LAPPEENRANTA-LAHTI UNIVERSITY OF TECHNOLOGY LUT

Mirka Viitala

# THE HETEROGENEOUS NATURE OF MICROPLASTICS AND THE SUBSEQUENT IMPACTS ON REPORTED MICROPLASTIC CONCENTRATIONS

ACTA UNIVERSITATIS LAPPEENRANTAENSIS 999

Mirka Viitala

# THE HETEROGENEOUS NATURE OF MICROPLASTICS AND THE SUBSEQUENT IMPACTS ON REPORTED MICROPLASTIC CONCENTRATIONS

Dissertation for the degree of Doctor of Philosophy to be presented with due permission for public examination and criticism in the Auditorium of the Mikkeli University Consortium at Mikkeli, Finland on the 10th of December, 2021, at noon.

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# Abstract

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During past decades, the awareness of plastic pollution has increased drastically, and small plastic particles, namely microplastics (MPs), have been found in the environment all over the world. However, microplastic research suffers from the variety of practices used in sampling, pre-treatment, and identification, each having their own limitations, which challenge the comparison and interpretation of reported results. In practice, these challenges limit the utilization of the explosively accelerating flood of monitoring data in assessing the impacts of various MPs in the environment.

This study aims to highlight the challenges related to the assessment of MPs from the environmental samples. This is done by evaluating the special features of different sampling, pre-treatment and identification methods that are commonly used in the MP research field. The main outcome is to underline the features and limitations behind the reported concentrations which should always be considered when reported MP concentrations are interpreted. In addition, this study focuses especially on the assessment of MPs from wastewater treatment plants (WWTPs) and in the aquatic environments receiving treated wastewater. To evaluate these issues, a case study was conducted at a local WWTP and its recipient lake area. The observations from the case study are discussed with the literature in this study.

Most of the variation in the reported MP concentrations is caused by the heterogeneity of the MPs in terms of their size, chemical composition, shape, and colour. The lower size limit of the reported MP concentrations is the most significant factor explaining the variation, and it is affected by all the selected methods from the sampling to the final identification of the MPs. The number of detected MPs is generally higher when smaller MPs are included in the examination, which highlights the importance of including the smallest MPs in future studies. This requires improvements in the purification and identification methods to allow the separation and detection of the smallest MPs. In addition, the variation in the size limits cause a gap between the reported environmental MP concentrations and the studied ecotoxicological effects of MPs. This, in turn, limits the assessment of the impacts caused by MPs detected in the environment.

Due to the variety and special needs of different sample types and the aims of MP studies, standardization of the methods themselves will probably not be the solution to improving the comparability of the MP studies. Therefore, this study suggests that standardized model MPs together with consistent recovery testing methods with selected sampling, pre-treatment, and identification methods would allow an assessment of the representativeness of the resulting MP concentrations. The standardized MPs should represent the heterogeneity of MPs in terms of shapes, polymer types, sizes, and colours. In this way, the reported results could also be compared more reliably. In addition, the limitations caused by the selected methods should be discussed openly along with the reported MP results to allow more reliable interpretations of MP pollution.

The conducted case study provides regionally novel knowledge about MP pollution in the studied WWTP and its recipient lake. The conventional WWTP removed MPs larger than 250 µm from wastewater effectively (98.3%) and directed them to other waste streams. Nevertheless, the impact of the discharged MPs from the WWTP (1 MP/L, >250 µm) or other urban activities was obvious in the bottom sediment of the recipient area compared to other parts of the lake. In addition, the detected MP pollution in the sediment showed a slight increase during the past 30 years.

The findings of this case study emphasise the effect of increasing plastic production on the plastic pollution entering the environment. The high proportion of PET fibres throughout the studied samples especially highlights the role of synthetic textiles as sources of MP pollution entering the environment via wastewater. These results highlight the importance of including microplastic fibres, and especially PET fibres, not only in the monitoring studies, but also in the validation of selected methodologies as well as in the ecotoxicity testing in the future.

As a conclusion of this study, due to the limitations related with the current methodologies, the reported MP concentrations should never be considered as accurate values. The details behind the reported concentrations should be discussed openly and considered during the interpretation both in the scientific and public debate.

Keywords: environmental pollution, freshwater, lake water, microplastic fibre, microplastic particle, microplastic, municipal wastewater treatment, plastic, sediment, sludge, wastewater, WWTP

# Acknowledgements

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Mirka Viitala (née Lares)
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Mikkeli, Finland

*Kaikki loputon kauneus*
*kaikki järjettömyys*
*kaikki ruoskivat toiveet*
*kaikki päättämättömyys*
*ovat lopulta tarkoituksen palasia*
*osa arvoitusta*
*valot pimeyksien reunoilla*

*Apulanta – Valot pimeyksien reunoilla*

# Contents

**Abstract**

**Acknowledgements**

**Contents**

# Nomenclature

| | |
|---|---|
| ABS | acrylonitrile butadiene styrene |
| ATR | attenuated total reflection |
| BAFF | biological aerated flooded filter |
| CAS | conventional activated sludge |
| DAF | dissolved air flotation |
| DF | disc-filter |
| dw | dry weight |
| FPA | focal plane array |
| FTIR | Fourier transform infrared |
| $H_2O_2$ | hydrogen peroxide |
| LOD | limit of detection |
| LOI | loss-on-ignition |
| LOQ | limit of quantification |
| MBR | membrane bioreactor |
| MP | microplastic |
| MPF | microplastic fibre |
| MPP | microplastic particle |
| OEP | oil extraction procedure |
| PA | polyamide |
| PAN | polyacrylonitrile |
| PC | polycarbonate |
| PE | polyethylene |
| PET | polyethylene terephthalate |
| PEVA | polyethylene vinyl acetate |
| PMMA | poly(methyl methacrylate) or acrylic |
| POM | polyoxymethylene or polyacetal |
| PP | polypropylene |
| PS | polystyrene |
| PTFE | polytetrafluoroethylene |
| PU or PUR | polyurethane |
| PVA | polyvinyl alcohol |
| PVC | polyvinylchloride |
| RB | Rose Bengal |
| RO | reverse osmosis |
| RSF | rapid sand filtration |
| RT | room temperature |
| SBR | styrene-butadiene rubber |
| SDS | sodium dodecyl sulphate |
| SF | sand filter |
| SS | stainless steel |
| TGA-DSC | thermogravimetric analysis with differential scanning calorimetry |
| UEPP | universal enzymatic purification protocol |

| | |
|---|---|
| UF | ultrafiltration |
| WAS | waste activated sludge |
| WPO | wet peroxide oxidation |
| ww | wet weight |
| WWTP | wastewater treatment plant |

# 1 Introduction

The mass production of plastics started in the 1940s, and production volumes of plastics have been accelerating ever since. In 2019, over 430 million tons of plastics were produced globally [1, 2]. Over 80% of the whole 70 million tons of produced synthetic fibres were made of polyester [1], most of which is polyethylene terephthalate (PET). In the EU, the most commonly used plastics are polypropylene (PP) (19%), low-density polyethylene (PE-LD) (17%), high-density polyethylene (PE-HD) (12%), polyvinylchloride (PVC) (10%), polyurethane (PU) (8%), PET (8%) and polystyrene (PS) (6%) [2]. Due to their cheap price and modifiable nature, plastics are used for countless purposes. In the EU, 40% of plastic is used for packaging and 20% for building and construction [2]. Other end-uses include transportation, electric devices, agriculture, household consumables and synthetic fabrics [2].

Due to the accelerating production and consumption rates of plastics, more and more plastics are consequently discharged into both the terrestrial and aquatic environment. Environmental plastic pollution was first noticed in the 1970s, when plastic particles were found in the North Atlantic [3, 4]. Later, plastics discharged into the oceans have been found to concentrate into five gyres [5]. In total, the number of plastic particles (>333 µm) floating in the sea has been estimated to be over 5 trillion and weighing close to 270,000 tons [5]. In addition, it has been observed that plastic items can be break down into smaller fragments during their use and due to weathering once they are exposed to environmental conditions. These small, weathered plastic particles along with the intentionally produced small plastics were then classified under one term—microplastics (MPs), i.e. plastic particles smaller than 5 mm [6].

Due to improved analytical methods, research interests and publicity, microplastic research has been explosively accelerating ever since and MPs have been detected in seawater [7–10], freshwater [11–16], sediments [8, 12, 15, 17–19], Arctic sea ice [20], inside organisms [15, 17], and in the air [21, 22]. Despite the wide range of microplastic studies conducted so far, scientists around the world have demanded a standardized methodology to compare results reported by different research groups. Overall, challenges with MPs originate from their diversity in terms of size, chemical composition, and shape together with the problematics in quantifying their presence in a reliable way. In the public debate, these features are often ignored and all reported results for MPs abundance are considered comparable and comprehensive. In addition, the current way of conducting microplastic research leaves a gap between the data received from environmental monitoring and the results reported by ecotoxicological studies. This leads to a loss of information as monitoring results describing the environmental pollution caused by microplastics cannot be directly interpreted by the knowledge received from the impact studies.

Overall, the understanding of the limitations and variation of the methodologies behind the reported MP concentrations is critical because these results are also used as a basis for the legislation and other restrictions concerning MPs. For example, in the EU, MP-related restrictions are currently under discussion. The European Commission is aiming to ban the use of intentionally added MPs and to reduce the discharges of unintentionally produced MPs in the near future. Hence, it is crucial that the limitations in the reported MP concentrations are interpreted and discussed openly.

This study aims to help underline the challenges related to the assessment of MPs from the environmental samples. This is done by evaluating the special features of different sampling, pre-treatment and identification methods that are commonly used in the MP research field. The main outcome is to underline the features and limitations behind the reported concentrations which should always be considered when reported MP concentrations are interpreted. In addition, this study focuses especially on the assessment of MPs from wastewater treatment plants (WWTPs) and in the aquatic environments receiving treated wastewater.

## 1.1 Research objectives

The main objectives of the thesis are following:

- To test and evaluate the suitability of commonly-used sampling, pre-treatment, and identification methods for assessing the level of microplastic pollution and characteristics of MPs in samples collected especially from WWTPs and related aquatic environments.
- To evaluate the representativeness and applicability of analysed samples to assess the environmental microplastic load. The evaluation is based particularly on the interference caused by the methods in question.
- To evaluate the special features related to the assessment of MP loads discharged from WWTPs and their possible impacts on the recipient environment.

These issues are discussed in the light of a case study conducted in Kenkäveronniemi WWTP and its recipient lake in Mikkeli, Finland, which aims to bring new knowledge about the microplastic pollution related to wastewaters on a local scale.

## 1.2 Impacts of the research

The main contributions of this thesis are:

- To provide an overview of which details behind the reported concentrations should be considered when reported microplastic results are discussed and how they should be considered during interpretation.
- To point out the weaknesses in the microplastics research field that limit the utilization of the explosively accelerating flood of research data in assessing the impacts of microplastics on the environment.

- To provide regionally novel information about the abundance of microplastics in a Finnish WWTP and in the lake area receiving treated effluents.

The results of this study may be used in the interpretation of the results of both past and future MP studies. The evaluation of the methodology may be utilized in the further development of sampling, pre-treatment, and identification of MPs not only in the aquatic environment, but also for other types of samples, such as soil, food, and industrial streams.

The results reported for the case study at Kenkäveronniemi WWTP and in the recipient lake area can be utilized in the future for studying the possible changes in MP pollution on a local scale. The studied WWTP utilized conventional activated sludge (CAS) process and it was replaced with a membrane bioreactor (MBR) based process in 2021. Hence, if the impacts of the advanced wastewater treatment process on the MP pollution of the recipient lake will later be assessed, the results from this case study offer important information about the past conditions.

## 1.3 Outline

A general view of microplastics together with their distribution and sources is provided in Chapter 2.

Chapter 3 includes descriptions of all sampling, pre-treatment, and identification methods that were used in this study, together with the descriptions of conducted recovery and contamination control measures. In addition, conducted measurements of the dry weight and cesium-137 activities for sediment samples are described.

In Chapter 4, the characteristics of different sampling, pre-treatment and identification methods are discussed in the light of the observations from this case study. In addition, suggestions for interpreting results from reported monitoring studies are provided.

Chapter 5 focuses on the special features and requirements that need to be considered, when the abundance of microplastics is studied in WWTP-related samples and the role of WWTPs as routes for MPs together with their possible impacts on the recipient water body are assessed. In addition, the results from the case study are discussed in the light of previously reported MP studies.

Finally, Chapter 6 summarizes the main conclusions of this work and provides suggestions for further studies.

# 2 Microplastics

Microplastics are a diverse group of particulate polymeric pollutants, which vary in multiple ways [23]. Originally, the description of MPs was based on their size and they were defined as plastic particles smaller than 5 mm [6]. An exact definition for MPs is still under discussion in the research community. Even if there is agreement on the size limits of MPs, the limitations in the analysis procedures cause variation in the sizes which are included in the reported results. Consequently, the lower size limits of MPs included in investigations vary widely from the micrometre level to millimetres [8, 16, 24–26].

In addition to their size, MPs vary by their shape. Thus, they can be separated into microplastic fibres (MPFs) and microplastic particles (MPPs), including fragments, films, and spheres. Still, the descriptions for different shapes are subjective and can also depend on the ways, preciseness, and on what basis microplastics are examined in each study. For example, the division of MPs into fibres and particles can be made according to their aspect ratio. Nevertheless, in previous studies, MPs have been considered to be fibres if their length to width ratio was higher than 3 [22] or 5 [27]. In addition, due to the variation in the shapes of MPs, different regulations are suggested for the sizes of MPPs and MPFs. For example, in the discussion related to the description of restricted MPs by the European Chemicals Agency (ECHA), size limits from 100 nm to 5 mm for MPPs and from 300 nm to 15 mm in case of MPFs have been suggested [28]. Thus, the criteria for classifying microplastics in terms of size and shape are still under debate in both research and legislation.

Furthermore, as plastics in general, MPs consist of a wide range of synthetic polymers with varying chemical structures. Different chemical structures affect the properties of MPs and the impacts that different polymers can cause in the environment either by themselves or by acting as vectors for other pollutants [29–32].

MPs can also be divided into primary and secondary MPs according to their origin. Primary MPs are purposely produced and consist of items such as plastic beads and pellets used in plastic production or plastic fragments added to cosmetics [33]. Secondary MPs in turn are formed from larger plastic items during their use or as a result of weathering and physical breakdown [6]. Organisms can also degrade MPs into smaller pieces through their normal feeding habits [34]. MPFs released from textiles are secondary MPs.

In addition to various compositions of polymers themselves, plastics can contain large numbers of additives, which are used to adjust their properties. For instance, differently coloured plastics are created with dyes and the modifiability of plastic can be adjusted with softeners, such as phthalates. These additives can in turn affect other characteristics, such as density, which in turn may alter the behaviour and environmental fate of MPs.

## 2.1 Sources and paths into the environment

Microplastics originate from various sources (Figure 2.1), of which traffic is considered one of the largest [35]. The abrasion of car tires and road-marking materials form MPs, which can either be buried in the soil close to the roads or be flushed away from impervious surfaces by stormwaters. Another source of MPs is improperly sorted plastic waste, which can break into smaller pieces due to weathering caused by UV radiation or mechanical abrasion [36, 37]. In addition, leakages and transport accidents cause discharges of MPs into the environment.

In addition to the direct sources of MPs, wastewater treatment plants act as significant routes of MPs from households and industry into the environment [18, 24, 38, 39]. WWTPs combine wastewater from different sources, treat it in different ways and separate remaining materials into various waste. Along with other pollutants, MPs can end up in solid waste fractions, such as grease or sludge, or enter a recipient water body with treated effluents.

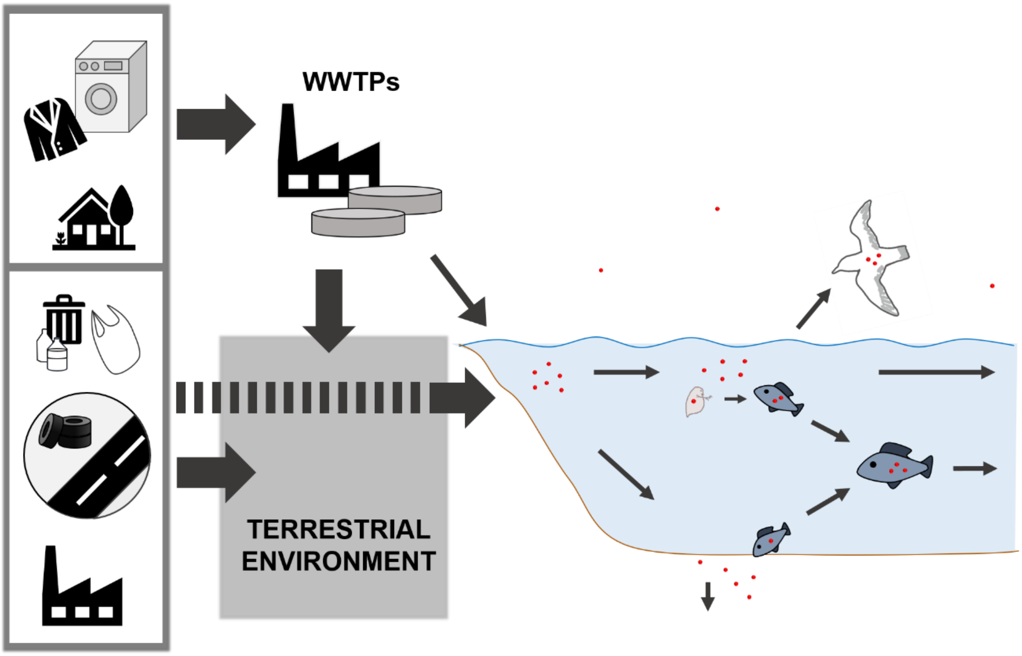

Figure 2.1: Sources of microplastics and their paths into the terrestrial and aquatic environment.

Different applications and uses of plastic products make some MPs more likely than others to be discharged into the environment. Hence, even though the production of plastic fibres covered only 16% of global plastic production in 2019 [1, 2], the regular use and washing of plastic textiles cause significant, continuous discharges of MPFs into the air and especially into wastewater. Overall, the relative quantities of discharged MPFs depends on the fabric type [40]. For example polyester fleece can release large quantities of MPFs; one square meter of fleece can discharge up to 7360 MPF/L during one wash [41]. In Finland, the annual release of polyester fibres from households via washing machines is estimated to be 154 tons [42].

Even though MP pollution has been studied over a decade, the removal efficiencies of MPs in WWTPs have started to attract attention only recently [39, 43–47]. The removal efficiency for MPs within a certain WWTP depends on the techniques that it utilizes. Primary treatment, including screening, grit removal and sedimentation, has been found to remove the majority (35–92 %) of MPs from wastewater, when microplastics larger than 20 to 63 µm are concerned [48–51]. Secondary treatment can further improve the removal of MPs. The most common type of secondary wastewater treatment process is the conventional activated sludge process. This consists of biological treatment with bacteria and protozoa in an aerated basin, and clarification, where excess sludge material is separated from the treated wastewater. Usually, the process is combined with primary treatment for removing larger and denser items and particles together with grease fractions. The removal efficiency for MPs larger than 20 µm in CAS-based plants have been reported to vary between 64 and 100% [46, 52]. However, even if only a small number of the incoming MPs pass the process with various effluents, the continuous discharge of low concentrations of MPs with treated effluent, such as 1.5 MP/L [53] or 10.7 MP/L (>25 µm) [54], can cause significant pollution in the environment, which might have an impact on organisms in the recipient lakes, rivers, and seas.

To enhance the removal of microplastics and other pollutants from conventionally treated WWTP effluents, advanced treatment technologies have been developed, and they are applied either separately as a main process or as a tertiary treatment after CAS process. The majority of the advanced technologies, such as membrane bioreactors (MBR), disc-filters (DF) and rapid sand filtration (RSF), are based on the separation of liquid and solid fractions by filtration using membranes or other physical separators. Dissolved air flotation (DAF), in turn, is based on the separation of suspended solids from wastewater by aeration and skimming. Talvitie et al. studied the removal of MPs larger than 20 µm with advanced technologies and reported removal efficiencies varying from 40.0–98.5% for DF to 99.9% for MBR in pilot-scale plants and 95.0 and 97.1% for full-scale DAF and RSF plants [55].

It needs to be noted that all the MPs that are removed from wastewater streams are concentrated to other waste streams, such as sludge and solid fractions separated during grit and grease removal. Depending on the reuse of municipal sludge and other solid wastes, the MPs can still be released into the terrestrial or aquatic environment. In Finland, 50% of municipal sludge was utilized in green construction, 40% in agriculture and the rest was stored or utilized for landscaping in 2016 [56]. The use of sewage sludge in soil cultivation increases the amount of MPs in the soil [57]. Due to the increased concern related to the retained MPs and other emerging pollutants in treated municipal sludge, the reuse of municipal sludge in agriculture is continuously under discussion. Hence, there is an urgent need for reliable ways to analyse MP concentrations in municipal sludge and to assess possible risks related with its utilization as a fertilizer in fields.

Overall, very few studies have been conducted in Finland or elsewhere in Nordic conditions focusing either on the efficiency of WWTPs to remove MPs from wastewater or on the impact of discharged MPs on the aquatic environment. The first studies in Finland focused on the Viikinmäki WWTP in Helsinki, where MPs and other micro-litter larger than 20 µm were analysed at different stages of the treatment process [24, 45]. Concentrations of micro-litter varied widely from 380 to 900 n/L in influent to 0.7 to 3.5 n/L in effluent [45].

## 2.2 Distribution in the aquatic environment

Because MPs are a wide group of differently shaped and sized particles and fibres consisting of varying polymers, each with their own specific properties, they can be unevenly distributed in the aquatic environment. Generally, MPs with a higher density than the surrounding water, such as PET (1.37–1.45 g/cm$^3$) or PVC (1.2–1.6 g/cm$^3$), are more likely to sink to the bottom [7]. In contrast, many commonly used plastics, such as PP, PE and PS, have a lower or similar density to water (0.9–1.1 g/cm$^3$) [7], and they are more prone to drifting with the water further away from their sources [58].

In addition, attached organic and inorganic material can alter the density of MPs, and, thus, cause even lighter polymers to be buried in the bottom sediment [9, 59]. Moreover, the shape of the particle affects its settling velocity in the water phase [60]. Furthermore, the ambient conditions in the aquatic system, such as water density, currents and wind may affect the distribution of MPs [61].

Consequently, sediments are significant sinks for MPs both in marine [8, 24, 62] and freshwater environments [15, 17, 25, 63]. For instance, PE concentrations in the bottom sediment of Tokyo Bay (6000 n/m$^2$) were four orders of magnitude higher than in the surface water (1.7–3.2 n/m$^2$) [8].

Due to the high variety in the environmental fate of microplastics, it is crucial to include different types of environmental samples in the investigations when the abundance of microplastics or their possible environmental impacts are assessed. To enable that, a wide range of sampling and pre-treatment methods has to be used, which leads to uncertainties in the reported results and challenges the comparison of results from different studies.

# 3 Materials and methods

The results of this study are based on a case study, which focused on Kenkäveronniemi WWTP and its recipient lake area near the city of Mikkeli in Finland. The samples for MP analysis were collected during years 2016–2019. The related sampling, pre-treatment, identification, recovery rate assessment and contamination control practices are presented in detail in Sections 3.1–3.6. In addition, several pre-treatment methods were compared to evaluate their suitability for assessing MP concentrations in wastewater and sludge. These methods are introduced in Section 3.4.1.

