<td></td>
</tr>
<tr>
<td>Disc filter</td>
<td>7.2</td>
<td>308.1</td>
<td>97.7</td>
<td>64.9</td>
<td></td>
</tr>
</tbody>
</table>

6.7. Microlitter at wastewater receiving environment

6.7.1. Microlitter in blue mussels (*Mytilus trossulus*)

The microlitter content of the receiving water body of a WTTP and blue mussels was studied and the microlitter content in mussels compared to a reference site. Microlitter was found from mussels at all study sites (Cage 1, Cage 2, source population, reference area) (IV). The average microlitter concentration in individual mussels from the wastewater receiving environment was higher compared to reference mussels (T-test: t= -5.605, p=0.000) while no difference in microlitter concentration per wet weight was found (T-test: p>0.05) (Fig. 6). This may be due to the difference in the size of the mussels collected from the wastewater receiving area and reference area (0.29 ± 0.01 g and 0.15 ± 0.01 g, respectively). When comparing microplastic concentrations, no significant difference was found between wastewater receiving and reference area mussels (T-test, p>0.05).
The analyses of mussel microlitter contents were done from individual mussel samples, so both the concentration of detected microlitter particles per individuals as well as per wet weight of mussel were determined. Wet weight data was received only from mussels that went through shell removal, and the average value was applied to all mussels from the study site. In the wastewater receiving area, the average microlitter concentration was $1.0 \pm 1.2$ ML ind$^{-1}$ ($3.5 \pm 4.4$ ML g$^{-1}$ ww) and the average microplastic concentration was $0.1 \pm 0.2$ MP ind$^{-1}$ ($0.4 \pm 1.9$ MP g$^{-1}$ ww). The corresponding figures for the reference site were $0.4 \pm 0.5$ ML ind$^{-1}$ ($2.6 \pm 3.5$ ML g$^{-1}$ ww) and $0.04 \pm 0.2$ MP ind$^{-1}$ ($0.3 \pm 1.3$ MP g$^{-1}$ ww).

![Figure 6. Average microlitter and microplastic concentrations in the wastewater receiving and reference area mussels per individual (A) and in g$^{-1}$ ww (B). Error bars show standard error.](image)

There are no other studies reporting microlitter concentrations in bivalves, thus the results cannot be compared to previous studies in this respect. However, the results presented here support previous studies that suggest that filter feeding mussels are efficient in ingesting microplastics, particularly synthetic fibers (Li et al. 2016, Mathalon and Hill 2014). The concentrations of microplastics found in blue mussels in this study ($0.3 – 0.4$ MP g$^{-1}$ww) are in good agreement with previous studies from e.g. the Belgian coastline ($0.26 – 0.51$ MP g$^{-1}$) (De Witte et al. 2014) and French and Dutch coastal waters ($0.2 \pm 0.3$ MP g$^{-1}$, Van Cauwenberghe et al. 2015). In some areas with high anthropogenic stress, the detected microplastic concentrations in bivalves can be even much higher. In the blue mussels from Chinese coastal waters values as high as $4.6$ MP g$^{-1}$ ww (Li et al. 2016) or even up to $10.5$ MP g$^{-1}$ww (Li et al. 2015) were detected.

Recent studies have investigated the accumulation of microplastics in marine bivalves, and aimed to assess the relationship between microplastic accumulation in bivalves and proximity to urban pressures. In laboratory studies it has been shown that increasing the level of exposure can increase the concentration of microplastic in marine invertebrates, including bivalves, i.e. the higher the concentration of microplastics, the more they are filtered and accumulate in bivalves (Cole et al. 2013, Setälä et al. 2016b). It has also been demonstrated that microplastics
may also accumulate in bivalve tissues after being ingested (Browne et al. 2008). In addition, Magnusson et al. (2016a) found more microplastics per individual mussel collected at the wastewater receiving environment ($2.7 \pm 0.7$ MP ind$^{-1}$) compared to a reference site ($0.5 \pm 0.2$ MP ind$^{-1}$) at the west coast of Sweden. The study reported here supports these findings by detecting more microlitter from wastewater receiving area mussels than at the reference site.