## 3.1 Sampling sites

Kenkäveronniemi WWTP was the main municipal WWTP in Mikkeli at the time of this study. The plant treated approximately 11,000 m$^3$ of municipal wastewater daily, covering the wastewater of roughly 42,000 inhabitants. The plant was built in 1962 as a biological treatment plant, and in 1971 parallel precipitation and sludge drying were added to the process. In 1986–1988, the plant was renovated and extended. During the last decades, the plant utilized a CAS process with step screening, 6-mm sieving, grit separation, primary clarification with grease separation, biological treatment with activated sludge, final sedimentation, and disinfection (Figure 3.1). Treated effluents were discharged into the bottom of Savilahti bay, close to the Pappilanselkä basin, via a 110-meter long pipe.

According to the monitoring records of the plant, the plant was operating normally during the sampling campaign at the plant. On average, the sludge volume index of the activated sludge was 62 mL/g. The total suspended solids for the effluent came to around 10.3 mg/L with a removal efficiency of 98.6%.

Kenkäveronniemi WWTP was replaced with an MBR-based WWTP in 2021. The new WWTP is located in Metsä-Sairila, and treated effluents are discharged into Pappilanselkä basin, about one kilometer south of the old discharge site.

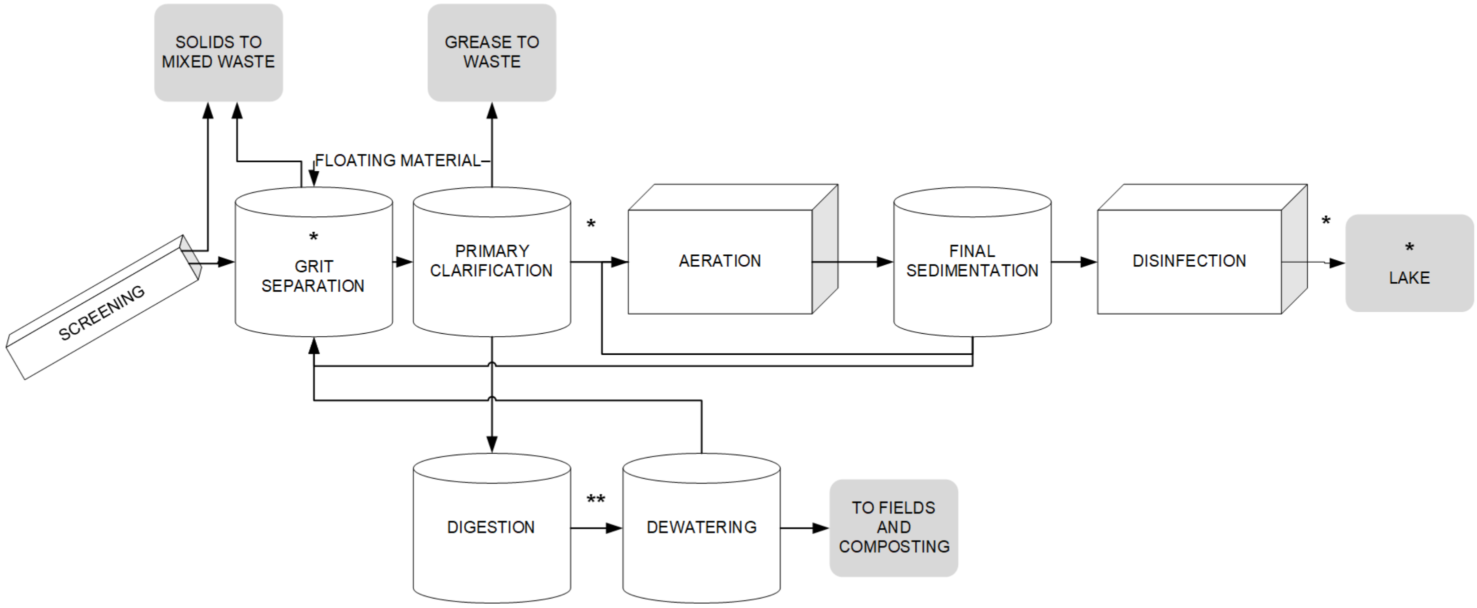

Figure 3.1: A graphical representation of the wastewater treatment process in Kenkäveronniemi WWTP, with sampling locations for water (*) and sludge (**).

Environmental samples were collected from a narrow part of Lake Saimaa, north of the Siikasalmi strait (Figure 3.2). The catchment basin consists of both an urban city centre, suburban, and industrial areas together with forests and agricultural land. The studied lake basin presents a moderate ecological state according to European Union Directive 2000/60/EC [64].

The Launialanselkä, Pappilanselkä, Annilanselkä and Kyyhkylänselkä basins were included in the MP examinations of this study. Sampling site 1 was in Launialanselkä, which is surrounded by a suburban area and is located 2 km north-east from the discharge site of the WWTP. Site 2 was in Pappilanselkä, the closest basin to the city centre of Mikkeli, and it was located within 600 m from the discharge site of the WWTP. Annilanselkä (site 4) and Kyyhkylänselkä (site 3) are surrounded by forests, suburban and agricultural areas, and the sampling sites were located 3 and 7 km downstream from the WWTP, respectively. In addition, water samples were collected from Mikkeli harbour and a sediment sample was collected from Pappilanselkä (site 5) for method and recovery testing.

According to the flow report by Ramboll Finland Oy [65], various wind conditions allow diluted WWTP effluents to migrate either towards the Launialanselkä or Annilanselkä basins.

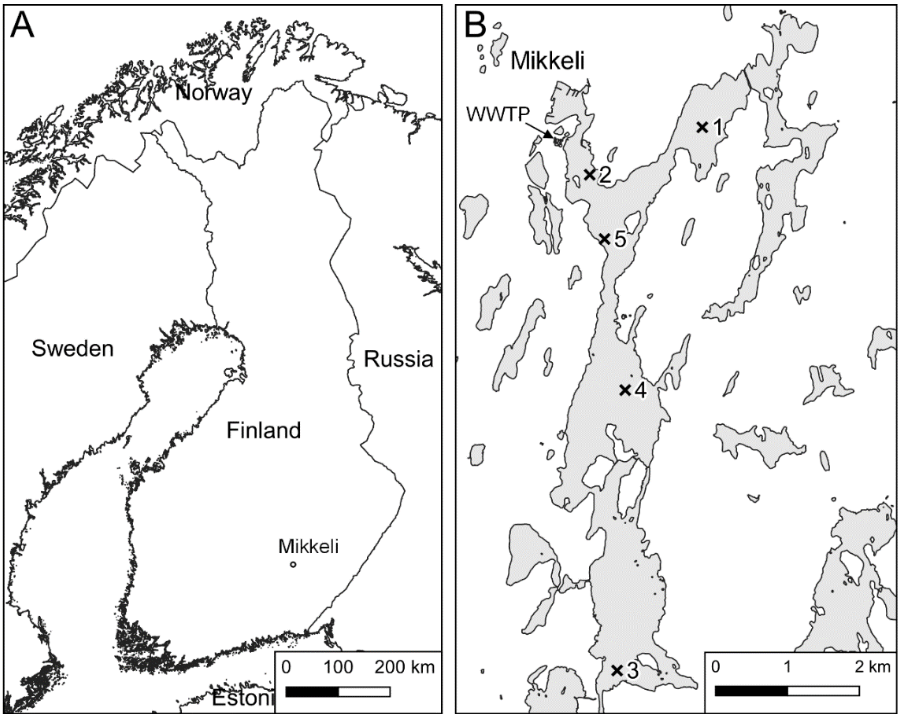

Figure 3.2: Maps of the sampling area; location of A) Mikkeli, and B) the sampling sites and Kenkäveronniemi WWTP.

## 3.2 Sampling

### 3.2.1 Wastewater treatment plant

In this work, wastewater and sludge samples were collected from different parts of wastewater treatment process in Kenkäveronniemi WWTP (Figure 3.1), as described earlier in our publications [66, 67]. Wastewater samples were collected from influent (after a 6 mm screen, at the beginning of the grit separation basin), after the primary clarification, and from the effluent after final disinfection. Sludge samples were collected from the digested sludge before dewatering. In addition, preliminary lake water samples were collected near the shore next to the WWTP to roughly estimate the concentration of MPs close to the effluent discharge site.

Sampling was conducted every other Monday during 10.10.2016–2.1.2017 between 8 and 12 am. Wastewater samples (4.0–30.0 L, Table 3.1) were collected with a stainless steel (SS) bucket and sieved on site with a cascade of two test sieves with mesh sizes of 0.25 and 5.0 mm. Material retained in the smaller mesh was rinsed with distilled water into pre-weighed beakers. For the influent, smaller volumes (4.0–8.5 L) were sampled to make the examination efficient with samples likely to contain higher concentrations of MPs. For method testing, wastewater samples were collected into 5-L PE containers without sieving. Sludge samples (150–200 mL) were collected with an SS cup and immediately poured into glass flasks.

The same set of equipment was used at each sampling point, and the sampling was always started from the samples which we expected to have the lowest MP concentrations to minimize the possibility of cross-contamination. All samples were stored in the dark at 4 °C until further treatment, for a maximum of two days.

Table 3.1: Collected volumes of wastewater and lake water from each sampling point during sampling campaign in 2016–2017 [67].

| Sampling point | Volume of collected sample (L) | Volume of examined sample (L) |
|---|---|---|
| Influent | 4.0–8.5 | 0.8–3.0 |
| After the 1st clarification | 16.0–30.0 | 16.0–30.0 |
| Final effluent | 17.5–30.0 | 17.5–30.0 |
| Lake | 18.5–30.0 | 18.5–30.0 |

### 3.2.2 Recipient lake

Lake water and bottom sediment were sampled from the Launialanselkä, Pappilanselkä, Annilanselkä (only sediment) and Kyyhkylänselkä basins (Table 3.2). Water samples were collected in triplicates 0.5 m below the surface at sites 1–3, or more precisely 0.35–0.65 m below the surface considering the height of the sampling device. In addition, samples were collected 1 m above the bottom, i.e. 5.0 m (4.85–5.15 m) below the surface, at site 2 to assess the effect of the effluent plume discharged to the bottom. The depths at the sediment sampling sites varied between 6.0 and 14.6 m.

Lake water and sediment samples were collected from a motorboat with different Limnos samplers (Figure 3.3) 11.–19.9.2019 and 13.–14.9.2018, respectively. Lake water samples were released from a Limnos water sampler on a metal filter (with an aperture of 20 µm, Ø 50 mm), placed on a glass filter holder. Each composite sample (10.4 L) consisted of five subsamples, and the filter was transferred into a glass bottle after the filtration of the last subsample. The inside walls of the Limnos were rinsed on the same filter with ultrapure water between subsamples. The samples were stored at 4 °C until further treatment. During the water sampling, the temperature, pH, and conductivity of the water were measured on-site with multi-meter (Palintest Micro 800 MULTI).

Each sediment core was divided into subsamples on site with the help of the ring structure of the sampler. The uppermost 10 cm was divided into 1-cm slices, whereas subsamples below 10 cm consisted of 2-cm thick slices. The samples were transferred with an SS spoon from the Limnos sediment sampler (Ø 94 mm) into glass jars. The samples were stored in the dark at 4 °C until subsamples for a loss-on-ignition analysis (LOI) and dating were collected, and then at -20 °C until further analysis.

Table 3.2: Details of the sampling sites and sampling dates for lake water and sediment samples during sampling campaigns in 2018–2019.

| Site | Lake basin | Coordinates | Sample type | n | Sampling depth (m) | Sampling date |
|---|---|---|---|---|---|---|
| 1 | Launialanselkä | 61°41'07.9"N<br>27°19'09.5"E | Water | 3 | 0.5 | 18.9.2019 |
| | | | Sediment | 1 | 13.5 | 13.9.2018 |
| 2 | Pappilanselkä | 61°40'48.2"N<br>27°17'22.2"E | Water | 3 | 0.5 | 19.9.2019 |
| | | | Water | 3 | 5.0 | 19.9.2019 |
| | | | Sediment | 1 | 6 | 14.9.2018 |
| 3 | Kyyhkylänselkä | 61°37'06.8"N<br>27°17'58.1"E | Water | 3 | 0.5 | 13.9.2019 |
| | | | Sediment | 1 | 13 | 13.9.2018 |
| 4 | Annilanselkä | 61°39'09.7"N2<br>7°18'03.4"E | Sediment | 1 | 14 | 13.9.2018 |
| 5 | Pappilanselkä | 61°40'17.8"N<br>27°17'44.5"E | Sediment | 1 | 14.6 | 14.9.2018 |

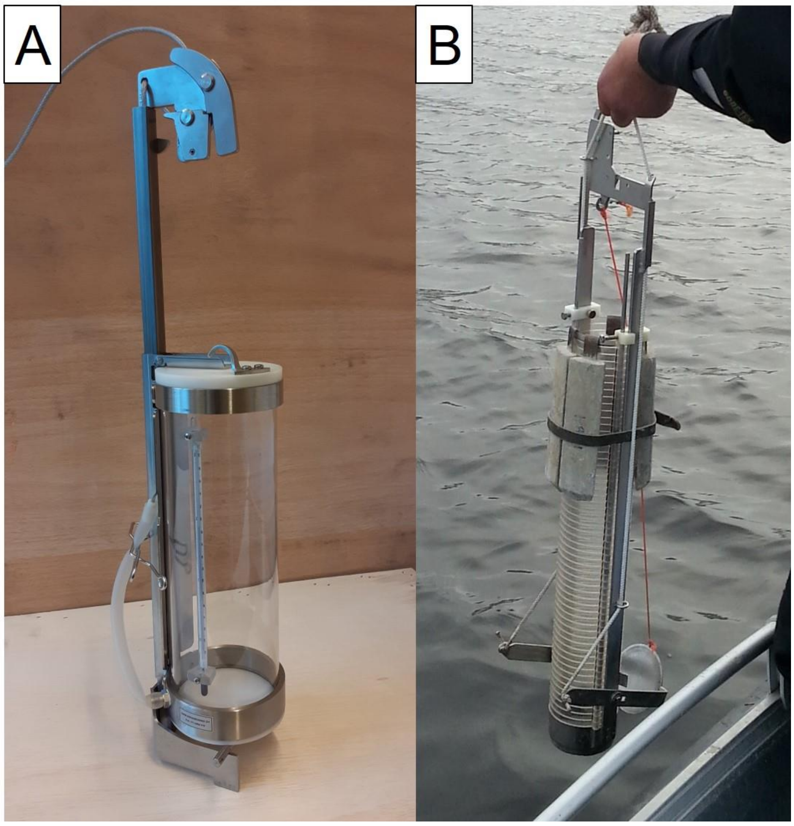

Figure 3.3: Sampling devices used for lake water and sediment sampling; A) a Limnos water sampler, and B) a Limnos sediment sampler with a ring structure.

## 3.3 Pre-treatment of samples before microplastics analysis

Before the identification of microplastics from the environmental samples, the amount of non-plastic materials had to be reduced to allow the visual and chemical identification of possible MPs. For that purpose, the samples were treated in various ways to remove organic and inorganic materials that could distract the identification of MPs. In the following sections, used purification and other pre-treatment methods are described in detail for each different sample type. All the chemicals and enzymes used in the present work are listed in Table 3.3.

Table 3.3: Chemicals and enzymes used in the present work.

| **Chemical** | **Product description** | **Application** |
|---|---|---|
| Acetic acid (glacial) ($CH_3CO_2H$) | CAS 64-19-7, Sigma-Aldrich, ≥99.5% | pH adjustment of NaOAc buffer |
| Citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) | CAS 5949-29-1, Sigma-Aldrich, ≥99% | Buffer for cellulase treatment of influent |
| Ethanol ($C_2H_5OH$) | CAS 64-17-5, Altia, ≥99.5% | Reagent alcohol for OEP |
| Hydrochloric acid (HCl) | CAS 7647-01-0, Sigma-Aldrich, >37% | pH adjustment of Tris buffer |
| Hydrogen peroxide ($H_2O_2$) | CAS 7722-84-1, VWR, 30% | Degradation of organic material |
| Iron(II) sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) | CAS 7782-63-0, Sigma-Aldrich, ≥99% | Catalyst for WPO |
| Isopropanol ($C_3H_8O$) | CAS 67-63-0, Sigma-Aldrich, ≥99.5% | Reagent alcohol for OEP |
| Methanol ($CH_3OH$) | CAS 67-56-1, Sigma-Aldrich, ≥99.9% | Reagent alcohol for OEP |
| Potassium hydroxide (KOH) | CAS 1310-58-3, Sigma-Aldrich, 90% | Degradation of organic material |
| Rose Bengal; 4,5,6,7-tetrachloro-2’ ,4’ ,5’ ,7’ -tetraiodofluorescein disodium salt ($C_{20}H_4Cl_4I_4O_5$) | CAS 632-69-9, Sigma-Aldrich, ≥80 % | Staining of other materials than plastics |
| Sodium acetate (anhydrous) ($C_2H_3NaO_2$) | CAS 127-09-3, Sigma-Aldrich, >99% | Buffer for cellulase and chitinase |
| Sodium dodecyl sulphate ($C_{12}H_{25}NaO_4S$) | CAS 151-21-3, VWR/Sigma-Aldrich, ≥98% | Macerating samples before enzymatic treatments |
| Sodium tungstate dihydrate ($Na_2WO_4 \cdot 2H_2O$) | CAS 10213-10-2, Merck, ≥98% | Density separation of sediment samples |
| Sulphuric acid ($H_2SO_4$) | CAS 7664-93-9, Sigma-Aldrich, 95-97% | Fe(II) solution for WPO |
| Tris(hydroxymethyl)aminomethane ($C_4H_{11}NO_3$) | CAS 77-86-1, Sigma-Aldrich, ≥95% | Buffer for protease |
| Trisodium citrate dihydrate ($C_6H_5O_7Na_3 \cdot 2H_2O$) | CAS 6132-04-3, Sigma-Aldrich, ≥99% | Buffer for cellulase treatment of influent |
| **Enzyme** | **Product description** | |
| Cellulase TXL | ASA Spezialenzyme GmbH, >80 U/mL | Degradation of cellulose from lake water |
| Cellulase from *Aspergillus niger* | Sigma-Aldrich, 0.8 U/mg | Degradation of cellulose from influent |
| Chitinase, chitodextrinase, 1,4-β-poly-N-acetylglucosaminidase, poly-β-glucosaminidase | ASA Spezialenzyme GmbH, >115 U/mL | Degradation of chitin from lake water |
| Protease A-01, subtilisin | ASA Spezialenzyme GmbH, 1100 U/mL | Degradation of protein from lake water |

### 3.3.1 Wastewater and sludge

The wastewater and preliminary lake water samples collected with 250-µm sieves were treated using wet peroxide oxidation (WPO) according to Masura et al. (2015) [68], as described earlier in our publication [67]. First, the on-site sieved samples were dried at 75 °C for at least 40 hours. Next, 20 mL of 30% hydrogen peroxide ($H_2O_2$) and 20 mL of aqueous 0.05 M Fe(II) solution were added to the dried samples (0.15 ± 0.06 g). The samples were let to settle for 5 min at room temperature (RT). Each beaker was covered with a watch glass and heated up to 75 °C using a hotplate with magnetic stirrer. In case of signs of over boiling, a small amount of distilled water was added to cool down the reaction. The reaction was allowed to continue for 30 min after additions of $H_2O_2$ or distilled water, i.e. for about 50 min for influent and 30 min for other water samples. The samples were cooled down to RT and vacuum filtered on gridded membrane filters (Sartorius, cellulose nitrate filter, Ø 50 mm, porosity 0.8 µm) with glass fibre filters (VWR, Grade 696, Ø 50 mm, porosity 1.5 µm) at the bottom for mechanical support. The filters were transferred onto petri dishes and allowed to dry for 24 hours at RT with loose aluminium foil covers.

Because the influent samples contained higher amounts of solid materials, mostly cellulose fibres originating from decomposed toilet paper, subsamples of a maximum 0.25 g dw were included in the examination and the samples were treated with WPO followed by enzymatic treatment with cellulase [69]. Due to the higher solid material content, also more hydrogen peroxide was used during the WPO treatment. First, 40 mL of $H_2O_2$ was added and then another 20 mL of $H_2O_2$ was added after 20 min to promote the degradation of the organic matter. After the WPO treatment, cooled samples were sieved in a 38-µm test sieve and rinsed into glass flasks with a small amount of distilled water, closed with foil and stored in the dark at 4 °C. Enzymatic degradation was conducted by adding 0.25 g of cellulase from *Aspergillus niger* per 0.25 g dw of sample, corresponding to 800 activity units per gram of substrate, together with 12.5 mL of sodium citrate buffer (pH 4.8), containing 0.1 M citric acid and 0.1 M trisodium citrate. Samples were incubated for 24 hours at 40 °C with 160 rpm and then filtered similarly as other wastewater samples.

Subsamples of well-mixed digested sludge (3 g in wet weight (ww), 0.1 g in dry weight (dw)) were poured into glass petri dishes, dried at 45 °C for 18 h with a pierced foil cover [39] and examined three times under a stereo microscope, as described later in Section 3.4.1.

### 3.3.2 Lake water

On-site filtered lake water samples were treated using the universal enzymatic purification protocol (UEPP) introduced by Löder et al. [70] with some adjustments. No partitioning or density separation was conducted. Amylase treatment was also excluded due to problems noticed during preliminary testing. Incubation with amylase caused the whole sample to precipitate, which made it impossible to filter the sample and continue the treatment.

The protocol included 1–3 d incubations with sodium dodecyl sulphate (SDS), protease, cellulase, 30% $H_2O_2$ and chitinase with buffers at varying temperatures. First, the samples were incubated at 50 °C for 24 h with 100 mL of 10% SDS solution and occasional manual mixing. The metal filter was removed from the bottle with tweezers, material attached to the filter was rinsed back into the bottle with ultrapure water, and the filter was placed on a glass filter holder. The sample was filtered on the same metal filter, and the bottle and filtration funnel were rinsed three times with ultrapure water. The filtration funnel was detached and rinsed with the next solution into the same bottle, after which the metal filter was also transferred into the same bottle and the remaining volume of the next solution was added together with the enzyme load. Each incubation step was conducted similarly, with solutions and enzymes changing according to the flow chart presented in Figure 3.4. The volume of added enzymes was based on the enzymatic activity of the used enzyme solutions, following the activities reported by Löder et al. [70]. After the last treatment step with $H_2O_2$, the samples were filtered on the same 20-µm meshes and stored in glass bottles with a small amount of ultrapure water. Finally, the samples were filtered on GF/C filters (Whatman GF/C, 1.2 µm, Ø 25 mm), and placed on glass microscope slides in petri dishes.