6.7.2. Microlitter in wastewater, surface water, and the water column

Wastewater effluent contained the highest concentrations of both total average microlitter and microplastics compared to the environmental samples that were collected at the water surface and water column (Table 9.). Altogether $3.1 \pm 0.8$ ML L$^{-1}$ and $0.4 \pm 0.2$ MP L$^{-1}$ were found in the secondary effluent in Suursuo WWTP. The microlitter was dominated by textile fibers (~88%) and after that next most common were irregular shaped PE particles (~11%). These concentrations and types of microlitter characterized from the effluent are consistent with previous studies from secondary effluents (e.g. II, III, Michielssen et al. 2016, Ziajahromi et al 2017a). With the measured microlitter concentration in the Suursuo WWTP effluent, the daily microlitter and microplastic discharge would add up to $\sim 1.7 \times 10^7$ ML and $\sim 2.3 \times 10^6$ MP, respectively, to the receiving environment.

Both the surface water and water column samples revealed higher microlitter concentrations at the cage 1, which was situated 700 meters away from the wastewater discharge pipe, compared to the sampling site at the cage 2, which was only 30 meters away from the discharge pipe (T-test: surface water: $t=4.246$, $p=0.013$, water column: $t=6.315$, $p=0.003$), while no significant differences for microplastics were found (T-tests: $p>0.05$) (Table 9.).
Table 9. Average microlitter and microplastic concentrations (particle m$^{-3}$, ± standard deviation) in seawater and wastewater effluent.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Microlitter</th>
<th>Microplastics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage 1, surface water</td>
<td>20 μm: 200 ± 67</td>
<td>20 μm: 22 ± 38</td>
</tr>
<tr>
<td></td>
<td>100 μm: 7.0 ± 5.3</td>
<td>100 μm: 0.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>300 μm: 1.3 ± 2.3</td>
<td>300 μm: 1.0 ± 1.0</td>
</tr>
<tr>
<td>Cage 1, water column</td>
<td>&gt; 100 μm: 57 ± 13</td>
<td>&gt; 100 μm: 11 ± 6.1</td>
</tr>
<tr>
<td>Cage 2, surface water</td>
<td>20 μm: 22 ± 19</td>
<td>20 μm: 11 ± 19</td>
</tr>
<tr>
<td></td>
<td>100 μm: 1.0 ± 1.0</td>
<td>100 μm: 0.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>300 μm: 0.7 ± 0.6</td>
<td>300 μm: 0</td>
</tr>
<tr>
<td>Cage 2, water column</td>
<td>&gt; 100 μm: 9.9 ± 0.6</td>
<td>&gt; 100 μm: 3.2 ± 2.8</td>
</tr>
<tr>
<td>Wastewater effluent</td>
<td>20 μm: 2170 ± 580</td>
<td>20 μm: 0</td>
</tr>
<tr>
<td></td>
<td>100 μm: 650 ± 150</td>
<td>100 μm: 290 ± 110</td>
</tr>
<tr>
<td></td>
<td>300 μm: 240 ± 150</td>
<td>300 μm: 160 ± 110</td>
</tr>
</tbody>
</table>

The results presented here were opposite to the expectations based on e.g. the findings of Magnusson and Norén (2014) where decreasing microplastic (synthetic fibers) concentration trend was found when sampling downstream from the effluent plume 20, 200, and 3 500 meters away from the discharge pipe. Our result suggest that while part of the wastewater-derived microlitter has potential to stay and maybe settle into the vicinity of discharge site, depending on the water dynamics of the receiving area, part of it can be also rapidly distributed and subsequently diluted over wider areas, making it difficult to identify the effect of certain point sources/pathways in the aquatic environment.

Further, the sampling protocol revealed higher microlitter concentrations in the water column compared to surface samples (size fractions 300μm and >100 - 300μm summed, T-test: t= -2516, p=0.049), while no significant differences for microplastics were found (T-test, p > 0.05). On average 5.0 ± 6.0 ML m$^{-3}$ and 0.8 ± 0.8 MP m$^{-3}$ was found in the surface, while 33 ± 27 ML m$^{-3}$ and 7.3 ± 6.2 MP m$^{-3}$ were found in the water column. According to the previous studies more microplastics and microlitter would be expected to be found in surface water (e.g. Reissner et al.2015, Bagaev et al. 2017). One possible explanation for the finding might be the source of the microlitter (wastewater discharge pipe) situated close to the bottom. As most of the microlitter in wastewater consisted of non-synthetic fibers (cotton, linen, viscose) with higher densities compared to surrounding brackish water of northern Baltic Sea, they do not necessarily reach the surface but remain submerged. The results indicate that collecting only surface water in wastewater receiving environment may lead to underestimation of the actual concentration of small anthropogenic litter.
Information on the abundance, distribution and type of microlitter in the Baltic Sea includes surveys and research on water surface and water column (Table 10.). However, as the applied methods for environmental sampling and analyzing the samples vary broadly, comparability of the results is limited. First studies from Baltic Sea report high abundances (up to 30,000 ML m\(^{-3}\)) of microlitter in surface waters (Norén 2007, Magnusson and Norén 2011). The main reasons for high values are the use of small mesh size filters (10 – 80 μm) and including black carbon in the studies. The black carbon is a common microlitter type found in sea water samples, particularly near the highly populated urban areas (Dubaish and Liebezeit 2013, Magnusson and Norén 2011). Later studies, focusing larger size (100 – 300 μm) microplastics report significantly lower abundances from 0 to 8 MP m\(^{-3}\) (Setälä et al. 2016a, Magnusson et al. 2016a, Gewert et al. 2017.)

<table>
<thead>
<tr>
<th>Concentration (ML m(^{-3}))</th>
<th>Sampling method</th>
<th>Sample volume (m(^{3}))</th>
<th>mesh size (μm)</th>
<th>Area/Sampling site</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>167 – 2400</td>
<td>plankton net</td>
<td>NA</td>
<td>&gt; 80</td>
<td>West coast, Sweden</td>
<td>Norén 2007</td>
</tr>
<tr>
<td>0.01 – 0.14</td>
<td>plankton net</td>
<td>72 – 141</td>
<td>&gt; 450</td>
<td>West coast, Sweden</td>
<td>Norén 2007</td>
</tr>
<tr>
<td>21</td>
<td>pump &amp; filter</td>
<td>1 – 2</td>
<td>&gt; 300</td>
<td>East coast, Sweden</td>
<td>Magnusson and Norén 2011</td>
</tr>
<tr>
<td>32400*</td>
<td>pump &amp; filter</td>
<td>0.004 – 0.01</td>
<td>&gt; 10</td>
<td>East coast, Sweden</td>
<td>Magnusson and Norén 2011</td>
</tr>
<tr>
<td>0.9 – 1.9</td>
<td>Manta trawl</td>
<td>NA</td>
<td>&gt; 333</td>
<td>West coast, Sweden</td>
<td>Magnusson et al. 2016a</td>
</tr>
<tr>
<td>0 – 0.8</td>
<td>Manta trawl</td>
<td>NA</td>
<td>&gt; 333</td>
<td>Gulf of Finland</td>
<td>Setälä et al. 2016a</td>
</tr>
<tr>
<td>0 – 1.25</td>
<td>pump &amp; filter</td>
<td>2</td>
<td>&gt; 300</td>
<td>Gulf of Finland</td>
<td>Setälä et al. 2016a</td>
</tr>
<tr>
<td>0 – 6.8</td>
<td>pump &amp; filter</td>
<td>2</td>
<td>&gt; 100</td>
<td>Gulf of Finland</td>
<td>Setälä et al. 2016a</td>
</tr>
<tr>
<td>0.2 – 8</td>
<td>Manta trawl</td>
<td>NA</td>
<td>&gt; 333</td>
<td>Stockholm archipelago</td>
<td>Gewert et al. 2017</td>
</tr>
<tr>
<td>0.1 – 0.5</td>
<td>Manta trawl</td>
<td>NA</td>
<td>&gt; 333</td>
<td>Central Baltic Sea</td>
<td>Gewert et al. 2017</td>
</tr>
<tr>
<td>510 – 9640*</td>
<td>Pump &amp; filter</td>
<td>1</td>
<td>&gt; 20</td>
<td>Helsinki archipelago</td>
<td>I</td>
</tr>
<tr>
<td>2 – 280</td>
<td>Pump &amp; filter</td>
<td>1</td>
<td>&gt; 20</td>
<td>South coast, Finland</td>
<td>IV</td>
</tr>
<tr>
<td>Water column</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 – 7500**</td>
<td>plankton net</td>
<td>0.3 – 9.2</td>
<td>&gt; 90</td>
<td>East coast of Sweden</td>
<td>Gorokhova 2015</td>
</tr>
<tr>
<td>100 – 900</td>
<td>Niskin bottles</td>
<td>0.01 – 0.03</td>
<td>&gt; 174</td>
<td>Baltic Proper basins</td>
<td>Bagaev et al. 2017</td>
</tr>
<tr>
<td>10 – 71</td>
<td>Plankton net</td>
<td>0.5 – 1</td>
<td>&gt; 20</td>
<td>South coast, Finland</td>
<td>IV</td>
</tr>
</tbody>
</table>