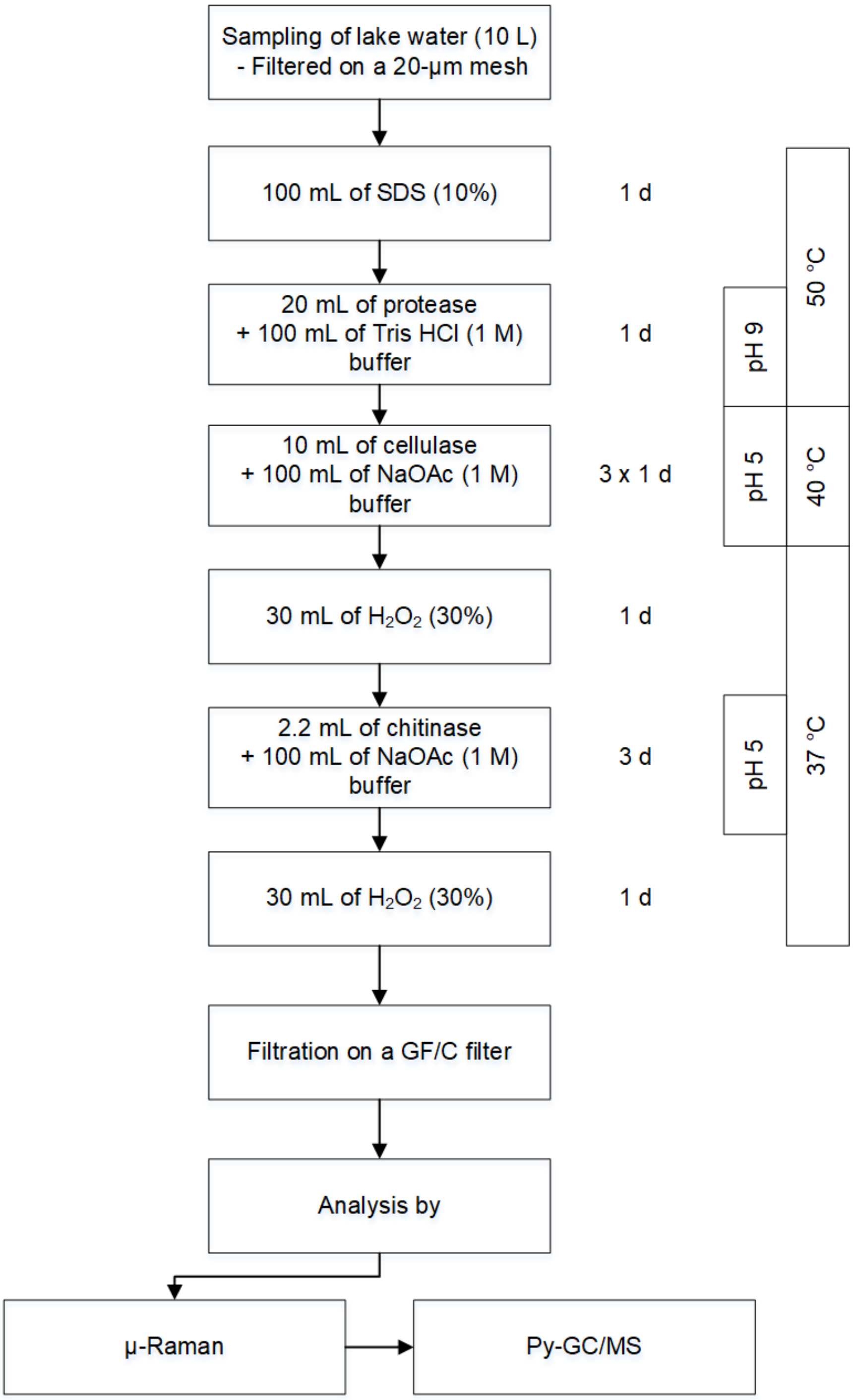

Figure 3.4: Procedure for lake water sample treatment, modified from Löder et al. [70].

### 3.3.3 Sediment

Sediment samples (29.6–74.1 g ww, 2.4–18.1 g dw) were purified according to the method by Frias et al. [71]; i.e. degrading organic material with $H_2O_2$ and separating MPs from denser material with sodium tungstate dihydrate ($Na_2WO_4 \cdot 2H_2O$). In contrast to 100 µm suggested as a lower size limit for monitoring purposes [71], the lower size limit of the sample pre-treatment was set to 63 µm by sieving.

First, 100 mL of 10% $H_2O_2$ was added to samples in two portions with a one-hour reaction time in between, mixed with glass rods after additions, and covered with aluminium foil. In case of excessive foaming, samples were mildly mixed, and a small amount of ultrapure water was added. After 18 hours of reaction time, the samples were sieved on a 63-µm test sieve, and the retained material was transferred into a 100-mL separatory funnel with a small amount of ultrapure water. Next, sodium tungstate dihydrate was added to the samples corresponding to a density of 1.4 g/cm$^3$ (550 g/L). In practice, 0.65 g of sodium tungstate dihydrate was added per 1 g of sieved sample material. The samples were mixed for 2 min and allowed to settle for 2 h, after which the settled material was discarded. Supernatant was vacuum filtered on a GF/C filter (Whatman GF/C, 1.2 µm, Ø 47 mm), which was transferred onto a microscope slide in a petri dish and dried in a desiccator.

The density (1.4 g/cm$^3$) of both the sodium tungstate solution and filtered supernatant was confirmed with a density meter (Rudolph Research Analytical DDM 2911).

## 3.4 Recovery rates of microplastics

The recovery of MPs was tested for each of the used pre-treatment methods with triplicate samples spiked with 10–20 pieces of different MPs. Detailed information about the used MPs is listed in Table 3.4 and representatives of each MP are presented in Figure 3.5. The spiked MPs represented the most widely-used polymers with densities varying from 0.9 g/cm$^3$ of PP to 1.4–1.5 g/cm$^3$ of PET and 1.2–1.6 g/cm$^3$ of PVC [7]. The density of SBR may vary a lot depending on the specific composition of the rubber in question [35]. The spiked MPs also varied in their size, the longest dimensions being mostly in the range of 500–1000 µm. The diameter of the MPFs varied from 20 to 30 µm. Unexpanded PS spheres were purchased from Sigma-Aldrich and SBR fragments were offered by Saltex Oy. Non-commercial MPs were self-made by cutting them with scissors from various plastic items, such as packages, fabrics, and ropes. All spiked MPs were imaged with stereo microscope (Zeiss, SteREO discovery.V8 with Axiocam 503 colour) before adding them to the samples, and their longest dimensions were measured manually from the images. Similarly looking MPs were not detected in our examinations at the WWTP or in the receiving lake area, and only MPs with a similar appearance with spiked ones were counted during the recovery testing.

Table 3.4: Spiked microplastics used in recovery testing. Densities according to Hidalgo-Ruz et al. [7] and Wagner et al. [35].

| **Type** | **Polymer** | **Source** | **Colour** | **Longest dimension (µm)** | **Density ($g/cm^3$)** | **Used in recovery testing with** | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | **wastewater and sludge** | **lake water** | **sediment** |
| Particle | PET | Beverage bottle | Blue | 360–880 | 1.4–1.5 | | | x |
| | PS | Sigma-Aldrich | White | 650–950 | 1.0–1.1 | x | x | x |
| | PE | Rope | Orange | 230–2900 | 0.9–1.0 | x | x | x |
| | PVC | Office folder | Transparent | 300–3100 | 1.2–1.6 | x | | x |
| | SBR | Saltex Oy | Black | 900–3400 | 0.9–1.2 | x | | x |
| Fibre | PET | Fleece | Yellow | 530–6700 | 1.4–1.5 | x | x | |
| | PA | Yarn | Green | 650–3600 | 1.0–1.1 | x | | x |
| | PP | Rope | Red | 300–3300 | 0.9 | x | x | x |

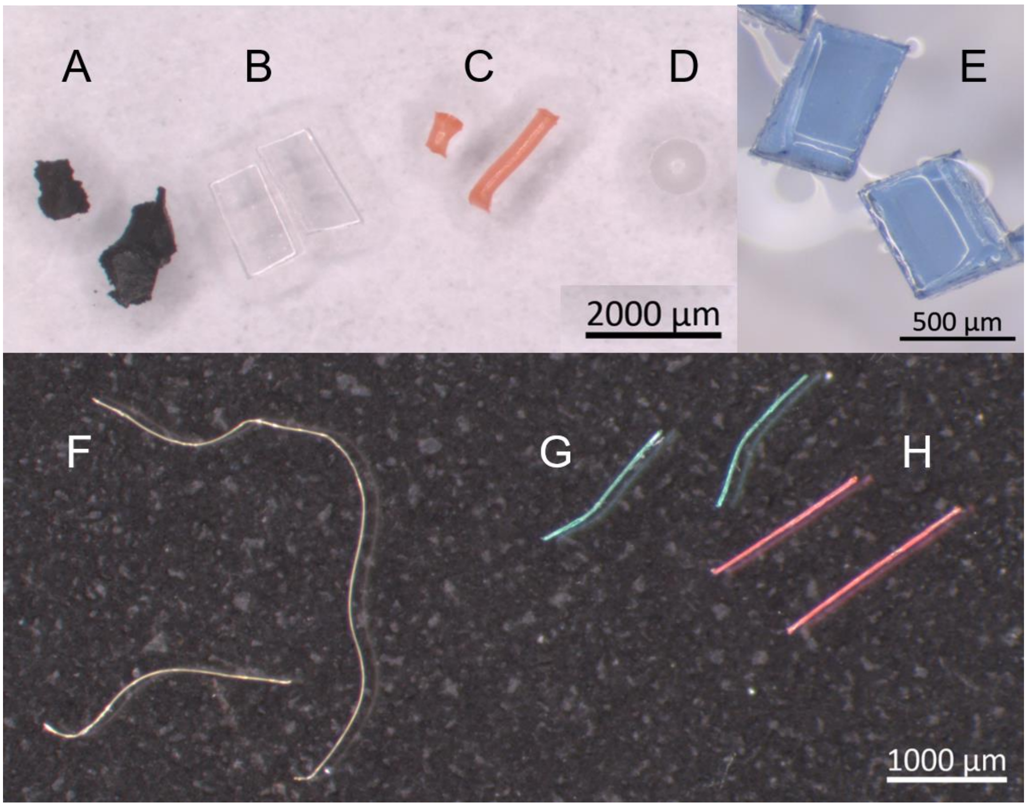

Figure 3.5: Appearance of the MPs used in the recovery testing: A) SBR, B) PVC, C) PE, D) PS, E-F) PET, G) PA, and H) PP.

### 3.4.1 Comparison of the recovery rates of various pre-treatment methods with wastewater and sludge samples

After the examination of wastewater and sludge samples, it was clear that selected methods, i.e. WPO for wastewater and the examination of dried sludge, were not efficient enough to purify the samples to allow feasible identification of microplastics. Hence, there was a need to improve the pre-treatment methods to allow more reliable detection of various types of microplastics. Thus, five previously-used pre-treatment methods were compared to evaluate their suitability for examining MPs in wastewater and sludge samples, as we presented in our previous publication [66]. The tested pre-treatment methods included filtration [24], WPO [67], degradation with potassium hydroxide (KOH) [72], an oil extraction procedure (OEP) [73], and a drying-based method [39], which was only tested with sludge (Figure 3.6).

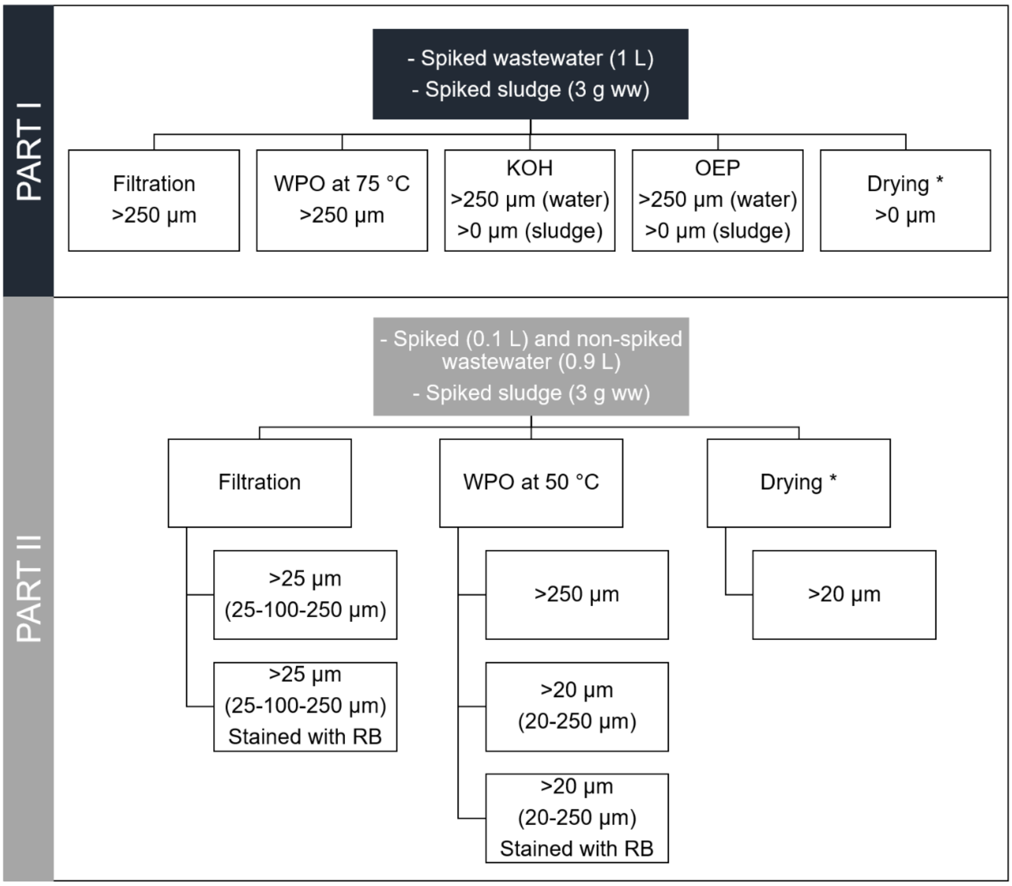

Figure 3.6: Representation of the conducted experiments in parts I and II of the comparison study. *Only tested with sludge samples.

In order to test the recovery of the methods, a selection of model MPs were inserted into samples (i.e. the samples were spiked with MPs). The recovery of spiked MPs was examined in triplicates with influent (1 L) and digested sludge (3 g ww) samples. To our knowledge, this was the first study to assess recovery of MPs with different pre-treatment methods by including both particles and fibres in the recovery testing.

A schematic diagram of the study is shown in Figure 3.6. First, five pre-treatment methods were compared and methods with highest recovery rates for spiked MPs were further adjusted in part II. In part I, the lower size limit was set to 250 µm in all pre-treatment methods for influent samples. For the sludge samples, the lower size limit varied depending on the studied method. The MPs were spiked in the whole volume of influent (1 L) and digested sludge (3 g ww) samples.

Adjustments in part II were made to the size limitations and the used temperatures. The MPFs had overall low recovery rates in part I (Table 4.2; 41–79%), which could probably be explained by their small diameter (20–30 µm) allowing them to pass through the 250-µm mesh lengthwise. Thus, the lower size limit during sample pre-treatment was reduced to 20–25 µm, depending on the pre-treatment method. To prevent clogging of the meshes during pre-treatment steps, the total volume of spiked influent was decreased to 0.1 L when smaller mesh sizes were used. In practice, 0.9 L of influent was first sieved with the 250-µm mesh, after which 0.1 L of spiked influent was sieved with the whole cascade of sieves.

In addition, staining with Rose Bengal (RB) was studied with filtered and WPO-treated samples. The main reason for the assessment of staining was the low recovery of yellow and flexible PET fibres, which were difficult to separate visually from cellulose fibres. Rose Bengal was selected over Nile Red, because Rose Bengal stains other materials than plastics, which allows recording the original colours of the MPs [12]. Staining was conducted by letting 5 mL of 0.2 mg/mL Rose Bengal solution react with filtered samples for 5 min, after which the sample was rinsed and dried [74].

*Filtration.* In part I, a filtration device introduced by Talvitie et al. [24] was built and equipped with nylon net with mesh size of 250 µm. Spiked samples were filtered, filters were transferred into petri dishes and allowed to dry at RT with a foil cover before examination. Sludge samples were diluted with 3 L of tap water before filtration [45]. In part II, two additional meshes (25 and 100 µm) were added in the filtration device [24].

*Wet peroxide oxidation.* For dried influent samples (0.26–0.28 g dw), WPO treatment and final filtration were conducted similarly as described in Section 3.3.1. For dried sludge samples (0.15–0.23 g dw), only 20 mL of $H_2O_2$ was added and the samples were heated for 30 minutes. In part II, the samples were first sieved on a cascade of test sieves (20 and 250 µm) and the retained material was rinsed into separate beakers. Samples were dried at 50 °C and further treated as described for part I, but the samples were heated to 50 °C instead of 75 °C to prevent the possible melting of MPs.

*KOH degradation.* The degradation of organic material was conducted with a 10% KOH solution according to Karami et al. [72] with small adjustments. Influent samples were sieved on a 250-µm test sieve and the retained fraction was rinsed into a glass bottle with a small amount of distilled water, whereas sludge samples were poured into the bottles without sieving. A KOH solution was added in the proportion of 1:10 (w/v). The samples were incubated at 40 °C for 48 hours and were then vacuum filtrated on glass fibre filters (VWR, Grade 696, porosity 1.5 µm).

*Oil extraction procedure*. OEP was first introduced by Crichton et al. [73] for seawater and sediment samples, and those procedures were used for the spiked influent (>250 µm) and sludge samples, respectively. A sieved influent sample was collected in a small beaker, rinsed with 200 mL of distilled water into a separatory funnel, and mixed for 30 s with 10 mL of canola oil. The sample was allowed to settle for 20 min and the water layer was discarded. Separation was repeated with another 200 mL of distilled water. The water layer was discarded, and the oil layer was released into a gridded membrane filter (Sartorius, cellulose nitrate filter, Ø 50 mm, porosity 0.8 µm) with a glass fibre filter (VWR, Grade 696, Ø 50 mm, porosity 1.5 µm) at the bottom. The separatory funnel was rinsed four times with 25 mL of 4% detergent solution (VWR, Deconex 22 LIQ-x) and vacuum filtered on the same filter. The filtered sample was incubated twice with reagent alcohol (90% ethanol, 5% methanol and 5% isopropanol) for 10 min, after which the filter was slightly wetted with water and transferred onto a petri dish. Sludge samples were first dried at 50 °C in Erlenmeyer flasks and mixed with 50 mL of distilled water, after which 5 mL of canola oil was added and mixed for 30 s and the sample was allowed to settle for 2 min. The sample was decanted into a separatory funnel, and water and oil were repeatedly added to the flask. Both fractions were poured into the same separatory funnel. The flask was rinsed four times with distilled water. The sample was mixed for 30 s and allowed to settle for 2 min and the water layer was discarded. Another 100 mL of water was added, and the mixing and settling was repeated, after which the water was discarded and the samples were filtered like the influent samples.

*Drying.* Only sludge samples were dried according to Murphy et al. [39], i.e. the sludge samples were dried at 45 °C for 19 hours. In part II, the sludge samples were first sieved with a 20-µm test sieve before drying to reduce the amount of solid material and to ease the examination. The retained material was rinsed into two petri dishes with distilled water. In contrast to the other methods, these samples were examined three times under an optical microscope. A small amount of distilled water was added to break down the sludge material during examination.

## 3.5 Identification of microplastics

All material retained on the filters was examined with a stereo microscope. Representative particles and fibres from the WWTP-related samples from 2016–2017 were identified using Fourier Transform Infrared (FTIR) microscopy and/or Raman micro-spectroscopy, as described in our publication [67]. In the case of lake water and sediment samples all visually suspected MPs were identified using Raman micro-spectroscopy. Both Raman and FTIR microscopy are commonly used methods for the identification of microplastics [75, 76]. In addition, mass concentrations of certain polymers in the lake water samples were analysed using a pyrolysis-gas chromatography-mass spectrometer (py-GC/MS).

For MPs in wastewater and preliminary lake water samples, the lower size limit was set to 250 µm due to the on-site sieving. Even though the lower size limit of included MPs was theoretically set to 0, 20 and 63 µm for sludge, lake water and sediment because of the pre-treatment procedures, respectively, manual sorting led to the exclusion of the smallest MPs. In addition, a significant amount of non-plastic material was left on the filters after the pre-treatment steps. Hence, the lower size limit was estimated to be around 100 µm, and this limit is used later in the discussion for these sample types.

### 3.5.1 Microscopy

First, all samples were visually examined under a stereo microscope (Zeiss, SteREO discovery.V8 with Axiocam 503 colour) with a magnification of 20–50x.

For filtered wastewater and preliminary lake water samples together with dried sludge samples from 2016–2017, all particles and fibres without a clear natural origin (e.g. seeds, cellulose fibres from toilet paper) were collected and placed in another petri dish. The sludge samples were slightly wetted with distilled water to break up the sludge material and examined three times using tweezers [39]. First two examinations were conducted with a white background and the third examination with a black background under a glass dish, to help recognize differently coloured MPs from the sludge samples. All the collected particles and fibres were subjected to a second round of visual identification, where all recovered particles and fibres were recorded by shape and measured by their largest dimension manually using ZEN 2.3 software. In addition, all the particles and fibres, approximately 5200 altogether, were classified in 54 groups according to their appearance. Considered features included for instance the helicity of the fibres, shapes of cross-section (round, oval or flat) and end of the fibres (clear cut, tapered or frayed), as well as hardness of fragments [77]. Representatives of each group were further analysed with FTIR and/or Raman microscopes.

For the lake water and sediment samples, all particles and fibres without a clear natural origin (e.g. water fleas and diatom shells) were recorded by shape and colour and the largest dimensions were measured as described earlier. In the case of lake water samples, the recorded particles and fibres were left on the original filter. However, particles and fibres were separated from the sediment samples on another GF/C filter due to the high amount of other material retained on the original filters.

### 3.5.2 Raman spectroscopy

The Raman method, first discovered in 1928 [78], is based on the excitation of the sample with monochromatic laser light and identifying the material according to the detected Raman scattering, which is characteristic to the certain bonds in the molecular structure of the material. Thus, the fingerprint spectra of different materials allow their identification.

When a laser beam is directed onto the sample, part of the light is either absorbed, reflected, or scattered. The majority of the scattered photons are scattered with the same frequency as the incident light. This elastic scattering is called Rayleigh scattering (Figure 3.7). A very small part of the scattered photons are emitted by inelastic scattering, which is called Raman scattering. In Stokes scattering, a molecule absorbs part of the energy leaving less energy for the scattered photon. Thus, the scattered light has a smaller frequency and the wavelength is longer than that of the incident light. In Anti-Stokes scattering, the molecule releases energy, causing the scattered light to have higher frequency and smaller wavelength than the incident light. [76]

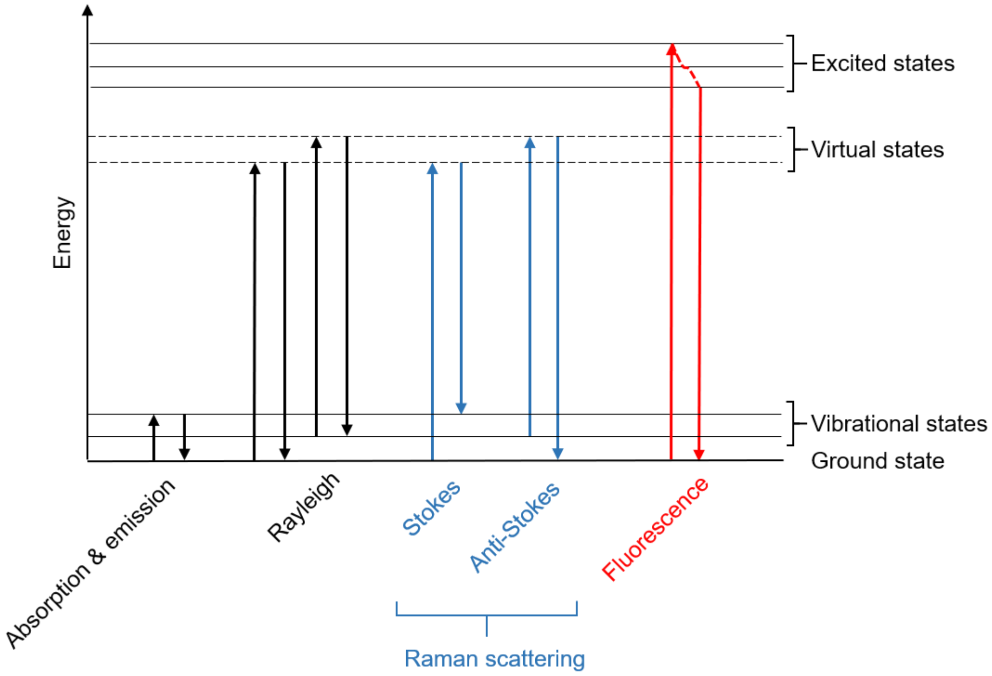

Figure 3.7: Different types of possible scattering.