*Including black carbon

**Data from zooplankton monitoring samples

The microplastic (>300 μm) concentrations from Baltic Sea are in the same order of magnitude as the microplastics (>300 μm) concentration found from elsewhere. From North Pacific Central Gyre (Moore et al. 2001) reported the concentration of 2.23 MP m\(^{-3}\) from and from coastal waters of California (Moore et al. 2002) reported the concentration of 7.25 MP m\(^{-3}\). Collignon et al. (2012) found the concentration of 0.116 MP m\(^{-3}\) from the Northwestern Mediterranean and Zhao et al. (2014) concentration of 0.167 MP m\(^{-3}\) from East China Sea.
6.7.3. Characteristics of microlitter in wastewater and wastewater receiving environment

Textile fibers dominated the samples in all matrices and most of the fibers were non-synthetic materials of cotton, linen and viscose. Non-synthetic textile fibers formed 68% of all microlitter found in the wastewater receiving area mussels and 55% in reference mussels (Fig. 7.). However, 35% of the fibers in recipient mussels were not successfully analyzed and hence the exact material composition is not known. In the effluent, non-synthetic fibers accounted 85% of the microlitter and in the surface water and water column 85% and 75% respectively. These results indicate that textile fiber is among the most common microlitter type in wastewater as well as in the aquatic environment and that the amount of non-synthetic fibers exceeds synthetic fibers.

Higher variety of microlitter types was found in wastewater receiving area mussels compared to reference mussels, which contained only fibers. In addition, wastewater receiving water environment contained different types of microlitter (fragments and flakes) and a variety of non-synthetic and synthetic materials. The result indicates a higher variety of microlitter sources in the wastewater receiving area. This can be expected since the area is not only under the influence of WWTP but also to other human activities.

Figure 7. Different types of microlitter found in the Wastewater receiving area (A) and reference area (B) mussels. NA = not available.

Textile fibers were the only microlitter types found in the recipient that could be directly linked to wastewater. Although wastewater effluent is one of the pathways for textile fibers to the aquatic environment (I, II, III, IV, Browne et al. 2011, Ziajahromi et al. 2017a), fibers were also found in reference mussels indicating that other pathways such as atmospheric input (Dris et al. 2015) may play an important role with fiber input to the environment.
6.8. Remarks of used methods

6.8.1. Sample contamination

The control samples for the in-situ pump and filtering method (including wastewater and seawater controls) and sequential sampling did not contain any microlitter, indicating that the risk of sample contamination from these sampling methods, their transportation and analyses was negligible. However, the method did not include sample processing steps (e.g. digestion, density separation, staining), which may impose the samples to airborne contamination or contamination from different equipment, chemicals, containers, etc.

Similar low contamination levels have been reported when using the in-situ pump and filtering method in other studies (e.g. Mason et al. 2016, Ziajahromi et al 2017a). However, Mintenig et al. (2017) found the control samples to be slightly contaminated with particulate and fibrous microplastics with a similar sampling method. One reason for the detected contamination might be the numerous sample processing steps required for the automated material analyses used in their study. On the other hand, the automated high quality analyses might be able to detect background contamination more reliably when compared to the analysis method based on visual selection used in this thesis and in other studies (II, III, Mason et al. 2016, Ziajahromi et al. 2017a).