Different wavelengths are used as light sources in Raman spectrometers. The most common wavelengths are 514, 532, 633 and 785 nm. The smaller the used wavelength is, the more energy is emitted onto the sample and thus more accurate data is collected. Due to the high sensitivity of the Raman method to non-polar structures, such as carbon-carbon bonds, it is a highly suitable method for identifying polymers, which commonly have long carbon chains in their structure [76, 79].

In the present work, the materials of the collected particles and fibres were identified with Raman micro-spectroscopy (Horiba Jobin Yvon, Labram HR) using a green laser (514 nm). LabSpec 5 software was used to present the spectral data collected for wavenumbers 200–3000 cm$^{-1}$.

For the WWTP-related samples and preliminary lake water samples from 2016–2017, on average two to three representative particles or fibres for each group were analysed with Raman micro-spectroscopy, covering 1.4% of all collected particles and fibres, as described in our publication [67]. For the lake water and sediment samples, all visually recorded particles and fibres without a clear natural origin were analysed using Raman micro-spectroscopy.

Spectral data collected for the particles and fibres from the WWTP-related samples from 2016–2017 were compared manually with reference libraries collected from previous publications [76, 80, 81]. In the case of lake water and sediment samples, the KnowItAll Informatics System software (Bio-Rad Laboratories, 2018) was utilized for comparing spectral data with reference libraries. Only particles and fibres with obviously matching peaks with reference to plastic spectra were counted as MPs, but no threshold was set for the similarity of the environmental and reference spectrum. During the automated comparison, some spectra matched with multiple polymers and, thus, they were marked as "plastic" without accurate identification. Examples of characteristic Raman spectra for common polymers are shown in Figure 3.8.

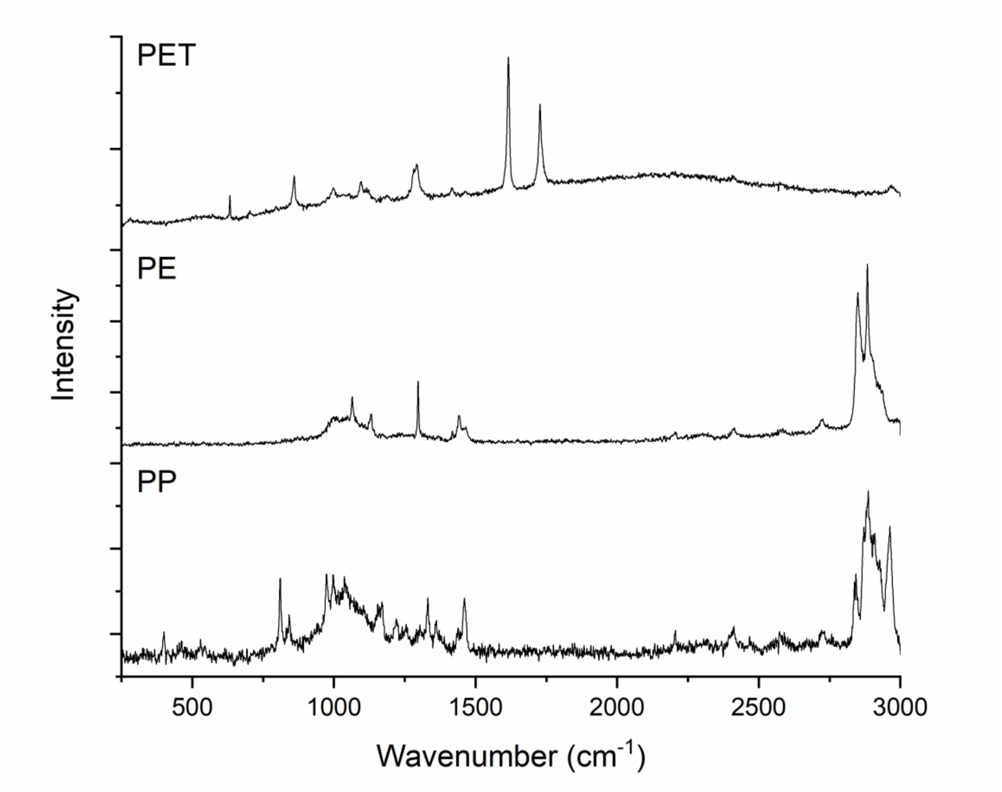

Figure 3.8: Examples of Raman spectra for commonly detected polymers.

### 3.5.3 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy is based on the irradiation of the sample with certain wavelengths of IR light (750 nm–1 mm) and measuring the transmitted or reflected light to examine the absorbed energy at each wavelength. The differences in the absorbed energy are converted into a spectrum by a Fourier transform. As different molecules bend and stretch differently with different wavelengths, presented molecules can be identified according to their fingerprint spectra, similarly to Raman spectroscopy.

FTIR microscopes utilize either transmittance, reflectance or attenuated total reflection (ATR) modes. The transmittance mode is suitable only for transparent particles, and hence, reflectance techniques are more often used for plastics. ATR-FTIR has the highest precision for detection of the fingerprint region of 1450–600 cm$^{-1}$, which corresponds to the stretching of C-H bonds, which are characteristic to many polymers. Examples of such characteristic peaks for PET, PE and PP in reflectance mode are shown in Figure 3.9. Unfortunately, in ATR, the crystal needs to be in contact with the surface of the sample to allow infrared radiation to penetrate the sample, which limits the use of ATR-FTIR microscopy to map the smallest MPs in environmental samples. FTIR microscopy with reflectance is used to map MPs from filtered samples, but it can be problematic with irregularly shaped MPs. In addition, a focal plane array (FPA) is a modification of FTIR microscopy, which measures multiple spots simultaneously and reduces the time needed for the chemical analysis of MPs [76].

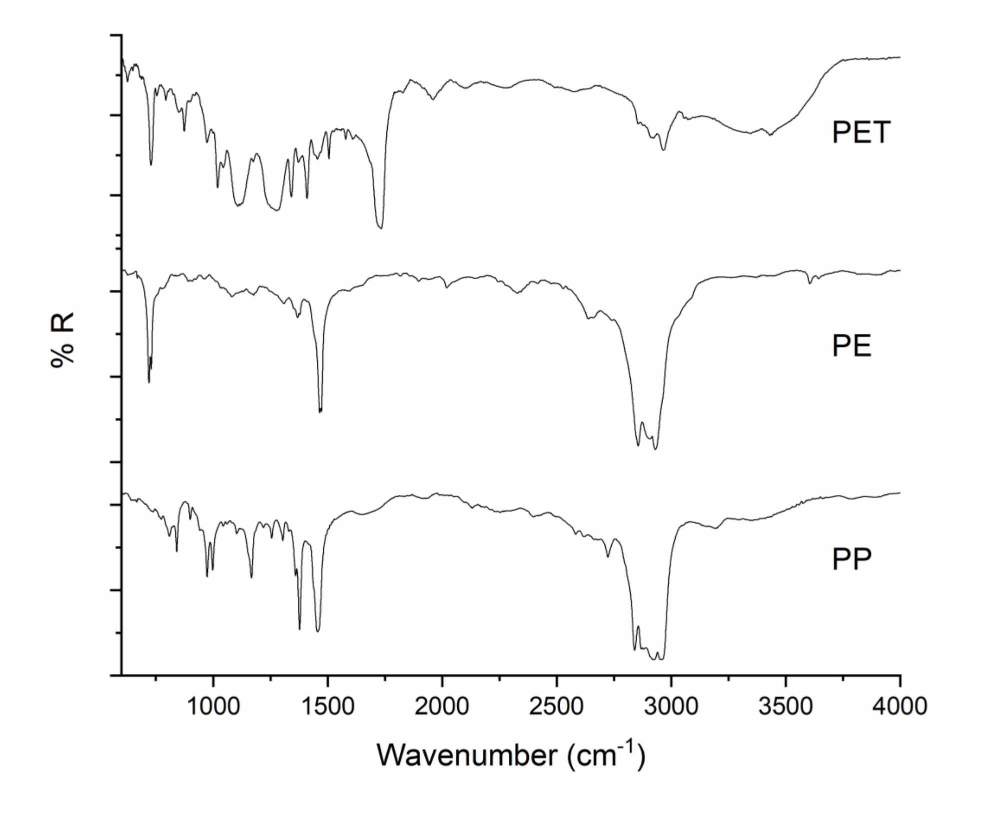

Figure 3.9: Examples of FTIR spectra for commonly detected polymers according to reflectance.

In this study, 1.3% of all collected particles and fibres from wastewater and sludge samples were identified using an FTIR microscope in addition to the Raman analysis discussed in the previous section. Spectral data was collected with an FTIR microscope (Spotlight 200i FT-IR microscope system equipped with Spectrum Two, PerkinElmer) in reflectance mode. Spectra were produced with 24 scans with wave numbers 600–4000 $cm^{-1}$ with a spectral resolution of 4 $cm^{-1}$. Baseline correction, data tune-up and normalization were done for the resultant spectra, which were then compared to spectra libraries supplied by PerkinElmer.

### 3.5.4 Pyrolysis gas chromatography mass spectrometer (py-GC/MS)

Analysis using a pyrolysis gas chromatography mass spectrometer (py-GC/MS) is based on the thermal degradation of molecules in an inert atmosphere into smaller degradation products which are identifiable by their mass [76, 82]. These pyrolysates can be traced to their precursor molecules and hence, the detection of certain pyrolysates can be used as a fingerprint of the initial molecule [82].

In this study, a py-GC/MS analysis was utilized for lake water samples after they were analysed using Raman micro-spectroscopy. The py-GC/MS analysis focused only on target polymers PE, PP and PS and their concentrations were quantified according to Steinmetz et al. [83]. Slightly wetted filters were placed into glass culture tubes and closed with polybutylene terephthalate caps with PTFE coated seals. The samples were dried at 60 °C, after which 2.5 mL of 1,2,4-trichlorobenzene containing 0.015% of butylated hydroxytoluene and 2.5 mL of p-xylene were added. Target polymers were extracted at 150 °C for 60 min, and after cooling down to RT, the supernatant was transferred into ND9 glass vials for analysis. Supernatants were pyrolyzed at 750 °C using a Pyroprobe 6150 filament pyrolyzer (CDS Analytical, Oxford, United States) coupled with a Trace GC Ultra with DSQII mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Concentrations of PE, PP and PS were determined according to their indicator pyrolysates 1,22-tricosadiene, 2,4-dimethyl-1-heptene and α-methyl styrene, respectively. Theoretical limits of detection (LODs) for the target polymers in lake water were 6.1 µg/L for PE, 7.9 µg/L for PP and 2.4 µg/L for PS. These values were calculated according to the original volume of lake water samples (10.4 L) and the analytical LODs of PE (12.76 mg/L), PP (16.41 mg/L) and PS (4.97 mg/L) for the 5-mL sample extracts.

## 3.6 Contamination control

In this study, multiple actions were conducted during sampling, pre-treatment, and analysis for preventing and assessing MP contamination. Because synthetic cloths are a significant source of MPFs [84], their use was avoided during sampling, pre-treatment and the analysis of the samples in order to prevent contamination. Dishes were rinsed three times with distilled or ultrapure water before use and after transferring the samples from one dish to another. In addition, plastic equipment was replaced with glass and metal equipment whenever possible, dishes were covered with aluminium foil to prevent aerial contamination and laboratory benches were wiped three times with wetted cellulose tissue before handling the samples. All used petri dishes and filters were examined under a stereo microscope before use to remove all visible particles and fibres. Pre-treatment of the wastewater and sediment samples was conducted in a fume hood, while lake water samples were treated in a laminar flow cabinet. In the case of sediment and lake water samples, $H_2O_2$ solutions and buffers were filtered through glass microfiber filters (Whatman GF/C, 1.2 µm, Ø 47 mm) and SDS solution through a SS filter (20 µm). Ultrapure water was not filtered, due to the final filtration of the water system (Sartorius, polyethersulfone, 0.45 µm) [71].

For assessing contamination during sampling, storing and pre-treatment steps, field blanks were collected for wastewater (n=7) and sludge (n=7), as well as for lake water (n=4) and sediment samples (n=4) and treated along with other samples. In practice, field blanks were collected by rinsing the sampling equipment with distilled or ultrapure water similarly as during the collection of the real samples and treating that water as a field blank sample. In the case of sediment samples, field blanks were empty glass jars, which were stored with sediment samples throughout the sampling. In addition, procedural blanks (n=6) were used to assess the contamination caused during the pre-treatment of sediment samples. The lowest subsamples from the sediment cores from sites 1, 2 and 4 were also examined for more information about possible contamination during sampling and pre-treatment, because those subsamples represented the time before the mass production of plastics, namely years 1864–1941, and should not have contained any plastics. Aerial contamination was also assessed by placing a GF/C filter on an open petri dish next to the working space during the pre-treatment steps of the lake water (n=7) and sediment samples (n=31). For all contamination samples, particles and fibres were identified directly from the filter.

## 3.7 Loss-on-ignition (LOI)

The dry weight and proportion of organic material was measured for all sediment samples using the loss-on-ignition method [85]. Well-mixed sediment (2.2–8.8 g ww) was dried in crucibles at 105 °C for 20 h, cooled in a desiccator and weighed to calculate the water content. Next, the samples were ignited for 2 h at 550 °C, cooled in a desiccator and weighed to calculate the proportion of organic material. LOI analyses were conducted during one week after sampling. In addition, the water content of sludge was measured similarly.

## 3.8 Dating of sediment cores

To estimate the age of collected sediment layers, the cesium-137 (Cs-137) activity was measured for dried subsamples (2.1–29.9 g dw) by the Geological Survey of Finland, using a gamma spectrometer BrightSpec bMCA-USB pulse height analyser coupled to a well-type NaI(TI) detector. Measurements were first taken for the topmost layers and continued until the Cs-137 level of the individual layer decreased close to zero. The

Chernobyl nuclear power plant accident in 1986 caused a high deposition of Cs-137 in Southern Finland, and it can be noticed in lake sediments as a peak in the Cs-137 activity [86, 87]. The sediment layer representing the year 1986 was located for each sediment core according to a clear increase in Cs-137 activity, and the sedimentation rates were calculated consequently for each sampling site. Subsamples representing the top 1 cm were excluded from the Cs-137 measurements due to the small amount of solid material left for MP analysis. According to the dating, only sediment layers representing years 1990, 2000, 2010 and the sampling year 2018, i.e. the uppermost layer, were included in the study.

## 3.9 Statistical testing and data processing

For wastewater and sludge samples, MP concentrations with standard errors (SE) were calculated for each sampling point according to Equations 3.1 and 3.2. The estimation for the number of discharged MPs via different flows was done according to Equation 3.3, based on the whole sampling campaign.

$$c = \frac{n}{V} \tag{3.1}$$

where *c* is the concentration of microplastics in the studied sample as n/L (water) or n/g dw (sludge), *n* is the detected number of MPs in the sample and *V* is the initial volume in litres (water) or mass in grams of the dry weight (sludge) of the examined sample.

$$SE = \frac{\sqrt{\frac{\sum(x - \bar{x})^2}{n-1}}}{\sqrt{n}} \tag{3.2}$$

where *x* is the observed value, $\bar{x}$ is the mean value of the samples and *n* is the sample size, e.g. the number of samples, for which the MP concentrations are calculated.

$$Daily\ amount\ of\ discharged\ MPs\ = c \times V_d \tag{3.3}$$

where *c* is the average concentration of MPs in the studied flow as n/L (water) or n/g dw (sludge) and $V_d$ is the average daily volume of the studied flow in litres (water) or grams of dry weight (sludge).

In the case of lake water and sediment samples, MP concentrations with SE were calculated for each sampling site and for all sites combined according to Equations 3.1 and 3.2. Statistical tests for MP concentrations in lake water and sediments together with all graphs were created using OriginPro 2018 (OriginLab Corporation). Differences between groups were tested using a 1-way ANOVA. For data sets which did not fulfil demands for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene test), a pairwise comparison with the non-parametric Mann-Whitney test with Bonferroni correction was conducted. When a data set was normally distributed with heterogenic variances, pairwise comparisons with Welch’s test were conducted. The limit for significance was set to 0.05 for all statistical analyses.

For each tested pre-treatment method, the average recovery rates were calculated for each MP type, MPPs, MPFs, and total MPs based on the numbers of recovered MPs according to Equation 3.4.

$$Recovery\ rate = \frac{n}{n_s} \times 100\% \tag{3.4}$$

where $n$ is the number of MPs detected in a treated sample and $n_s$ is number of MPs spiked into the sample.

Maps were created with QGIS 3.12.3 using open data from DIVA-GIS (administrative boundaries), the Finnish Environment Institute (Ranta10) and the National Land Survey of Finland (Ranta10).

# 4 Evaluation of available methodology and interpretation of the results

Throughout this case study, it was obvious that many issues cause challenges both in the examination of MPs from environmental samples and the interpretation of the results obtained. Part of the challenges are probably caused by the rapid development of the research field and the related lack of standardization, while others originate from differences of sample types, selected sampling and pre-treatment methods or limitations of analytical methods. Nevertheless, differences in the sampling, pre-treatment, and analysis of MP samples result in challenges in the comparison of reported results [88]. These issues are discussed separately in the following sections together with the related findings of our case study and their implications in the interpretation of reported results.

## 4.1 An overview of detected microplastics in this case study

In this case study, a wide variety of MPFs and MPPs were detected in different environmental samples (Figure 4.1). The polymeric composition, shapes and sizes of the MPs varied between sample types (Figure 4.2 and Figure 4.3), which also represents the variety of MPs in general. Nevertheless, the composition of the detected MPs was also probably affected by the selected methods from sampling to analysis. These limitations are discussed in detail in Sections 4.2 to 4.6.

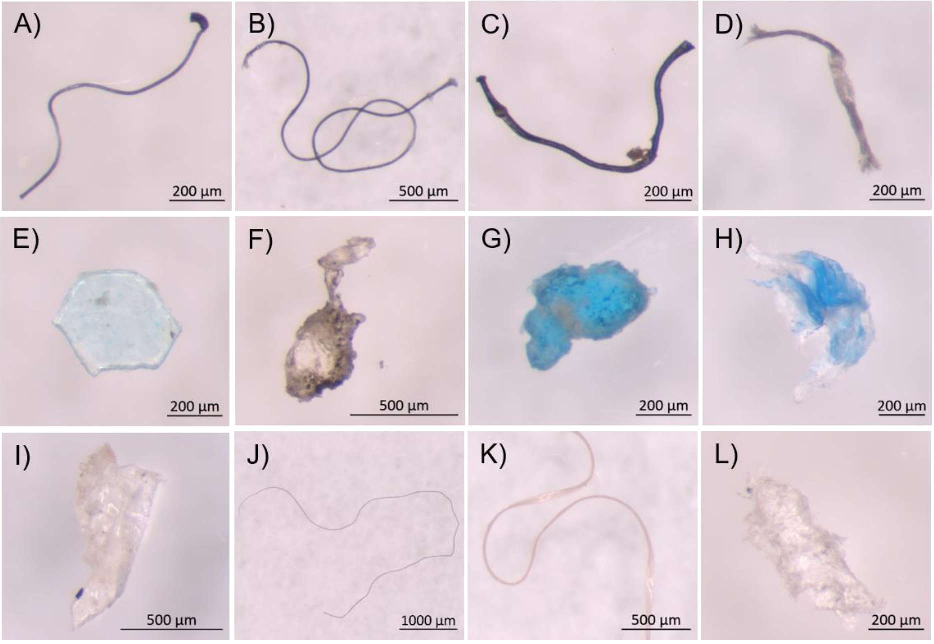

Figure 4.1: Examples of microplastics detected in different stages of the WWTP and the recipient lake. (A–E) PET, (F–I) PE, (J–K) PA and (L) PP [67].

Overall, MPFs were most abundant types of microplastics in the wastewater, sludge, and sediment samples (Figure 4.2 and Figure 4.3A). Correspondingly, in the WWTP-related samples, the majority of the detected MPs were PET fibres, whereas PE was most detected polymer for MPPs. In this study, PET counts also included matches with polyester in the comparison with reference spectra. Additionally, in the sediment of the recipient lake, PET covered the greatest share of the detected MPs (58%) (Figure 4.3B). In the lake water, PP (33%) and PET (29%) were the most detected polymers. These findings are in line with the fact that PET is more likely to be found in the sediment than PE or PP, due to its higher density than water [7].

Throughout this case study, the majority (55–87%) of the detected MPs were smaller than 1 mm in size, and, as expected, the size of the detected MPs was relatively smaller, when the lower size limit was reduced (Figure 4.3C). In the WWTP, 22–48% of the detected MPs were smaller than 500 µm. Relatively largest shares of smallest MPs (<250 µm) were detected in the lake water samples collected during both sampling campaigns.

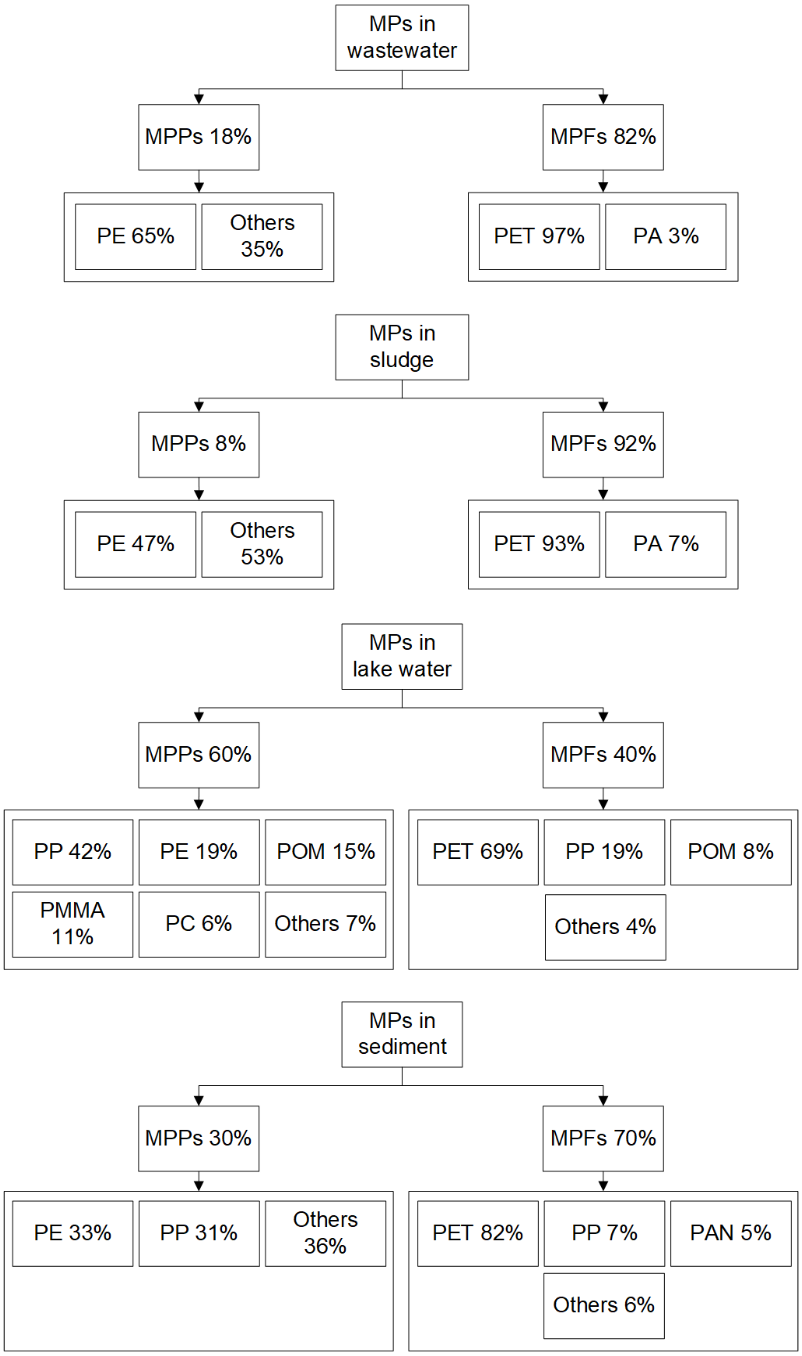

Figure 4.2: Relative shares of polymers detected as microplastic particles and fibres in wastewater (>250 µm), sludge (>100 µm), lake water (>100 µm) and sediment (>100 µm).

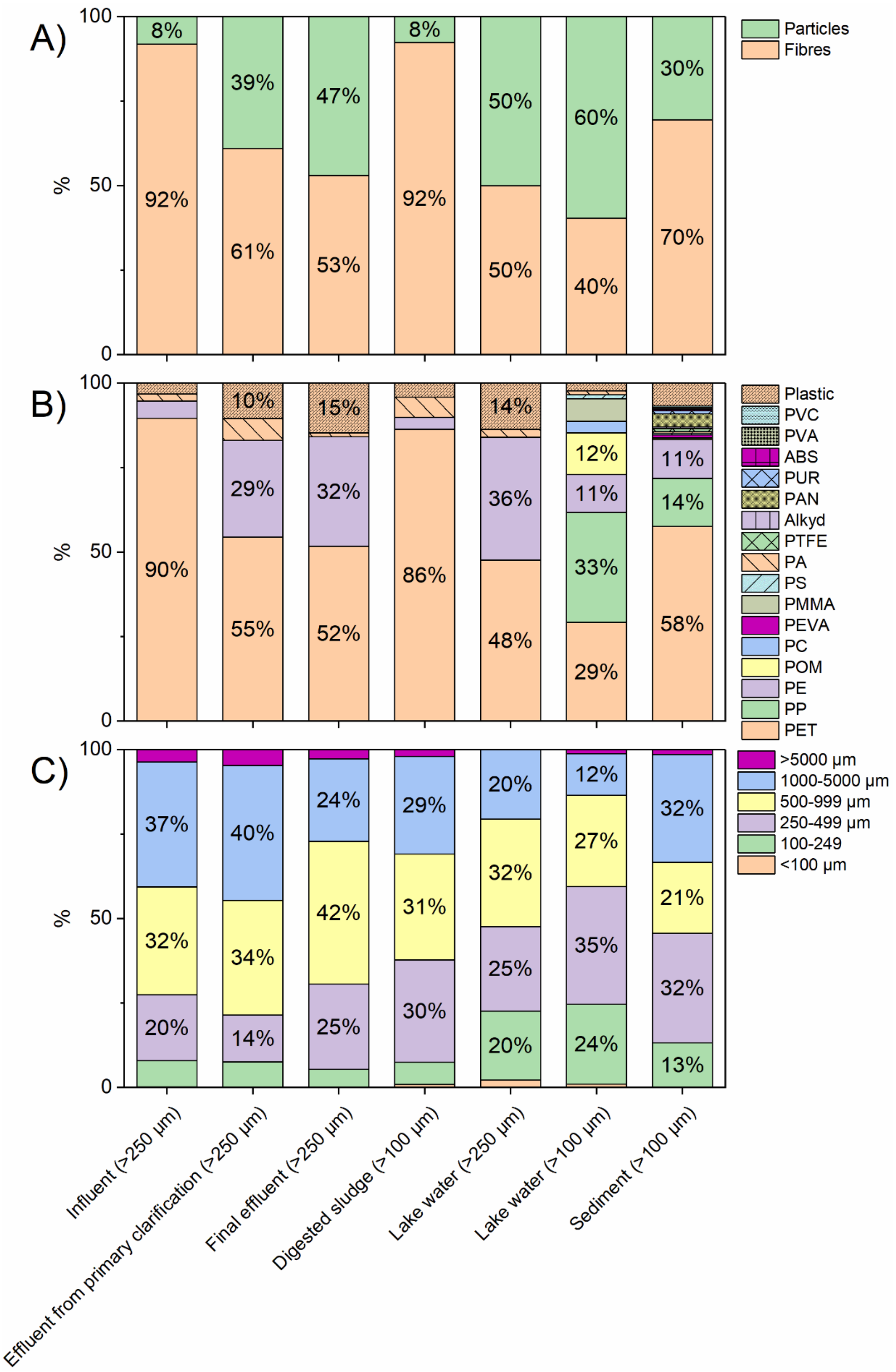
A)
B)
C)
%
100
50
0
8%
92%
39%
61%
47%
53%
8%
92%
50%
50%
60%
40%
30%
70%
Particles
Fibres
10%
15%
14%
90%
29%
55%
32%
52%
86%
36%
48%
12%
11%
33%
29%
11%
14%
58%
Plastic
PVC
PVA
ABS
PUR
PAN
Alkyd
PTFE
PA
PS
PMMA
PEVA
PC
POM
PE
PP
PET
37%
32%
20%
40%
34%
14%
24%
42%
25%
29%
31%
30%
20%
32%
25%
20%
12%
27%
35%
24%
32%
21%
32%
13%
>5000 µm
1000-5000 µm
500-999 µm
250-499 µm
100-249
<100 µm
Influent (>250 µm)
Effluent from primary clarification (>250 µm)
Final effluent (>250 µm)
Digested sludge (>100 µm)
Lake water (>250 µm)
Lake water (>100 µm)
Sediment (>100 µm)

Figure 4.3: Relative portions of detected MPs according to A) shape, B) polymer type, and C) size classes in different points of Kenkäveronniemi WWTP [67] and the recipient lake. The lower size limit used is mentioned separately for each sampling point. In the case of wastewater, sludge and lake water (>250 µm) samples [67] "plastic" refers to groups with representatives of different polymers and in case of lake water and sediment samples (>100 µm), it stands for MPs with high matches to various polymers by their Raman spectra.

## 4.2 Sampling methods

The selected sampling method has a significant influence on the properties of the collected MPs and on the reported MP concentrations. Overall, multiple sampling techniques have been utilized, each of them causing varying limitations on the sizes of detected MPs together with the different units resulting from the sampling method [89]. For examining MPs in different types of water bodies, neuston nets, such as a Manta trawl [5, 11, 12, 90–92], pumping with or without on-site filtration [11, 24] and other bulk samplers, such as Niskin bottles [16], have been used. The Manta trawl usually sets the lower size limit of collected MPs to 300–333 µm, while pumps and other bulk samplers allow MPs of basically any size to be collected.

The selected sampling method and following size limitations play a key role in the interpretation of the results. According to previous studies, reducing the lower size limit for collected MPs causes an exponential increase in the detected concentrations of MPs in environmental samples [11, 13, 93–98]. For example in the study by Uurasjärvi et al. MPs were detected from lake water in over 600-fold concentrations when the lower size limit was reduced from 333 µm (0.27 MP/m$^3$) to 20 µm (168.8 MP/m$^3$) [11]. Another example of this phenomenon is presented for the MP concentrations in the CAS effluents in Figure 4.4, where the increase in reported MP concentrations is obvious when the lower size limit is reduced. In this case study, MPs in the lake water samples had roughly the same relative abundancies for different size classes, no matter whether the lower size limit was set to 100 or 250 µm (Figure 4.3C). This might be caused due to the identification method, which still depended on manual and optical sorting and the relatively small difference in the used aperture sizes.

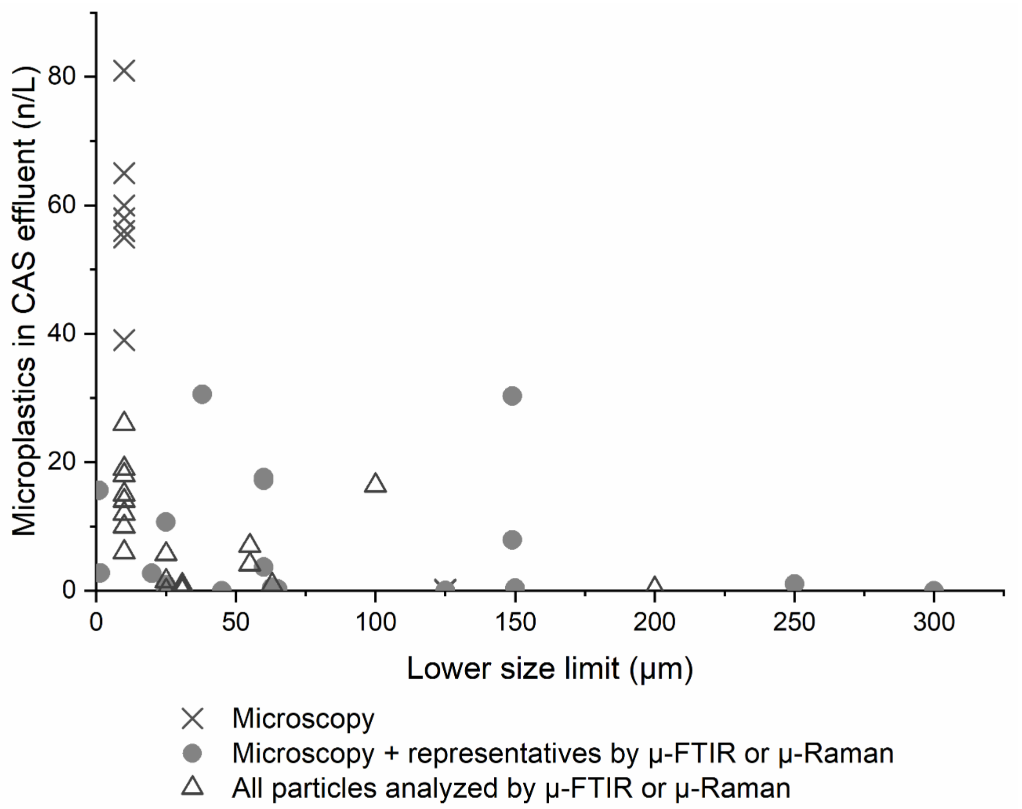

Figure 4.4: Previously reported MP concentrations in CAS effluents (n/L) in relation to the lower size limit according to identification practices [18, 39, 43, 46–48, 50, 52–54, 67, 74, 94, 99–112].

In relation to the used mesh aperture, various shapes of MPs need specific attention. Due to the elongated shape of the fibres, they might pass through meshes even though their longest dimension would be larger than the aperture. Although MPFs are often found to be the most abundant types of MPs in aquatic environments [18, 38] and wastewater [18, 24, 42], their recovery is less efficient, when large meshes are used. This was confirmed in this case study, as later discussed in the comparison of pre-treatment methods shown in Table 4.2. Therefore, even though the predominance of MPFs over MPPs has also been reported for other WWTPs [18, 24, 50, 51, 74, 94, 100, 113–115], the reported results are still likely to underestimate the abundance of MPFs. Furthermore, even though the wastewater samples in this case study were filtered on a 250-µm mesh, i.e. with an aperture much larger than the common diameter of MPFs, and the recovery of MPFs with the used pre-treatment method was only 68%, fibres still covered over 90% of the detected MPs in the influent samples (Figure 4.3A). This highlights the importance of including fibres in the microplastics studies.

In addition, no matter what the aperture is, the mesh can start to clog due to retained material, which will enhance the collection of particles smaller than the aperture. Clogging can also limit the maximum volume of the collected sample. Therefore, when the lower size limit is reduced, also the volume of the collected sample must usually be reduced. On the other hand, MPs with smaller dimensions than the mesh size can also be collected, if they are entangled with larger particles during sieving [116]. This was recognized also in this case study, when MPs smaller than the used mesh size (250 µm) were detected in wastewater samples (Figure 4.3C). As a consequence, the number of detected and reported MPs always reflects only the retained fraction of MPs, not the exact number of MPs larger than the aperture [90, 117]. Hence, the amount of small MPPs (<250 µm) and varying sized MPFs would probably have been much higher in this case study, if a smaller aperture had been used [74, 118].

Another issue varying within the past MP studies utilizing different sampling techniques, are the units in the reported results. Usually, MP concentrations are reported per volume or mass of the sample matrix. On the other hand, results for water samples collected with a neuston net or beach sediment samples have also been reported as MPs per area [119]. Therefore, sufficient details of the sampling should be reported along with the MP concentrations to allow the results to be converted to different units for comparison, when necessary. In practice, that would mean also providing the sampled depth of the water with a neuston net, depth of the included beach sediment layer and water content of the sediment, if concentrations are calculated per wet weight. Likewise, due to the layered structure of bottom sediments, the examined depth of the sediment core should be provided with possible information about accumulation rates to estimate the age of the sample.

## 4.3 Pre-treatment methods

After the samples have been collected, they usually need to be purified from non-plastic materials before the quantification of possible microplastics. The aim of the pre-treatment stage is to separate microplastics so that they can be identified and counted reliably with the selected size limitations. Purification can be based on the degradation of disrupting materials, such as organic material, or separation of microplastics from other materials, such as dense mineral particles. In addition to purification steps, pre-treatment can include drying of the sample, which can be necessary to relate the number of MPs to the dry weight of the sample or to allow higher efficiency of the chemical treatments.

Likewise as with sampling techniques, numerous pre-treatment methods have been used to remove non-plastic material from samples [7, 120, 121]. The main problem is that selected chemical treatments need to be efficient enough to remove the majority of the distractive material from the samples, but gentle enough not to attack MPs. Due to the varying chemical compositions of MPs, also their resistance to different pre-treatments can vary. Especially PA is more sensitive to acidic and oxidative treatments than other commonly used polymers [122, 123]. In this case study, no visible effects were noticed concerning PA fibres during treatments with hydrogen peroxide at 10–15% dilution, 10% KOH solution, canola oil or potassium formate solution. To study the possible effects of chemical treatments on different polymers, analyses utilising scanning electron microscopy (SEM) could provide useful information about the changes on the surface of MPs [124, 125]. In addition, if the MP concentrations are based on the number of particles and fibres, the pre-treatment steps should not cause any fragmentation of the plastics due to weathering or mechanical abrasion [126].

The advantages and disadvantages observed for different pre-treatment methods during this study are listed in Table 4.1. It needs to be highlighted that some limitations might not be valid for those sample matrices which were not included in our study. For example, cellulose fibres were problematic in the WWTP-related samples, but they are not that abundant in other types of environmental samples. Overall, both sampling and pre-treatment should always be optimized according to the sample type and the aim of the study [127].

The methods used in this study were selected according to their reported use at the time when experiments were conducted, and according to their advantages compared to other methods. Because the field of MP research is constantly developing, some methods, such as WPO conducted with additional heating, are no longer recommended.

Table 4.1: Advantages and disadvantages for different pre-treatment methods observed during this study.

| **Treatment method** | **Tested sample types** | **Advantages** | **Disadvantages** |
|---|---|---|---|
| Filtration with detachable filters | Wastewater<br>Sludge | + Possible to treat samples immediately after collection<br>+ Simple, chemical-free procedure<br>+ No visible deformations on MPs<br>+ Size limits easy to change | - Cellulose fibres not removed<br>- Possible loss of MPs during transfer of the filters<br>- Small MPs might pass the sieve while drying; difficult to examine both side of filters<br>- Possible contamination from mesh (PA) and device (PVC)<br>- Chemical treatments necessary if small MPs are studied or automated identification conducted |
| Enzymatic treatment (UEPP) | Lake water | + No toxic chemicals<br>+ No visible deformations on MPs | - Time-demanding, multi-step procedure<br>- Incomplete degradation of some natural materials, e.g. diatom shells |
| Wet peroxide oxidation (WPO) | Wastewater<br>Sludge | + Rapid procedure | - Cellulose fibres only slightly degraded<br>- Harmful chemicals<br>- Aggressive foaming might cause loss of MPs<br>- Heat can alter the appearance of MPs; water bath is recommended<br>- PS beads had shinier surfaces after WPO |
| Degradation with $H_2O_2$ | Lake water<br>Sediment | + No toxic chemicals | - Aggressive foaming might cause loss of MPs |
| Degradation with KOH | Wastewater<br>Sludge | + Simple procedure<br>+ No visible deformations on MPs | - Cellulose fibres and other organic material not degraded<br>- Attached organic material may affect identification of MPs<br>- High chemical consumption |

Table 4.1: (continued)

| **Treatment method** | **Tested sample types** | **Advantages** | **Disadvantages** |
|---|---|---|---|
| Oil extraction procedure (OEP) | Wastewater<br>Sludge | + Canola oil cheap and harmless<br>+ No visible deformations on MPs | - Multistep procedure<br>- Pre-sieving is needed if sample contains large fragments and separation funnel is used<br>- Oil residuals might interfere identification<br>- Cellulose fibres not removed<br>- MPs may attach glass surfaces<br>- Toxic chemicals (methanol)<br>- High static electricity of MPs (especially PE and PS), possibly due to reagent alcohol |
| Density separation | Sediment | + Allows high removal of dense materials<br>+ Many options for separation solutions<br>+ Simple procedure without toxic chemicals (NaCl, $Na_2WO_4$, $KHCO_2$)<br>+ No visible deformations on MPs with $Na_2WO_4$ | - Denser polymers are excluded, if light solutions (water, NaCl) are used<br>- Different densities challenge comparison of results<br>- Cellulose fibres (1.5 g/cm$^3$) not removed with solutions with same or higher density, e.g. $KHCO_2$, $ZnCl_2$<br>- Pre-sieving is needed if sample contains large fragments and separation funnel is used<br>- Attached organic material may affect identification of MPs<br>- Some of the solutions (e.g. $ZnCl_2$) are harmful<br>- High chemical consumption |
| Drying | Sludge | + Simple, chemical-free procedure | - Examination subjective and time-consuming<br>- MPs may attach on the walls of the petri dish during drying<br>- May cause changes in the colours of MPs<br>- Not suitable for matrices with low MP concentrations<br>- Not suitable for automated identification |

Generally, organic material is often degraded by oxidation with hydrogen peroxide in multiple variations [62, 70, 71]. Even though treatments with hydrogen peroxide have been very commonly used in MP studies, not all organic material is easily degraded with these treatments, as was noticed during this case study. Especially cellulose fibres were not fully degraded from the wastewater samples with WPO within the reaction time of 30 to 50 min and with the selected volumes of hydrogen peroxide (20–60 mL) in relation to the dry weigh of the samples (≤0.25 g). Increasing the reaction time or volume of the used $H_2O_2$ or Fe(II) solution could also increase the degradation of organic material. In addition, during the aggressive reaction caused by hydrogen peroxide, temperatures over 60 °C should be avoided to prevent MPs from melting, if the appearance of the MPs is to be examined [128]. In addition, possible losses of MPs due to aggressive foaming should be prevented by covering samples loosely during the treatment, and by rinsing the cover into the sample after the treatment.

Organic material can also be degraded by enzymatic treatments [44, 70], as was done with the lake water samples in this case study. With enzymatic treatments, samples are not treated with harsh chemical treatments, which could affect the appearance of MPs. As a drawback, enzymatic degradation is a time-demanding process, which also requires high precautions due to the multiple filtration steps.

Samples with a higher load of mineral material, for instance sediment samples, are commonly treated via density separation, where denser material is separated and polymers lighter than the solution are retained for examination with the supernatant. Challenges arise when studies are conducted with different separation solutions with varying densities. The separation of other materials is more efficient when lighter solutions are used, but, on the other hand, denser polymers, such as PET, can be excluded from the examination. Common density separation solutions vary from water (1.0 g/cm$^3$) [25] and sodium chloride (NaCl, 1.2 g/cm$^3$) [129] to sodium polytungstate (2.1 g/cm$^3$) [130]. In addition, the time of settling varies widely from just 10 minutes [63] to multiple days [114, 131]. Because both the density and the shape of the particles affect their settling velocity [60], the settling time may play a crucial role in the extraction of MPs by density separation. Furthermore, centrifugation can be utilized to improve the separation of denser material [132]. One more varying practice is related to the amount of supernatant that is included in the analysis of MPs. Some researchers have included the whole supernatant [102, 103, 131], while some have only collected the uppermost part of it [133].

During this case study, density separation was tested with sediment samples by using sodium tungstate in a density of 1.4 g/cm$^3$. It needs to be pointed out that the studied sediments were treated with $H_2O_2$ before density separation. Because possibly attached organic material was already degraded, this approach probably allowed better separation of denser mineral material. In addition, the studied sediments consisted of very fine fragments, which allowed thorough mixing of the sample with the density separation solution as well as the use of density separation funnel with a small outlet. In addition, the settled sediment material was discarded after two hours, after which the whole supernatant was filtered. If the settling would have lasted longer, the supernatant could have been clearer from disruptive, non-plastic particles. Furthermore, the selection of the density separation solution has a large impact not only on the included polymers, but also on the excluded non-plastic materials. For example, cellulose fibres have a density around 1.5 g/cm$^3$, and they would not be removed from wastewater samples with $ZnCl_2$, which is often used in densities greater or equal to 1.5 g/cm$^3$ [129, 133].

Compared to the harmful and expensive chemicals often used in density separation, the advantages of oil-extraction procedure lie in the use of mainly non-toxic and inexpensive chemicals. As a drawback, cellulose fibres can also attach to the oil layer, which limits the suitability of OEP for WWTP-related samples. It might still be a suitable method for separating MPs from other types of samples. For example, Scopetani et al. adjusted this method for soil samples by freezing the samples before separating the MPs attached to the oil layer [134].

Thus, different pre-treatment methods can either leave different amounts of interpreting material on the sample, exclude part of the MPs, or chemically or physically attack MPs, distorting the results and challenging the comparison of differently treated samples. In addition, one pre-treatment step is rarely able to remove all distractive materials from the sample, and, thus, the pre-treatment procedure for certain samples often consists of a couple of steps. For example, density separation does not remove organic material from the samples, and, thus, it is often combined with other pre-treatment steps degrading the light-weighed non-plastic material.