The contamination was relatively high in composite samples (0.6 ± 0.2 ML L⁻¹). Contamination comprised mainly for textile fibers, most likely airborne. Contamination values were subtracted from microlitter concentrations from each sampling site (influent, primary effluent, secondary effluent). Many WWTPs include automated 24-hour composite samplers for monitoring different parameters from wastewater. These samplers can provide a relatively easy and fast microlitter sampling protocol including intra-day variation. However, when the microlitter concentrations are low, as they often are in final-stages of wastewater treatment processes, composite samples with relatively high contamination levels and low sample volumes (max. 14.5 L in this study) may distort the results.

The procedural blanks contained on average 0.6 ± 0.9 ML ind⁻¹ across all mussel batches. Contamination values are subtracted from each mussel’s total microlitter content by matching its type (material and shape). The open-air dissection of mussel samples has been suggested as posing a risk of contamination (Catarino et al. 2017). The results presented here verify this assumption, since mussels that went through shell removal had significantly higher fiber concentration than those digested with their shells on (0.8 ± 0.9 and 0.5 ± 0.7 fibers ind⁻¹, respectively, independent samples T-test, p=0.008). On average, the mussels that had their shells dissected contained 29% more fibers. Dissecting of mussels is a commonly used protocol in many microplastic studies (De Witte et al. 2014, Farrell and Nelson 2013, Rochman et al. 2015). Hence, it is possible that some of the results in other studies may have contained overestimations of the true fiber contents. The digestion of the whole mussel is therefore recommended.
6.8.2. Sampling and sample processing of wastewater and sludge

The filtering set-up designed to sample wastewater-derived microlitter is based on in-situ fractionation. The fractionation (filtration through different mesh sized filters) offers the opportunity to collect different microlitter size-classes automatically. This process helps to estimate the size distribution of the particles. In situ fractionation also increases the sample volume of the smaller mesh-size filters. Simultaneous pump and filtering combined with in-situ fractionation is the most commonly used method when sampling microlitter from wastewaters (Carr et al. 2016, Michielssen et al. 2016, Mason et al. 2016, Ziajahromi et al. 2017a). However, in other studies, the filtration is usually performed with a stack of sieves (Carr et al. 2016, Michielssen et al. 2016) or with some other larger custom made filter assembly (Mason et al. 2016, Ziajahromi et al. 2017a). After filtering, the sieves/larger filters are rinsed in separate containers, and the steps of sample processes are usually performed, e.g., digestion, density-based separation, and staining (Mason et al. 2016, Mintenig et al. 2017, Ziajahromi et al. 2017a). These steps are useful when the focus is on microplastics, as both digestion and density-separation can reduce the risk of any misidentification of microplastics. Larger dimensions of the filtering device can also increase the sample volumes (Carr et al. 2016). In order to get representative samples across the treatment plant, multiple replicates, as well as sampling of significant volumes of influent, should be conducted. The sample processing steps, such as density separation and digestion, could probably help increase the influent sample volume.

The composite sampling can offer solutions for taking intra-day variation into account. However, the sample volume is often too low for accurate microlitter estimations, particularly in low concentration matrices like secondary and tertiary effluents. Composite samplers that are commonly utilized in WWTPs take a maximum of 20 – 30 L of water (e.g. ISCO samplers). While the sample volume is an adequate when examining the classical wastewater quality parameters of organic matter, nitrogen, phosphorus and SS, it is too low when estimating the microlitter levels in the secondary and/or tertiary effluent. Low sample volume, together with the low microlitter concentration of effluents and sensitivity of the sampling method to contamination (II) can lead to false estimations of microlitter concentration in effluents (III). Hence, it would be optimal to be able to combine composite sampling technique with larger sample volumes.
6.8.3. Sampling and sample processing in wastewater recipient

Digestion of mussel tissue
The analysis of microlitter from biota samples requires effective extraction methods. Acid digestion has often been used to isolate the microplastics from bivalves. However, concentrated acid (e.g., HNO₃) can have a detrimental effect on textile fibers and result in the total destruction of this type of microlitter during extraction (Claessens et al. 2013). The enzyme digestion method applied to mussels in this thesis was a safe, inexpensive, and gentle method that did not damage microlitter particles, not even non-synthetic textile fibers. Several different digestion solutions were tested on a variety of textile materials, and the enzymatic approach was the only one not to cause discoloration or destruction of the non-synthetic fibers (IV).