Regardless of the selected methodology, its suitability should be assessed according to the recovery of various MPs. However, recovery testing should be conducted with MPs representing different shapes, sizes, and densities, to make sure that the selected sampling, pre-treatment and characterization methods allow the collection and detection of environmentally relevant MPs. In addition, it should be noted that in the case of MPs, the recovery rates depend highly on the used mesh sizes and the recovery of larger MPPs tends to be higher than the recovery of smaller MPPs or MPFs. This was also noticed both in our comparison study as well as in the environmental study, where recoveries were lower for MPFs than for MPPs (Table 4.2). The higher exclusion of MPFs was clear when different pre-treatment methods were compared using a lower size limit of 250 µm. While larger MPPs (230–3400 µm) had recovery rates of 96–99%, the recovery of MPFs varied from 41 to 79% (Table 4.2). Furthermore, when the lower size limit was reduced from 250 µm to 20–25 µm in the case of filtration and WPO, the total recovery rates were overall slightly higher. Altogether, 0–19% of spiked MPs were detected in size fractions 25–250 µm (filtration) or 20–250 µm (WPO). The use of 20 and 25-µm meshes especially allowed the collection of fibres with diameters of around 30 µm. As a drawback, the addition of one or two filtration steps to each method increased the time consumption due to the increased number of subsamples. In addition, shorter and flexible PET fibres seemed to be excluded more easily than longer and inflexible PA and PP fibres with the tested pre-treatment methods. Of the model fibres used in this case study, PET fibres were similar to the fibres which are likely to be released from textiles and, thus, which would also be likely to enter the environment.

Table 4.2: Recoveries of MPPs, MPFs and total MPs for used and tested pre-treatment methods. RB=Staining with Rose Bengal. Lower size limits are listed according to physical limitations during pre-treatment steps.

| **Pre-treatment method** | **Sample type** | **Adjustment** | **n** | **Number of spiked MPs** | **Lower size limit (μm)** | **Recovery (range) (%)** | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | **MPPs** | **MPFs** | **MPs** |
| UEPP | Lake water | | 3 | 80 | 20 | 97 (93–100) | 85 (80–95) | 91 (86–98) |
| $H_2O_2$ + density separation with $Na_2WO_4$ | Sediment | | 3 | 140 | 63 | 99 (97–100) | 84 (80–88) | 95 (94–96) |
| Filtration | Wastewater | | 3 | 70 | 250 | 98 (95–100) | 64 (57–73) | 83 (79–87) |
| | | | 3 | 70 | 25 | 98 (98) | 83 (77–90) | 91 (89–94) |
| | | RB | 3 | 70 | 25 | 98 (98–100) | 82 (70–93) | 91 (86–97) |
| | Sludge | | 3 | 70 | 250 | 96 (95–98) | 41 (33–53) | 72 (70–77) |
| | | | 3 | 70 | 25 | 100 (100) | 83 (80–90) | 93 (91–96) |
| | | RB | 3 | 70 | 25 | 98 (95–100) | 80 (67–87) | 91 (83–94) |
| WPO | Wastewater | At 75 °C | 3 | 70 | 250 | 98 (98–100) | 68 (60–73) | 85 (81–89) |
| | | At 50 °C | 3 | 70 | 250 | 98 (98–100) | 71 (67–80) | 87 (84–91) |
| | | At 50 °C | 3 | 70 | 20 | 98 (98–100) | 73 (67–80) | 88 (84–91) |
| | | At 50 °C +RB | 3 | 70 | 20 | 100 (100) | 78 (77–80) | 91 (90–91) |
| | Sludge | At 75 °C | 3 | 70 | 250 | 98 (98–100) | 79 (77–80) | 90 (89–91) |
| | | At 50 °C[b] | 3 | 70 | 250 | 99 (98–100) | 76 (71–83) | 89 (86–93) |
| | | At 50 °C[b] | 3 | 70 | 20 | 99 (98–100) | 80 (73–90) | 91 (89–96) |
| | | At 50 °C + RB | 3 | 70 | 20 | 99 (98–100) | 76 (67–93) | 89 (84–97) |
| KOH | Wastewater | | 3 | 70 | 250 | 98 (95–100) | 64 (60–67) | 83 (83–84) |
| | Sludge | | 3 | 70 | 0 | 98 (98–100) | 59 (53–63) | 81 (80–83) |
| OEP | Wastewater | | 2 | 70 | 250 | 99 (73–100) | 63 (43–67) | 84 (60–86) |
| | Sludge | | 3 | 70 | 0 | 88 (78–95) | 77 (73–83) | 83 (76–90) |
| Drying | Sludge | Without sieving[b] | 3 | 70 | 0 | 100 (100) | 66 (50–74) | 85 (79–89) |
| | | With sieving[b] | 3 | 70 | 20 | 98 (95–100) | 68 (57–80) | 85 (79–91) |

[a] One replicate had nine PA fibres.
[b] One replicate had 11 fibres of PA or PP.

During the pre-treatment of lake water and sediment samples of this case study, a total of 91 and 95% of the spiked MPs were recovered with the selected procedures, respectively (Table 4.2). It needs to be noted that the reported recovery rates are always valid only for the MPs with similar kinds of properties with the MPs used in recovery testing. Therefore, smaller MPs were probably recovered to a smaller extent from the WWTP-related samples as well as from the environmental samples, because they could have passed through the meshes more easily or more probably ignored during visual examination. Hence, the reported MP concentrations were not recalculated according to these recovery rates, which probably causes an underestimation of the reported MP levels.

In conclusion, even though a wide range of pre-treatment methods are currently used and most probably will also be used in the future, it would be important to standardize the means of evaluation of the used methods. If the polymer types, shapes, and sizes of spiked MPs were consistent throughout the research field, differences caused by the various pre-treatment methods could be better evaluated and the results gained by implementing those methods could be compared according to the results from the recovery testing. This should be further supported by a more heterogeneous selection of commercial model MPs, which should also include a selection of MPFs.

## 4.4 Identification of microplastics

Different techniques have been utilized for recognizing MPs from environmental samples and identifying their polymer types. The identification and classification of microplastics is based on their chemical composition, size, shape, and colour. Visual characterization provides information about the appearance of particles without confirmation of their chemical composition. In turn, chemical characterization methods are used to confirm the polymeric composition of the examined particles. These methods are divided into non-destructive spectroscopic techniques, including different variations of FTIR and Raman spectroscopies, and thermal techniques such as py-GC/MS, which are based on the degradation of chemical structure and the following detection of the degradation products. The advantages and disadvantages of different identification techniques are discussed in the following sections, and they are also summarized in Table 4.3.

Table 4.3: Commonly used identification methods with their advantages and disadvantages based on the observations from this case study and the literature [11, 26, 76, 79, 83, 98, 120, 130, 135–138].

| **Identification technique** | **Method** | **Information about** | | | | **Advantages** | **Disadvantages** |
|---|---|---|---|---|---|---|---|
| | | **chemical composition** | **size** | **shape** | **colour** | | |
| Visual | Microscopy | | x | x | x | + Non-destructive | - Not suitable for small MPs (<100–500 µm)<br>- Subjective |
| Spectroscopic | FTIR micro-spectroscopy | x | x | x | x | + Non-destructive<br>+ Suitable for particles >10 µm | - Identification of black tire-wear particles is challenging<br>- Humidity and $CO_2$ cause interferences |
| | Raman micro-spectroscopy | x | x | x | x | + Non-destructive<br>+ Suitable for samples with any thickness<br>+ Suitable for particles >1 µm | - Fluorescence<br>- Identification of black tire-wear particles is challenging<br>- Dyes can overwrite the spectrum of the polymer<br>- Analysis of the surface; attached biofilm of other material can distort identification |
| Thermo-analytical | py-GC/MS | x | | | | + Mass-based concentrations<br>+ No need for manual or visual selection of MPs | - Natural organic material can cause interferences and overestimation of the polymer<br>- Destructive |

So far, the most used method is visual examination by microscopy. One benefit of microscopic examination of MPs is the information about their colours. The eco(toxico)logical background for recording the colours of MPs is the similarity of specifically coloured MPs with the natural prey of different animals [139]. However, the classification of colours has also been criticized due to their possible bleaching during harsh chemical treatments [8] and the subjectivity in their classification. Hence, the comparison of colour distributions can be misleading, and they were not discussed during this case study.

In this study, some natural materials were retained on the filters along with collected MPs after all the conducted pre-treatment steps, which made the visual separation between MPs and natural material challenging. In addition, the abundance of transparent MPs was probably underestimated, because they were ignored more probably than brighter colours during microscopic examinations.

To improve the visual detection of microplastics, staining has been suggested as one option to distinguish MPs from natural materials [12, 74]. The most commonly used stains include Rose Bengal and Nile Red, which stain other materials than plastics or particularly attack plastics, respectively. However, in this case study, Rose Bengal did not stain cellulose fibres in WWTP-related samples. Hence, microplastics, especially yellow PET fibres which are easily confused with cellulose fibres, were not identified more efficiently. The average recovery rates of PET fibres were not higher in stained (50–67%) than in non-stained (30–90%) samples, when lower size limit was set to 20–25 µm (Table 4.2). In contrast, the colour of some PS and PVC fragments were slightly modified due to staining, especially after WPO treatment. These findings were inconsistent with a previous study, where Rose Bengal was reported to be suitable for PS [74]. Therefore, staining could be a helpful method for pristine plastics, but it might have limitations with weathered MPs.

Another option for confirming the plastic nature of particles and fibres is a hot needle test [140, 141], which, however, is not suitable for small MPs due to the challenges in their manual handling. Still, none of these visual methods offer any information about the polymeric type of MPs.

As presented in Figure 4.4, previously reported MP concentrations for CAS effluents were higher when their identification was based on microscopy alone compared to the studies, where at least part of the MPs were confirmed using spectroscopic methods. Thus, conducting only the optical classification with a microscope poses a high risk of not only a false negative, but also a false positive identification of MPs. Therefore, the polymeric composition of the MPs should always be confirmed with other characterization methods, such as FTIR, Raman, or thermo-analytical techniques. Nevertheless, only 28 and 14% of MPs studies published in 2015–2017 utilized either FTIR or Raman in confirming the plastic nature of suspected MPs, respectively [75]. In a couple of recent studies conducted of WWTPs, only 32 [48] and 77% [142] of suspected

MPs were confirmed to be plastics by FTIR, which highlights the possibility of overestimating of MP concentrations without confirmation of the polymer type.

Unfortunately, spectroscopic methods also have limitations. The spectra from FTIR or Raman analyses for environmental MPs rarely provide high matches with the reference libraries, which can be caused by the remaining organic material, different additives used in the plastics [79] or by changes in the surfaces of the MPs due to weathering or thermochemical pre-treatments. These challenges especially concern Raman spectroscopy, because it characterizes materials based on the chemical structure of the surface. In this case study, it was not possible to define the exact polymer of some MPs collected from lake water and sediment by their Raman spectra. In some cases, all suggested matches were various polymers, even though the peaks of the detected spectrum did not match obviously with any of them. Hence, those particles were counted as MPs and marked only as "plastics" without a clear definition of their polymer type. One reason for this might have been the existing variety of copolymers. If the comparison with spectral libraries does not recognize the copolymeric nature of the MPs and matches the MP with only one of the monomeric structures of the copolymer, there might be uncertainties in the concentrations of MPs consisting of different polymers. In some cases during this case study, the correlation of the unknown spectrum with the reference polymer was weak, but specific peaks for the certain polymers were obvious and, thus, the MP was identified accordingly. Poor matches with reference spectra can also be caused by the comparison of weathered, environmental MPs with the pristine polymers used for creating spectral libraries [143]. In some studies, the lower limit for acceptance in library searches has been set at 60–80% [27, 52, 103, 140]. This kind of threshold is necessary when an automated library search is needed, but it can also cause exclusion of MPs which do not provide a good quality spectrum during the chemical analysis. Hence, the results gained with limited match percentages can lead to underestimations of the MP levels.

Furthermore, the identification of some polymers, such as PA or SBR, might be challenging with spectroscopic techniques [11]. In this case study, some particles and fibres were excluded from the results, because their Raman spectra did not distinguish PA from natural materials. In addition, no tire-wear particles made of SBR were identified using FTIR or Raman techniques in any of our samples, even though car tires are considered to be one of the largest sources of MPs [35]. Their absence can be explained by the light absorbing nature of black rubber [11, 135], which hinders their identification with spectroscopic methods. Other reasons could be their small size [11] together with their high density [26], which may have excluded tire-wear particles from our examinations.

Moreover, because Raman scattering is usually very weak, other types of scattering easily overwrite it. Scattering interference can be caused by the dyes used in plastics or by fluorescence, which leaves material unidentified when applying Raman spectroscopy [26, 76, 130, 135]. Fluorescence can be diminished by using wavelengths over 700 nm. These problems were encountered for the MPs collected from the WWTP and lake as well as for the colourful MPFs used in the recovery testing of wastewater and sludge samples. In addition, only the dye was recorded by Raman spectroscopy for 17 and 13% of all analysed particles and fibres from the lake water and sediment, respectively. Thus, those particles and fibres may have been plastics, but they could not be identified with the selected identification method.

In the case of FTIR, one limitation in the identification of microplastics is caused by the used wavelength itself. The use of IR radiation presenting wavelengths from 750 nm to 1 mm limits the spatial resolution to 10 µm [136, 137]. Hence, FTIR microscopy cannot be utilized to characterize the smallest microplastics, whereas Raman microscopy is able to identify MPs down to 1 µm in size due to the shorter wavelength used in the excitation laser [98, 137]. This is not only relevant to the smallest MPs, but also to fibres, which often have their diameter around the spatial resolution limits of FTIR microscopy. According to this study, MPFs accounted for 40–92% of the detected MPs, depending on the sample type and size limitations (Figure 4.3A). Hence, it is important to select identification methods that truly allow the identification of MPs throughout the size range the study aims to focus on. An FTIR microscope can also be equipped with an FPA detector, which allows the detection of smaller MPs with a pixel resolution of 5.5 µm [27].

In the WWTP-related part of this case study, representatives of all visually classified and grouped particles and fibres were selected for Raman (1.4%) and FTIR analysis (1.3%). According to our microscopic and spectroscopic analysis, some natural and plastic fibres had similar kinds of appearance. Therefore, the whole group with similar appearance was excluded from the reported results. Unfortunately, this approach included a risk of both false positive and false negative MP counts. In addition, specific polymers might have been over- and underestimated, if they shared a similar appearance with other polymers, because all possible MPs were classified according to their appearance before any analysis. Hence, even though the results based on identification of representatives alone would offer accurate MP concentrations in general, the relative shares of polymers might be distorted. This was later considered for the environmental samples collected in 2018–2019, where all suspected MPs were characterized with Raman spectroscopy.

In addition, thermo-analytical techniques, including py-GC/MS and thermogravimetric analysis with differential scanning calorimetry (TGA-DSC), have been utilized for analysis of MPs to a smaller extent. Unfortunately, thermo-analytical techniques might have problems detecting a wide range of polymers. For example, TGA-DSC has been reported to be suitable only for PE and PP [144], while py-GC/MS has been found to work without interference at least for PE, PS and PP [83]. Furthermore, residuals of natural organic materials can cause interference in the quantification of different polymers using py-GC/MS as pyrolysates may be mixed with synthetic polymers. In the case of PE, interference can originate from natural long aliphatic chains such as fats or waxes [83, 138], while chitin, wool and wood can cause interference in the detection of PS and PVC [138]. Furthermore, because thermo-analytical techniques are destructive methods, they do not offer any information about the size, shape or colour of the detected plastics [120].

Another challenge arises from the different units of reported MP concentrations based on the selected identification technique. MP concentrations resulting from microscopic and spectroscopic analysis are usually reported as particulate counts per volume or mass of a sample. However, thermo-analytical techniques offer results as mass concentrations. Different types of concentrations cannot be directly compared, but both are useful in relating the abundance of MPs to their ecotoxicological risks. Nevertheless, if the volume of MPs detected using spectroscopic techniques could be measured reliably and connected to the density of the detected polymer, spectroscopic techniques could also offer mass concentrations. That would bring these techniques closer to each other and allow comparison of the results gained with different kinds of techniques.

In the lake water samples of this case study, target polymers (PE, PS and PP) were detected with py-GC/MS in concentrations higher than the LOD in five samples (Table 4.4). Detected concentrations varied between 7.9 and 39.8 µg/L for PE, 2.5 and 16.3 µg/L for PS and 246.9 µg/L for PP. All three polymers were found in sample 3S1, for which MPs consisting of those same polymers were also detected via Raman micro-spectroscopy.

Some of the before-mentioned limitations with Raman micro-spectroscopy and py-GC/MS were also noticed in this case study, when the results gained with these two identification techniques were compared. Some target polymers were only detected with either one of the techniques (Table 4.4). It needs to be pointed out that differently sized MPs were included in different chemical analyses. For Raman analysis, MPs smaller than 100 µm were excluded due to visual and manual selection, whereas all particles and fibres retained on the filter after 20-µm filtration were included in the py-GC/MS analysis. Hence, samples such as 2B1 and 3S2 might have contained multiple small PS particles or fibres, which were excluded from the visual selection of potential MPs with Raman micro-spectroscopy. The mass of very few microplastics in the samples might also been so small that they did not cause concentrations higher than the LODs for the py-GC/MS method. Another explanation for the differences could be the similar appearance of MPs with natural organic material remaining on the filter, which could have caused them to be excluded from the Raman analysis. PP was detected in most lake water samples by Raman micro-spectroscopy. Nevertheless, py-GC/MS had a low detection rate for PP, which may be explained by the higher LOD compared to PE and PS. Due to the before-mentioned overwriting of polymeric spectrum by dyes or fluorescence, some PE, PP or PS particles or fibres may have been excluded from the Raman-based MP counts, but later identified with py-GC/MS, which does not suffer from similar interference. On the other hand, improperly removed organic material might have caused interference in the quantification of target polymers, especially PE and PS, and, hence, may have led to the overestimation of those polymers using py-GC/MS. This is further supported because none of the polymers were detected using py-GC/MS in blank samples which did not contain any natural organic material, but which contained PE and PP according to a Raman analysis.

Hence, the mass concentrations caused by the detected particles and fibres consisting of PE and PP did not exceed LODs of 6.1 µg/L and 7.9 µg/L, respectively.

Table 4.4: Concentrations of PE, PS and PP in lake water based on their detection with Raman micro-spectroscopy (n/L, >100 µm) and py-GC/MS (µg/L, >20 µm). - = below limit of detection

| **Site** | **Depth (m)** | **Sample** | **Polymers detected with Raman (n/L)** | | | **Polymers detected with py-GC/MS (µg/L)** | | |
|---|---|---|---|---|---|---|---|---|
| | | | **PE** | **PS** | **PP** | **PE** | **PS** | **PP** |
| 1 | 0.5 | 1S1 | 0.1 | - | 0.3 | - | - | - |
| | | 1S2 | - | - | 0.8 | - | - | - |
| | | 1S3 | - | - | 0.1 | - | - | - |
| 2 | 0.5 | 2S1 | 0.1 | - | 0.3 | - | - | - |
| | | 2S2 | - | - | - | - | - | - |
| | | 2S3 | 0.2 | - | 0.1 | - | - | - |
| | 5.0 | 2B1 | 0.2 | - | 0.2 | - | 6.0 | - |
| | | 2B2 | - | - | 0.1 | 39.8 | - | - |
| | | 2B3 | 0.1 | - | 0.1 | - | - | - |
| 3 | 0.5 | 3S1 | 0.1 | 0.1 | 0.2 | 23.5 | 16.3 | 246.9 |
| | | 3S2 | 0.1 | - | 0.6 | - | 2.5 | - |
| | | 3S3 | 0.1 | - | 0.1 | 7.9 | - | - |
| | | | **(n/sample)** | | | **(µg/L)** | | |
| | | BLANK1 | 1 | - | 6 | - | - | - |
| | | BLANK2 | - | - | 11 | - | - | - |
| | | BLANK3 | 1 | - | 2 | - | - | - |
| | | BLANK4 | 2 | - | 7 | - | - | - |

Based on these observations, it would be crucial to assess the recovery rates of various MPs not only by the selected pre-treatment methods, but also by the exact same identification protocol that is used for actual samples. In addition, the use of real environmental samples as a sample matrix would allow the assessment of possible errors caused by distracting materials.

## 4.5 Contamination control

Contamination is another aspect to be highlighted and considered both during the sampling, pre-treatment, and identification, as well as when reported results are interpreted. Nevertheless, different practices are followed for the interpretation of the results related with the contamination control samples. Some researchers have subtracted detected contamination from their results [17, 48, 97, 108], some have reported detected contamination without subtracting it from the results [15, 45, 145], while some have not reported any contamination measurements at all [14, 63].

Despite the efforts to avoid contamination in this case study, described in Section 3.6, some MPs were detected from the blank samples and the aerial contamination samples related to all sample types (Figure 4.5). Because field and procedural blanks were mostly conducted by rinsing the sampling equipment with distilled or ultrapure water in a volume smaller than the actual samples, detected contamination is presented as the number of MPs per blank sample and these cannot be directly related to the detected MP concentrations in environmental samples (Table 4.5). In the case of aerial contamination, detected MPs were counted per one aerial contamination sample, which were related to the pre-treatment and analysis of a various number of environmental samples.

Table 4.5: Microplastic contamination (n/blank sample) detected in the field blanks (FB) and procedural blanks (PB) together with aerial contamination (AC) samples related to the WWTP-related samples together with lake water and sediment samples.

| **Sample type** | **Pre-treatment method** | **Type** | **n** | **Lower size limit (µm)** | **n/blank sample ± SE** | | |
|---|---|---|---|---|---|---|---|
| | | | | | **MPPs** | **MPFs** | **MPs** |
| Wastewater | WPO | FB | 7 | 250 | 2.3 ± 0.9 | 2.3 ± 0.6 | 4.6 ± 1.3 |
| Sludge | Drying | FB | 7 | 100 | 0.0 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| Lake water | UEPP | FB | 4 | 100 | 7.3 ± 2.1 | 2.3 ± 0.8 | 9.5 ± 1.6 |
| | | AC | 7 | 100 | 0.1 ± 0.1 | 0.4 ± 0.3 | 0.6 ± 0.3 |
| Sediment | $H_2O_2$ + density separation | FB | 4 | 100 | 14.3 ± 3.6 | 1.8 ± 0.8 | 16.0 ± 3.9 |
| | | PB | 6 | 100 | 14.8 ± 1.2 | 4.5 ± 1.3 | 19.3 ± 1.9 |
| | | AC | 31 | 100 | 0.1 ± 0.00 | 0.1 ± 0.1 | 0.2 ± 0.1 |

In the WWTP-related blank samples, 4.6 ± 1.3 and 0.1 ± 0.1 MPs were counted per blank sample for water and sludge, respectively. The highest level of contamination was detected for the first sampling event, and some evidence of cross-contamination, such as planktonic organisms, was found in the field blank samples. It was also noteworthy that the MP concentrations in wastewater during the first sampling event were the highest compared to the other sampling events (Figure 5.1).

For the lake water and sediment samples, 9.5 ± 1.6 and 16.0 ± 3.9 MPs were detected per field blank sample, respectively. With both lake water and sediment studies, the majority (76–89%) of MPs in blank samples were MPPs. Hence, the reported environmental MPP concentrations were more probably distorted by contamination than the concentrations of MPFs. On the other hand, a third of the MPs in the sediment blanks were identified as Kevlar (Figure 4.5B), which was not detected in any of the sediment samples. Other commonly detected polymers in the blank samples were PP and PE. Their sources may have been the chemical packages and plastic wash bottles, which were used during the sampling and pre-treatment.