Pump and filtering v.s plankton net
More microlitter was found in the water column (plankton net method) than in the surface water (pump and filtering method) when comparing the same size fraction > 100 µm of microlitter. This finding is probably due to the fact that the majority of the microlitter material detected in our study were composed of materials that were denser than water, such as cotton, linen, viscose (density ~ 1.5 g cm⁻³) and PES (density ~ 1.4 g cm⁻³). These microlitter materials are not necessarily floating on the surface water, but are located, at least for a while, in the water column. The result indicates that microlitter sampling performed solely from surface water may greatly overlook the actual microlitter contamination in the water environment.

When a 20 µm filter was included in the pump and filtering results, more microlitter was detected. This finding is largely explained due to those fibers that were clearly more numerous in the 20µm filters. Since the diameter of many textile fibers were small (~ 20 µm) they can be probably retained more reliably by using 20 µm filters. If the sampling method must be able to measure fibers, also smaller mesh size filters (≤ 20 µm) should be included in the sampling.
6.8.4. Microlitter characterization

The identification of microlitter in all of our studies was based on a visual pre-selection. Some of the particle types can be identified visually relatively accurately, and due to that visual information, their sources can be estimated. These particles include textile fibers and certain microbeads from the cosmetics. However, with analyses based solely on visual inspection, the actual materials (polymer) of the particles will remain unknown. Additionally, without any instrumental analysis, those particles determined as microplastics show error values up to 70% (Hidalgo-Ruz et al. 2012). All particles determined as microplastics in this thesis went through a FTIR analysis. However, the microplastics were first visually identified from the samples (filters). Due to visual pre-selection, it is possible that parts of the microplastics can be left identified visually, and hence the microplastic results presented in this thesis do contain the risk of underestimation.

In the FPA-based, micro-FTIR analysis technique, the visual identification step is excluded, and the samples are automatically analyzed from the filters (Tagg et al. 2015, Löder et al. 2015b, Mintenig et al. 2017). The automated analyses offer a clear advantage compared to methods that are based on visual pre-identification. In the future, microlitter and microplastics identification will most probably develop in an automated direction.

However, that method still has to solve some challenges. For example, the automated whole-filter analysis appears to be time consuming, especially when analyzing wastewater samples that contain a high number of particles. This issue may further lead to compromises like performing only a partial analysis of the samples in order to process the analysis within reasonable time. Further, the analyses of textile fibers, a very common microlitter type in environmental as well as wastewater samples, appears to be challenging when using the automated methods (Mintenig et al. 2017). Upon visual examination, textile fibers can be identified relatively well and selected for further analyses. Visual information can be also useful when estimating microlitter sources, like textile fibers and microbeads from cosmetics, as these particles are relatively recognizable.
7. Conclusions

The results obtained for this thesis show that conventional wastewater treatment can remove most (> 99%) of microlitter (> 20 μm) from wastewater during its processes. The pre-treatment has the most fundamental effect on microlitter removal, by removing ~ 98% of the incoming microlitter. The AS process can further remove ~ 88% of the microlitter concentration. Only < 0.5% of the incoming microlitter is then left in secondary effluent. Despite this seemingly high removal, wastewater effluent can still act as a pathway for microlitter, as large volumes of wastewater are discharged into aquatic environments constantly. The removal of microlitter through sedimentation processes resulted in the majority of microlitter being retained in the sewage sludge. Hence, land-applied sludge offers a pathway for microlitter to enter the environment.