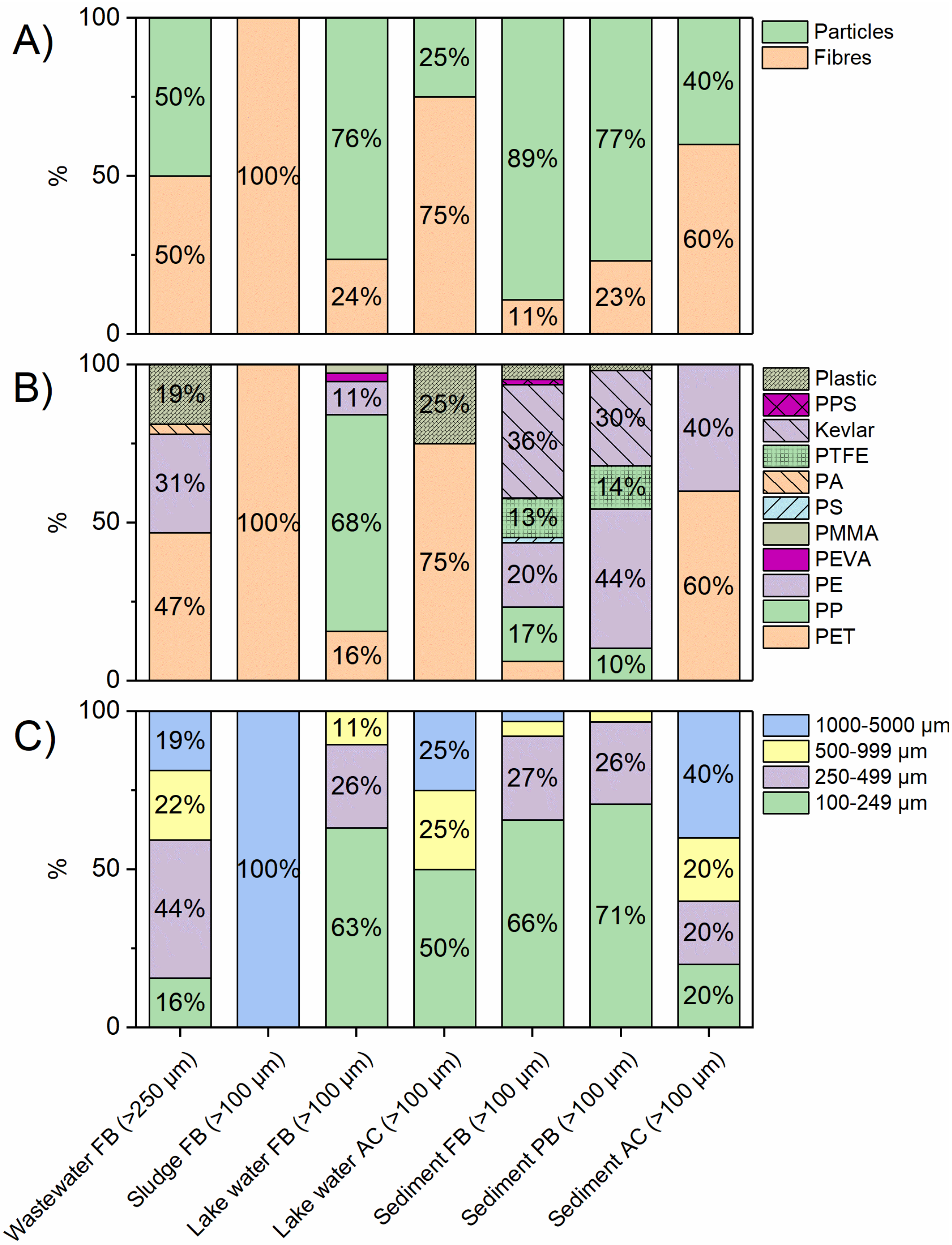

Figure 4.5: Relative portions of A) MPFs and MPPs, B) detected polymers, and C) sizes in field (FB) and procedural blanks (PB) together with aerial contamination samples (AC) collected along with wastewater, sludge, lake water and sediment samples. The used lower size limit is mentioned separately for each sample type.

Overall, the MPs in the blank samples (Figure 4.5C) were smaller than in the related lake water and sediment samples (Figure 4.3C). As an example, the size distributions of detected MPs are presented for lake water samples and the related contamination control samples in Figure 4.6. This difference may be explained by the fact that in the blank samples, smaller MPs were easier to recognize during optical sorting due to the absence of distracting natural material. Therefore, even though the number of MPs were relatively high in the blank samples, contamination was unlikely to cover an equal proportion of the reported MP concentrations in the environmental samples. Nevertheless, contamination may have increased the reported MP concentrations by some level. For the future studies, it would be crucial to not only process but also to analyse blank samples in a same way as the environmental samples to allow a better comparison of the results.

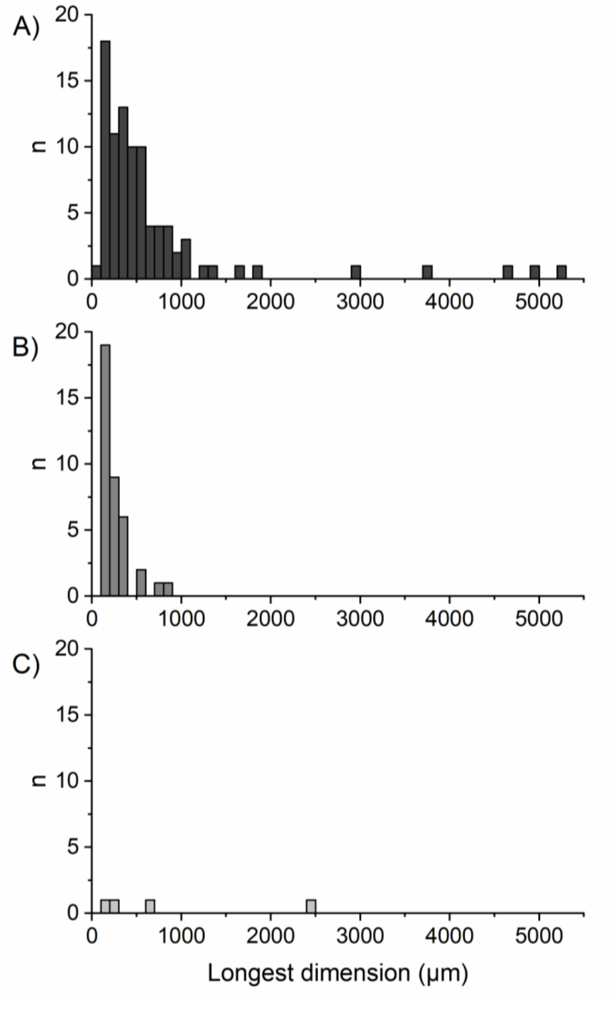

Figure 4.6: The size distribution of detected MPs in A) lake water samples, related B) blank samples and C) aerial contamination samples in this case study.

Aerial contamination was less common; only four and five MPs were detected in all the aerial contamination samples related to the pre-treatment and identification of lake water and sediment samples, respectively. Most of the detected aerial contamination was caused by PET fibres. For the WWTP-related samples, aerial contamination was not followed.

In addition to contamination caught with the blank samples, some possible contamination can also be pointed out from the actual samples in this case study. The Limnos samplers used in both lake water and sediment sampling consisted of polycarbonate (PC), and in the case of water samplers, also of polyoxymethylene (POM). Thus, they may have caused some contamination in the collected samples. In the case of sediment samples, PC was not detected at all. Hence, the Limnos sampler itself did not have an impact on the reported MP concentrations for our sediment samples, at least for MPs larger than 100 µm. In contrast, PC (3 MPPs) and POM (8 MPPs and 3 MPF) detected in lake water samples might have originated from the Limnos water sampler. In the case of other commonly used sampling devices, such as Manta trawls (PA) or pumps with plastic tubing, the possible contamination caused by the device itself should also be discussed along with the detected MPs.

Due to the high variation in the MPs detected in the blank samples in this case study, reported MP concentrations were not corrected according to them. This kind of approach can cause an overestimation of the environmental MP concentrations. On the other hand, as mentioned earlier, MPs detected in the blank samples were smaller than the ones detected in the environmental samples. Hence, the exclusion of the reported contamination from the environmental MP concentrations would probably have distorted the results.

The risk of contamination and loss of sample can increase with the number of pre-treatment steps [44]. This was confirmed also in this case study, where more contamination was detected in the blank samples related to wastewater samples than the less-treated sludge blanks. On the other hand, more contamination was detected for sediment samples than for lake water samples, which were treated with multiple steps and had a smaller lower size limit during UEPP. The smaller contamination of the lake water samples might be due to conducting the treatment steps in a laminar flow hood instead of the fume hood, which was used for the sediment samples. Thus, the sensitivity of the sample collection and pre-treatment to contamination depends on many practices, which should be considered thoroughly when MP studies are conducted.

To improve the contamination control and to enable the accurate comparison of results from environmental samples and contamination control samples, the volume of the control sample and the time used for sample collection should be comparable to the actual samples. However, for example in the case of neuston net samples, it would not be possible to filter several cubic meters of pure water to mimic the possibility of MPFs or small MPPs retaining on the net [11]. In addition, sampling devices can be kept open to mimic the aerial contamination during sampling, but in some cases, it might not correspond to the aerial exposure of the actual samples.

Additionally, in an ideal case, contamination samples should be treated and examined with similar size limitations as other samples. If blank samples are collected in the same way, with the same volume and within the same duration as environmental samples, i.e. they totally mimic the collection of environmental samples, the detected MP counts could be reliably subtracted from the reported results. Even then, it would still be questionable, whether the subtraction of the results should be done separately for each detected polymer and size class.

One solution for this issue could be the procedure introduced recently by Horton et al. [108]. They presented an example of how contamination could be corrected and how limits for detection (LOD) and quantification (LOQ) could be set. First, they calculated the resolution of the analysis, or, in other words, how high the original MP concentration needs to be in order to detect at least one MP (equal to LOD), or 3 particles (equal to LOQ) in the final filter. In addition, they calculated the initial LOD and LOQ by multiplying the standard deviation of procedural blanks by 3.3 and 10, respectively. Then, the highest values for each polymer, i.e. either resolution-based LOD/LOQ or initial LOD/LOQ, were set as the final LOD and LOQ. All the calculations were suggested to be conducted separately for each detected polymer. In addition, they extracted the number of MPs detected on procedural blanks from the result that was compared to the final LOD and LOQ. They also underlined that blank correction would be more significant for samples with a lower abundance of MPs. According to this view, the reported MP concentrations of this case study would be most reliable for the influent, sludge, and sediment samples. The highest concentrations of MPs were detected in those samples, and, thus, the blank correction would not be as significant as for other wastewater and lake water samples.

## 4.6 Interpretation of microplastic results

There are multiple points that should always be considered, when interpreting reported MP results. As discussed before, differing measurement practices can cause significant disparities between the actual environmental MP concentrations and the reported MP concentrations after all the sampling, pre-treatment, and identification steps (Figure 4.7). The most important issue are the lower size limits used throughout the study. Another issue is the inclusion of MPFs in the reported results. Additionally, the uncertainty of the results is higher if reported concentrations are not based on the identification of all particles and fibres. If the identification is conducted only for visually suspected MPs or representatives of possible MPs, the uncertainty of the results is even higher. Moreover, if some colours are excluded or under-represented in the results, the reported MPs concentrations and relative shares of various polymers can be distorted. Hence, the recovery of realistically sized and shaped MPs should be tested, and related information should be provided with reported MP concentrations. Moreover, the practices to prevent and control possible contamination should be informed. Even though sampling, pre-treatment, and identification methods develop constantly, reported microplastic concentrations should never been considered as exact information, but rather as rough estimations of the actual MP concentrations in the studied area.

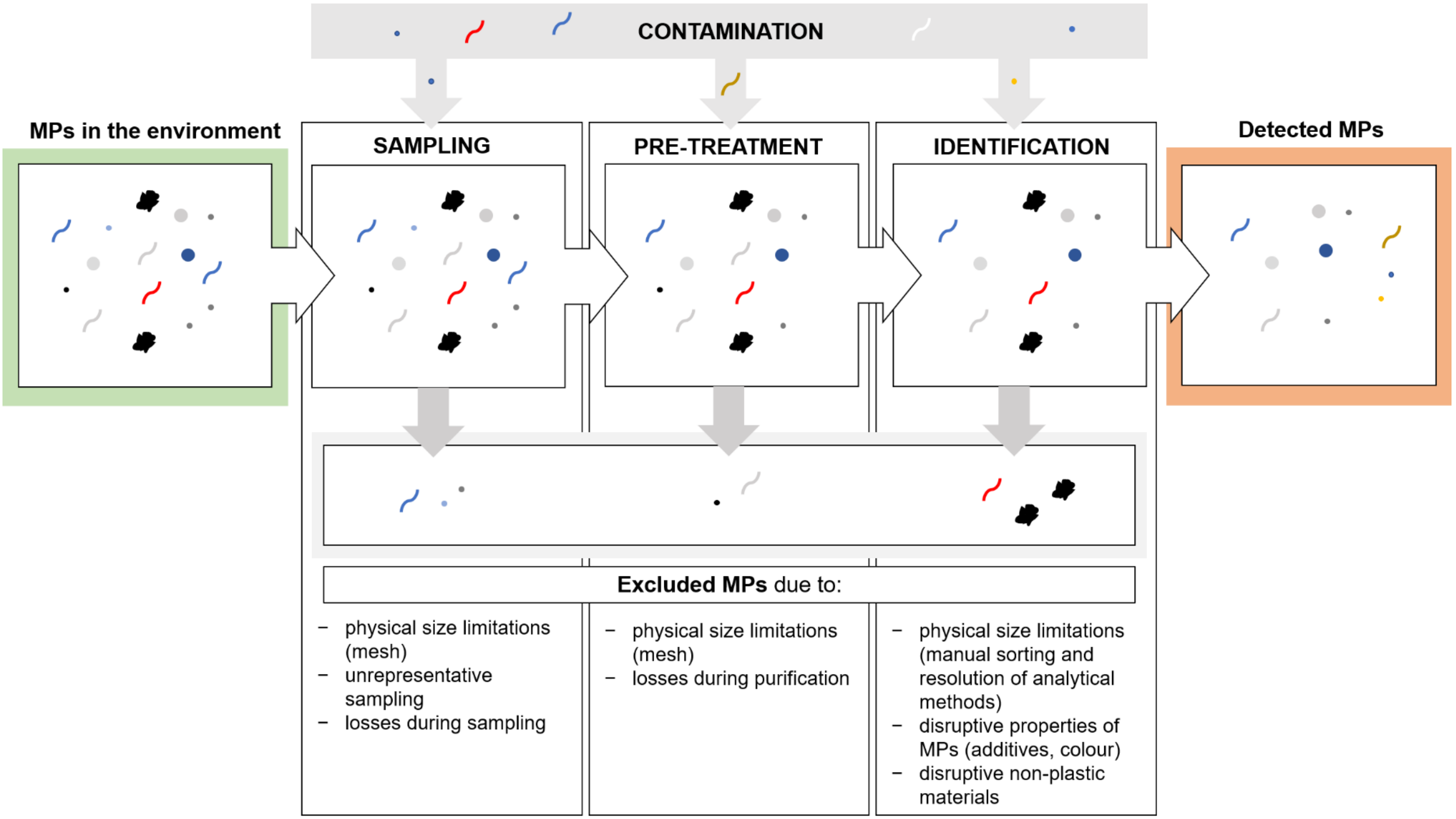

Figure 4.7: The variables affecting detected and reported MP concentrations. The size of excluded part of each variable depends on the selected sampling, pre-treatment, and identification methods. In addition, the means of contamination mitigation, monitoring and possible subtraction affect the reported concentrations.

Overall, in the microplastic research field, more attention should be paid to the reporting of the results. Researchers should provide information about the limitations that selected methods cause in their results. For example, if density separation is used, it would be a good practice to underline that polymers with a higher density than the used separation solution are probably excluded from the results. Likewise, it would be crucial information to be reported with results if the selected characterization method has limitations in detecting some polymers or if the resolution of the analytical method does not allow the detection of the smallest MPs. For example, in studies by Ben-David et al. [94] and Bayo et al. [142], the only information about the lower size limit of the studied MPs is the aperture of the final filters (0.45 µm). Nevertheless, the identification of representative MPs with µ-Raman [94] or tweezer-picked MPs with FTIR most likely did not allow the inclusion of the smallest MPs. In general, these kinds of improved actions in reporting would not only help the research field to develop the methodology for covering a wider range of MPs, but they would also help to underline the differences behind the reported results.

As an example of the suggested practice, the limitations and other necessary information for the interpretation of the environmental MP concentrations of this case study are presented in Table 4.6. These issues were also discussed earlier in Sections 4.2–4.5.

Table 4.6: An example of the practice for reporting limitations in the reported MP concentrations. The limitations reported here are related with the MP concentrations of this case study. Recoveries are valid only for the polymers and size ranges listed in Section 3.4.

| **Results for sample type** | **Possibly excluded polymers** | **Included size range** | **Recovery (range) (%)** | | | **Other limitations** |
|---|---|---|---|---|---|---|
| | | | **MPPs** | **MPFs** | **Total MPs** | |
| Wastewater | - SBR<br>- Polymers with colours covering the Raman spectrum of the material at 514 nm<br>- Polymers with fluorescence covering the Raman spectrum of the material at 514 nm | >250 µm | 98 (98–100) | 68 (60–73) | 85 (81–89) | - Only 1.3% and 1.4% of collected particles and fibres analysed with µ-FTIR or µ-Raman<br>- Generalization may have caused misidentification between similarly looking particles and fibres<br>- Concentrations of MPs with similar appearance to natural particles might be underestimated due to visual examination<br>- Concentrations not corrected according to blank samples or recovery tests<br>- Impact of cellulase treatment on the recovery rates for influent samples not assessed<br>- The solutions used during purification were not filtered before addition into samples<br>- Influent samples collected from the grit separation basin<br>- Only a subsample of 0.25 g dried influent included in examination |
| Sludge | - SBR<br>- Polymers with colours covering the Raman spectrum of the material at 514 nm<br>- Polymers with fluorescence covering the Raman spectrum of the material at 514 nm | >100 µm | 100 (100) | 66 (50–74) | 85 (79–89) | - Only 1.3% and 1.4% of collected particles and fibres analysed with µ-FTIR or µ-Raman<br>- Generalization may have caused misidentification between similar looking particles and fibres<br>- Concentrations of MPs with similar appearance to natural particles might be underestimated due to visual examination<br>- Concentrations not corrected according to blank samples or recovery tests |

Table 4.6: (continued)

| **Results for sample type** | **Possibly excluded polymers** | **Included size range** | **Recovery (range) (%)** | | | **Other limitations** |
|---|---|---|---|---|---|---|
| | | | **MPPs** | **MPFs** | **Total MPs** | |
| Lake water, analysed with Raman | - SBR<br>- Polymers with colours covering the Raman spectrum of the material at 514 nm<br>- Polymers with fluorescence covering the Raman spectrum of the material at 514 nm | >100 µm | 97 (93–100) | 85 (80–95) | 91 (86–98) | - Concentrations of MPs with similar appearance to natural particles might be underestimated due to visual examination<br>- Concentrations not corrected according to blank samples or recovery tests |
| Lake water, analysed with py-GC/MS | - Other polymers than PE, PP and PS | >20 µm | - | - | - | - Concentrations not corrected according to blank samples or recovery tests<br>- Recovery tests were not conducted with py-GC/MS |
| Sediment | - SBR<br>- The abundance of polymers with density over 1.4 g/cm$^3$ (e.g. PET, PVC, PTFE) might be underestimated<br>- Polymers with colours covering the Raman spectrum of the material at 514 nm<br>- Polymers with fluorescence covering the Raman spectrum of the material at 514 nm | >100 µm | 99 (97–100) | 84 (80–88) | 95 (94–96) | - Concentrations of MPs with similar appearance to natural particles might be underestimated due to visual examination<br>- Concentrations not corrected according to blank samples or recovery tests |

After all, the main reason for estimating microplastic pollution in the environment is to determine their possible ecological and (eco)toxicological effects. Due to the wide variation in the properties of the MPs themselves and their varying distribution in the environment, organisms can be exposed to different types and quantities of MPs depending on where they live [146]. Hence, in order to assess the effects of MP pollution in a certain area, different compartments, such as sediment and water, should be examined [17]. In addition, it needs to be considered that MPs are able to transfer between trophic levels [147], and, hence, MPs present in one compartment of the environment may cause effects also in the organisms living in other compartments of the ecosystem.

From an ecotoxicological point of view, the effects of MPs on organisms can be divided into three categories. First, MPs can cause physical and chemical effects to organisms. The most often reported effects include reduced growth and activity together with decreased survival rates [146]. In addition to the direct effects of MPs themselves, different additives in plastics, such as plasticizers and bisphenol A, can cause their own various effects [32]. Thirdly, MPs can act as vectors for pathogens and pollutants, such as polychlorinated biphenyls, polyaromatic hydrocarbons and metals, allowing them to disperse in the environment, and probably affect organisms more easily [29–31].

Many problems occur in the ecotoxicological interpretation of environmental concentrations of MPs, and these problems are related with the wide chemical properties of MPs together with the wide variation in their sizes. Firstly, ecotoxicological studies are often conducted with MPs smaller than 100 µm and even to a greater extent with nanoplastics (NP) in sizes below 1 µm [146, 148]. This leaves a gap between the results gained from environmental monitoring studies and the available information about their possible effects. Hence, to assess the ecological risks of MPs, the smallest MPPs as well as MPFs, should be included in monitoring studies [93]. Secondly, PS has been the dominant polymer in previous ecotoxicological studies, covering half of the conducted ecotoxicity studies [146]. This means the knowledge about the possible ecotoxicological effects of other commonly detected polymers is relatively scarce. Hence, the ecotoxicological studies considering a wider range of polymers needs to be conducted to assess the possible effects of environmentally relevant MPs. Thirdly, mass-based concentrations are often used in ecotoxicological testing, which challenges the assessment of possible effects with particulate concentrations that are often reported for environmental samples analysed with spectroscopic methods. Furthermore, ecotoxicological testing is often conducted in unrealistically high concentrations, which do not represent the actual exposure occurring in the environment, and, moreover, acute effects are examined more often than chronic effects [35, 149].

Consequently, the assessment of the ecotoxicological and ecological effects of detected MP concentrations needs to be discussed in the light of present knowledge, in relation to the detected polymers and within the size range that they have been detected in the studied environment. Lastly, in the real environment, organisms are simultaneously exposed to a wide range of MPs, their additives, and other chemicals, and, thus, real ecotoxicological and ecological effects may differ from the effects detected during single-compound exposure studies.

# 5 Assessment of microplastic loads from WWTPs and their distribution and impacts in recipient water bodies

## 5.1 Microplastics abundance at WWTPs

In addition to the previously discussed challenges faced in MP studies in general, many other issues need to be considered when MP concentrations are studied at different points of WWTPs.

Firstly, when process streams, such as wastewater and sludge, are considered, it needs to be recalled that bulk samples represent only a blink of the overall flow of wastewater and, correspondingly, MPs in the wastewater. Fluctuations of the incoming stream also cause variations in MP concentrations, which challenges both the sampling and comparison of the results even from different points of the same process. Therefore, the presented point of time and duration of sample collection needs to be considered to gain representative results. In addition to the diurnal variance in MP concentrations in wastewater, the seasonal variation of MP concentrations has also been discussed [94, 101]. Reasons for proposed seasonal fluctuations could involve variation in the amount and types of clothing for locations with high annual fluctuation in temperatures, such as Finland. Nevertheless, it is still unknown, whether MP loads in wastewater vary within the cold and warm seasons in Nordic conditions. In South Carolina, in the USA, Conley et al. noticed a 2.7 to 4.8-fold variation in the MP concentrations in 24-hour composite effluent samples collected during one year in three WWTPs, but without any clear seasonal trends [101].