The pre-treatment also had the greatest effect on microlitter size and shape distribution by efficiently removing larger size classes (> 300 and 100 – 300 μm) and textile fibers. After the pre-treatment, the most common size-fraction was the smallest one (20 – 100 μm). Despite this efficient removal of fibers in pre-treatment, it remained the most common microlitter type for the treatment processes. The most commonly detected polymers in the effluent were cotton (39%), PES (32%) and linen (14%), derived mainly from textile fibers. The most commonly detected plastics were PES, PE (4%), polypropylene (1%), and polystyrene (< 1%).

The removal of microplastics was substantially enhanced with advanced wastewater treatment technologies. However, the current wastewater treatment technologies are not particularly designed to remove microplastics from the wastewater and hence do not necessarily do so. As the large volumes of effluents are discharged into the aquatic environments continuously, microplastic pollution should be taken into consideration when designing advanced wastewater treatment technologies and implementing them into WWTPs. For example, by upgrading 15 largest WWTPs in Finland with more advanced treatment technology (e.g. sand filtration), the total microplastics emissions from WWTPs could be significantly (~ 60%) decreased.

According to my estimations approximately 480 billion microplastics are discharged via wastewater effluents into the aquatic environments every year in Finland. Although, the estimation is coarse, it can be used as first-hand information when evaluating the role of WWTPs as pathways for microplastics.

Elevated microlitter concentrations and higher variety of microlitter types were detected in the filter feeding blue mussels in the wastewater recipient area compared to reference area with low anthropogenic impact. These results indicate that wastewater treatment plants may influence the microlitter abundance and composition of biota in recipient area. However, the microlitter abundances detected in aquatic environment is affected by various factors, and hence understanding the relationships between the sources of microlitter and consequent contamination levels in aquatic environment is challenging.
In conclusion, this thesis provides profound understanding of removal of microlitter in different wastewater treatment processes, which is a prerequisite to further evaluate the role of WWTPs as pathways of microlitter into the environment.

8. Future research needs

In a future, when evaluating microlitter and microplastic emissions from WWTPs, the variations in microlitter concentration in wastewater should be taken into account. Thus to provide better understanding of WWTPs as a pathway for microlitter to the aquatic environment, monitoring studies in WWTPs applying different treatment technologies are recommended. Further, the comparability of the results between the studies must also be improved. This requires development of standardized and applicable methods for the detection of microlitter in wastewaters.

According to results presented here and elsewhere (Magnusson and Norén 2014, Carr et al. 2016, Murphy et al. 2017), most (~ 99%) of the microlitter entering the WWTPs is retained in the sludge and hence the land-applied sewage sludge offers a major pathway for wastewater-derived microlitter to the environment. Therefore, the future focus on microlitter in wastewater is recommended to be directed to the sludge fraction.

Further, the results of this thesis and in Mahon et al. (2017) suggest that some sludge treatments may affect the microlitter abundances in sewage sludge. However, these results lack a systematic approach to examine the full effect of the treatments. Future research, including extensive sampling before and after the examined sludge treatments, should be performed to better estimate the possibilities for sludge treatments to impact the microplastic load entering the environment with land applied sewage sludge. It is also important to note here that the extraction of microplastics from sewage sludge is extremely challenging, so further development of sludge sample processing is needed to reach the desired higher quality results.

To further evaluate the relative importance of the role of WWTPs as pathways for microlitter to the aquatic environment, information about other pathways like stormwater run-off, atmospheric input and in situ degradation of larger litter items are needed.
9. References


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Implications of Single and Mixture Exposures. Environmental Science & Technology 51(22), 13397-13406.
Wastewater treatment plants (WWTPs) have been suggested to act as one of the pathways for microlitter and microplastics to the aquatic environment. Wastewaters contain microlitter like textile fibers and plastic microbeads from personal care products. As vast volumes of wastewaters are discharged continuously into aquatic environments and the amounts are expected to grow due the population growth and urbanization, the role of WWTPs as a pathway of microlitter to aquatic environments may be significant. At the same time, WWTPs can offer solutions to reduce the input. This thesis examines the removal of microlitter in conventional as well as in advanced wastewater treatment processes. Also the annual microplastic load discharged from the Finnish WWTPs to the aquatic environment is evaluated. Further, the potential effects of microlitter discharge via wastewaters on the receiving water body and its biota is assessed.