Within the sampling campaign at Kenkäveronniemi WWTP, large variations were found in the detected MP concentrations of both wastewater and sludge (Figure 5.1). The sampling campaign consisted of seven sampling events covering almost three months, but the samples were collected on Monday mornings and the sampling covered only a part of the autumn and winter. Hence, any possible diurnal variation and variation within days of week or warm and cold seasons were not captured in the reported results. These observations highlight both the need for multiple sampling occasions and a larger sample size to estimate the concentrations of MPs at WWTPs more reliably. Another solution to covering the diurnal variation could be the use of composite samplers, which would allow the representation of average MP concentrations [45, 101]. Nevertheless, to overcome the variation in wastewater and sludge, the average MP concentrations should be reported based on multiple sampling events rather than many samples collected within a short period. In addition, considering the hydraulic retention time of the wastewater treatment process could allow a more reliable assessment of the removal efficiency of MPs. In practice, the load of MPs in the incoming water could be followed according to the retention time of the WWTP and samples could be collected accordingly from latter parts of the treatment process.

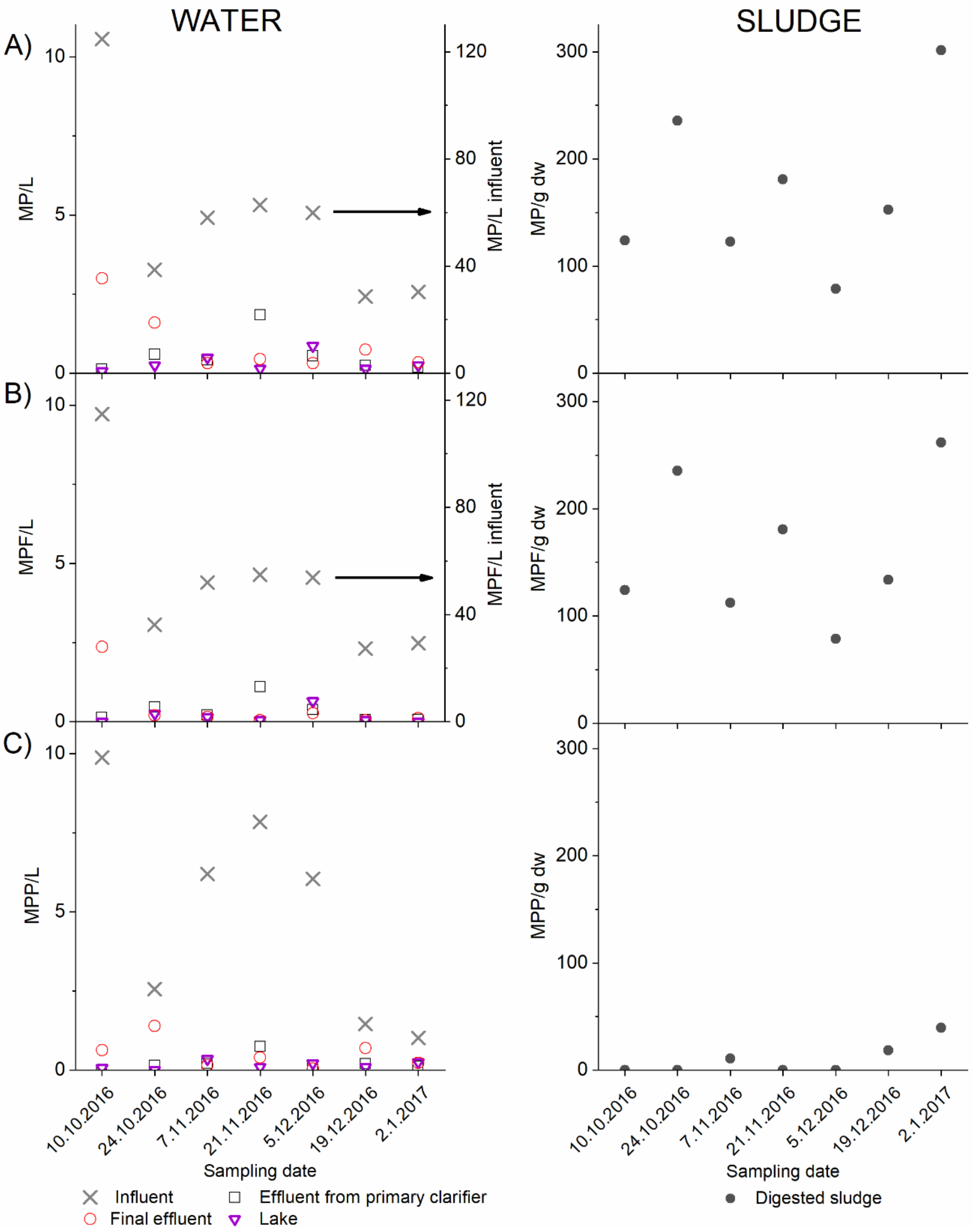

Figure 5.1: Concentrations of A) MPs, B) MPFs and C) MPPs in water (n/L, >250 µm) and sludge samples (n/g dw, >100 µm) on different sampling dates. The results for influent samples (x) in (A) and (B) have their own scale on the right Y-axis. The figure is modified from our previous publication [67].

The sampling of process streams can also cause other kinds of variation in the detected MPs. During this case study at the WWTP, PE fragments were only detected in effluent and shore water samples. The reasons for not detecting them in the influent might be the smaller volume collected from the influent stream, the diurnal variation in the contents of the wastewater stream or the more stable flow at the effluent sampling point, possibly allowing light PE fragments to concentrate on the surface and to be collected during the sampling. The latter could also explain the lower MP concentrations detected in the effluent from the primary clarifier (0.6 ± 0.2 MP/L) compared to the final effluent (1.0 ± 0.4 MP/L). Salmi et al. reported similar results for the Nenäinniemi WWTP, where concentrations of MPs (>20 µm) were lower in the primary effluent (3 MP/L) than in the secondary effluent (13 MP/L) [145]. In this case study, the sample from the primary clarifier represented the whole flow of the wastewater in an exact point of the process, while samples of the final effluent were collected from the upper part of the effluent stream. Other reasons for the higher MP concentrations in the final effluent could be the breakdown of the MPs during the wastewater treatment process or during the conducted pre-treatment steps before the identification of the MPs. In addition, the smaller amount of distractive materials in the final effluent might have allowed more accurate identification of MPs, allowing better recognition of PE fragments.

In addition, many streams are recycled back to the beginning of the process in WWTPs, which highlights the importance of the representativeness of the sampling points [145]. At Kenkäveronniemi WWTP, these flows include reject water from the sludge dewatering, water from rinsing of removed solids and grease, floating material from the primary sedimentation and waste activated sludge (WAS) from the final sedimentation. In a study conducted at Nenäinniemi WWTP, very high concentrations of MPs larger than 20 µm were detected in the reject waters from gravimetric thickening (475 MP/L) and centrifuge (10400 MP/L) treatments [145]. In addition, 69–79% of MPs (>25 µm) were already removed during screening and grit separation at two WWTPs in Australia [50]. Therefore, our influent samples, collected from the grit separation basin, consisted of both incoming, screened wastewater and the before-mentioned recycled flows (Figure 3.1). It can be assumed that the MP concentrations would have been lower in the initial, screened influent than the concentrations now reported for samples collected from the grit separation basin. On the other hand, if the influent samples would have been collected before any screens, the MP concentrations would probably have been higher. To avoid this kind of problem in the future, the selected sampling points should be chosen and described more precisely than usually has been done in microplastic studies. Especially the location of the sampling points in relation to the screens and internally-recycled flows should be considered when removal rates of MPs are calculated for WWTPs. Overall, when the MP budgets or total removal efficiency of MPs at a certain WWTP is assessed, influent samples should be collected before any primary treatments.

In this case study, the overall highest MP concentrations were still predictably detected for influent from the grit separation basin (57.6 ± 12.4 MP/L) and digested sludge (170.9 ± 28.7 MPs/g dw) (Table 5.1) [67]. Fibres covered the majority of the detected MPs in both of those sample types. The final effluent contained 1.0 ± 0.4 MP/L, half of which were fibres.

Table 5.1: Detected concentrations of MPPs, MPFs and total MPs (n/L (>250 µm) or n/g dw (>100 µm) ± SE) in different stages of the wastewater treatment process in Kenkäveronniemi WWTP [67].

| **Sample type** | **Sampling point** | **n** | **MPP/L ± SE** | **MPF/L ± SE** | **MP/L ± SE** |
|---|---|---|---|---|---|
| Water | Influent from grit separation | 7 | 5.0 ± 1.3 | 52.6 ± 11.3 | 57.6 ± 12.4 |
| | Effluent from primary clarifier | 7 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.6 ± 0.2 |
| | Final effluent | 7 | 0.5 ± 0.2 | 0.5 ± 0.3 | 1.0 ± 0.4 |
| | | | **MPP/g dw ± SE** | **MPF/g dw ± SE** | **MP/g dw ± SE** |
| Sludge | Digested sludge | 7 | 9.8 ± 5.7 | 161.0 ± 25.5 | 170.9 ± 28.7 |

Our findings were consistent with the previously reported MP concentrations in effluents of secondary WWTPs varying from 0 to 30.6 MP/L (Table 5.2). The large variation in reported MP concentrations can be explained by the differences in initial MP concentrations of influents and in the detailed processes of WWTPs [150] together with differences in the used lower size limitations (1–300 µm) and the sampling, pre-treatment, and identification methods [126], which were discussed earlier in Chapter 4.

Table 5.2: Previously reported concentrations of MPs (n/L) in municipal WWTP effluents together with removal efficiencies of MPs (%) for primary and secondary WWTPs together with WWTPs with disinfection as a tertiary treatment. Only results, which were based on a spectroscopic confirmation of subsamples or all particles and fibres are included. The lower size limit is based on the physical limit via filtration or the limit caused by identification methods.

| Studied WWTP | Ref. | Volume of sampled final effluent (L) | No. of replicates per WWTP | Overall lower size limit (μm) | MP concentration of effluent (n/L) | Removal efficiency of MPs (%) | Identification method |
|---|---|---|---|---|---|---|---|
| **WWTPs with primary treatment** | | | | | | | |
| Stonecutters Island WWTP, Hong Kong | [151] | 192 | 4 | 54 | 1.4–10.8 | | μ-Raman2) |
| WWTP in Australia | [74] | 3 | | 25 | 1.54 | | μ-FTIR1) |
| **WWTPs with secondary treatment, incl. CAS and other biological treatments and disinfection** | | | | | | | |
| Långeviksverket WWTP, Sweden | [47] | 1000 | 4 | 300 | 0.01 | 99.9 | FTIR-ATR1) |
| *Kenkäveronniemi WWTP, Finland* | *This study* [67] | *17.5–30* | *7* | *250* | *1.05* | *98.3* | *μ-Raman, μ-FTIR1)* |
| WWTP in Cartagena, Spain | [103] | 4 | | 200 | 0.31 | 90.3 | FTIR2) |
| WWTP in Valencia, Spain | [105] | 5 | | 150 | 0.44 | | μ-ATR-FTIR1) |
| 2 WWTPs in Wuhan, China | [111] | 10 | 2 | 149 | 7.9–30.3 | 62.7–66.1 | μ-Raman1) |
| 23 WWTPs in Korea | [46] | 1000 | 1 | 100 | 0.001–0.13 | 96.7–100.0 | μ-FTIR1) |
| WWTP in Cadiz, Spain | [112] | 15–35 | | 100* | 16.4 | 97.2 | ATR-FTIR2) |
| WWTP in Glasgow, UK | [39] | 50 | 2 | 65 | 0.25 | 98.4 | μ-FTIR1) |
| WWTP in Northern Italy | [52] | 30 | 3 | 63 | 0.9 | 64.0 | μ-FTIR2) |
| WWTP in Vancouver, Canada | [48] | 30 | 6 | 63 | 0.50 | 98.3 | μ-FTIR1) |
| 3 WWTPs in Charleston Harbor watershed, USA | [101] | 3.6–30 | 10–16 | 60 | 3.70–17.60 | 85.2–97.6 | μ-FTIR-ATR1) |
| 2 WWTPs in Seyhan and Yüregir, Turkey | [102] | 5 | 6 | 55 | 4.11–7.00 | 73.0–79.0 | μ-Raman2) |

Table 5.2: (continued)

| **Studied WWTP** | **Ref.** | **Volume of sampled final effluent (L)** | **No. of replicates per WWTP** | **Overall lower size limit (µm)** | **MP concentration of effluent (n/L)** | **Removal efficiency of MPs (%)** | **Identification method** |
|---|---|---|---|---|---|---|---|
| Sha Tin WWTP, Hong Kong | [151] | 192 | 4 | 54 | 0.6–3.5 | | µ-Raman[2] |
| WWTP in USA | [43] | | | 45 | 0.00 | 99.9 | FTIR[1] |
| WWTP in Harbin, China | [107] | 20 | 3 | 38 | 30.6 | 75.7 | µ-Raman[1] |
| 5 WWTPs in China | [110] | 20 | 2 | 30.8 | 0–0.85 | | µ-Raman[2] |
| WWTP in East Midlands, UK | [53] | 10 | 4 | 25 | 1.5 | | FPA-µ-FTIR[2] |
| WWTP in UK | [108] | 360 | 2 | 25 | 5.7 | 99.9 | µ-FTIR[2] |
| WWTP in Madrid, Spain | [54] | 25 | 3 | 25 | 10.7 | 93.7 | µ-FTIR[1] |
| 3 WWTPs in Australia | [50] | 68–110 | 1 | 25 | 0.18–0.96 | 98.3–99.8 | µ-FTIR[1] |
| WWTP in Australia | [74] | 27 | | 25 | 0.48 | | µ-FTIR[1] |
| 12 WWTPs in Lower Saxony, Germany; 4 WWTPs have also tertiary treatment | [44] | 390–1000 | | 20 | 0.01–9.05 | | FPA-µ-FTIR[1] |
| WWTP in Karmiel, Israel | [94] | 3–100 | >52 | 20 | 2.72 | 95.8 | µ-Raman, FEG-SEM[1] |
| 9 WWTPs in China | [109] | 2 | 1 | 10* | 6–26 | 35.0–98.0 | µ-Raman[2] |
| WWTP in Hunter Region, Australia | [104] | 60 | 2 | 1.5* | 2.76 | 76.6 | ATR-FTIR[1] |
| WWTP in Karmiel, Israel | [94] | 3–100 | >52 | 1 | 15.62 | 88.2 | µ-Raman, FEG-SEM[1] |

[1] Only representative particles and fibres or subsamples identified.

[2] All particles and fibres identified.

* The lower size limit is probably higher due to the limitations of the identification method.

Similarly, previously reported MP concentrations in sludges vary widely, which may be caused by the collection of the samples from different points of sludge treatment processes together with the variation in the incoming load of MPs, in the pre-treatment methods and in the size limitations. In Finland, other studies have reported higher MP concentrations (>20 µm) for the final dried sludge than detected in this case study for the digested sludge before drying (170.9 MP/g dw; >100 µm). At Viikinmäki WWTP, 186.7 micro-litter/g of dried sludge was detected [45], while 9379 MP/g dw was reported for the dried sludge at Nenäinniemi WWTP [145]. In the UK, only 0.8 MP/g of centrifuged sludge (>0 µm) was reported [39]. In China, a study of 28 WWTPs revealed average MP concentration in dried sludge to be around 23 MP/g dw (>37 µm) [113]. In other Chinese studies, 2.9 MPs/g dw (>80 µm) [114] and 46.3 MP/g dw [107] were detected for dewatered sludge. For the latter study, no information of the lower size limit was provided.

In the current case study, digested sludge was collected before dewatering. At Viikinmäki WWTP in Helsinki, Finland, 20% of the micro-litter was returned to the treatment process with the reject water from sludge dewatering [45]. If the MPs at Kenkäveronniemi WWTP behave similarly during dewatering, the concentration of MPs in the final discharged sludge would be around 137 MP/g dw. Even the corrected MP concentration would have been relatively high compared to the majority of previously reported results, considering the higher size limitations than in other studies [39, 45, 113, 114].

## 5.2 Removal efficiency of microplastics in WWTPs

According to the results of the present study, the removal efficiency of MPs at Kenkäveronniemi WWTP was 98.3% when a lower size limit of 250 µm was used [67]. For MPPs and MPFs, the removal efficiencies were 89.8% and 99.1%, respectively. These efficiencies were based on the detected MP concentrations in the influent from the grit separation basin, thus, including the recycled flows. Because the MP concentrations would still likely have been higher in the non-screened influent compared to the MP concentrations measured from the grit separation basin, the total removal efficiency at Kenkäveronniemi WWTP was likely underestimated by this study.

However, these findings were in line with recently reported MP removal rates for WWTPs with secondary treatments; varying between 62.7 and 100.0%, when lower size limits over 100 µm are used (Table 5.2) [46], [47], [111]. When smaller MPs are also included in the examinations, the reported MP removal efficiencies in secondary WWTPs vary widely between 35 and 100%, as can also be seen in Figure 5.2 [39, 43, 45–50, 52, 54, 55, 67, 94, 101–104, 107–109, 111, 112, 114, 142]. The previously-discussed impacts of the exact locations of the influent sampling sites together with the possible variation in the incoming flow of MPs on the calculated removal efficiency of MPs can explain part of this variation.

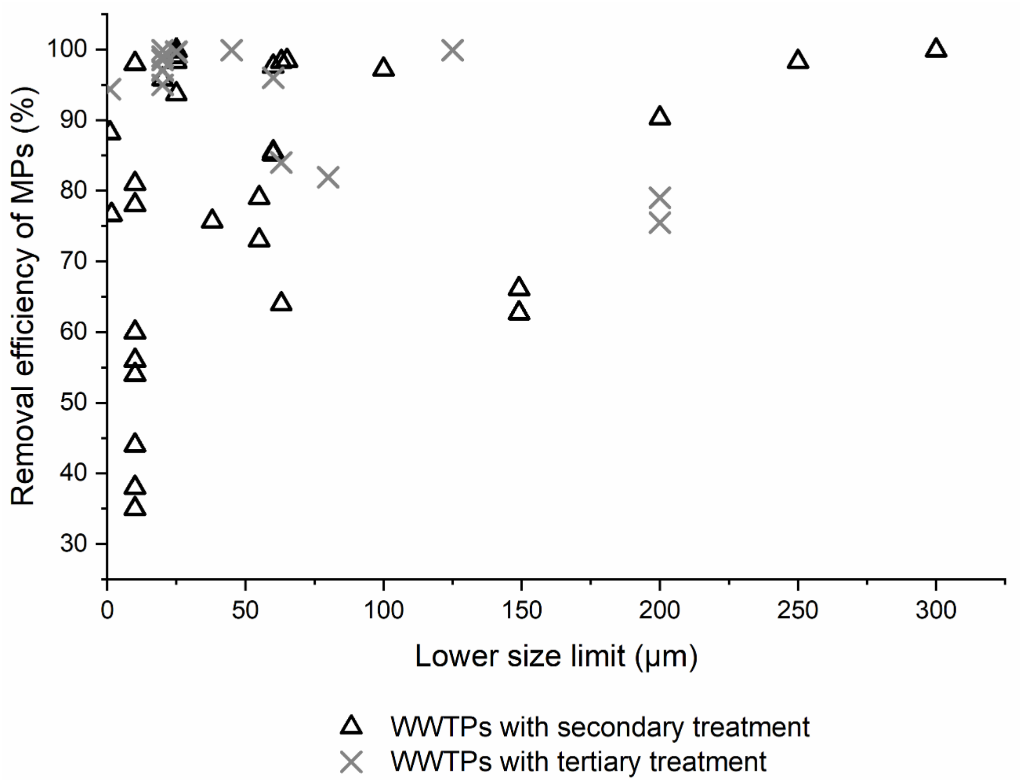

Figure 5.2: Previously reported removal efficiencies of MPs (%) in various WWTPs in relation to the lower size limit. Only results, which were based on spectroscopic confirmation of representative MPs, subsamples or all particles and fibres are included. [39, 43, 45–50, 52, 54, 55, 67, 94, 101–104, 107–109, 111, 112, 114, 142]

In general, tertiary or advanced wastewater treatments are utilized to improve the removal of nutrients or emerging pollutants from wastewater. They can also improve the removal of MPs from wastewater (Table 5.3), but the removal efficiencies vary for different treatment methods. One of the most promising tertiary treatment is the MBR process. It has been reported to remove even up to 99.9% of MPs, when MPs over 20 µm [55] and 100 µm [46] were included in the examinations. In contrast, Bayo et al. reported MPs removal rate of 79.0% for an MBR-based process in a study, where only MPs larger than 200 µm were detected [142]. These results need to be critically interpreted. Because the membranes used in the studied MBR unit had pore size around 0.2 µm, they should not let any MPs larger than that enter the treated effluent. If the MBR process was working properly and membranes were intact, MPs detected in the effluent could be originated from the system itself, from aerial fallout or from contamination during the sampling, pre-treatment, or identification. In addition to MBR, DF has been reported to remove 99% of

MPs over 20 µm [145]. It needs to be pointed out, that some tertiary treatments have been reported to remove MPs less efficiently than secondary CAS-based processes. For example Bayo et al. observed MPs removal rate of 75.5% for RSF in their study, where only MPs larger than 200 µm were detected [142]. Thus, advanced treatments are not always automatically better solutions for removing MPs from wastewater compared to the more conventional processes.

Table 5.3: Previously reported concentrations of microplastics (n/L) in municipal WWTP effluents treated with tertiary or advanced treatments (incl. MBR, DF, RSF, DAF, ultrafiltration (UF), reverse osmosis (RO), cloth filter, trickling filter, sand filter (SF), and biological aerated flooded filter (BAFF)) together with removal efficiencies of MPs (%). Only results, which were based on a spectroscopic confirmation of subsamples or all particles and fibres are included. The lower size limit is based on the physical limit via filtration or the limit caused by identification methods.

| **Studied WWTP** | **Ref.** | **Process** | **Volume of sampled final effluent (L)** | **No. of replicates per WWTP** | **Overall lower size limit (µm)** | **MP concentration of effluent (n/L)** | **Removal efficiency of MPs (%)** | **Identification method** |
|---|---|---|---|---|---|---|---|---|
| EDAR Águilas WWTPs, Spain | [142] | MBR, RSF | 4.04–4.43 | 17–18 | 200 | 0.92–1.08 | 75.5–79.0 | FTIR2) |
| WWTP in USA | [43] | Gravity filter | 196000 | | 125 | 0.00 | 99.9 | FTIR1) |
| 8 WWTPs in Korea | [46] | MBR, DF, filtration | 1000 | 1 | 100 | 0.003–0.07 | 99.5–100.0 | µ-FTIR1) |
| WWTP in Zhangzhou, China | [114] | Filtration | 20 | 3 | 80 | 2.90 | 81.9 | µ-Raman1) |
| WWTP in Northern Italy | [52] | SF | 30 | 3 | 63 | 0.4 | 84.0 | µ-FTIR2) |
| WWTP in Scotland, UK | [49] | Trickling filter | 5 | 5 | 60 | 2.00 | 96.0 | FTIR-ATR1) |
| 6 WWTPs in USA; detailed information for only 1 WWTP | [43] | Gravity filter | 189000–229000 | | 45 | 0.00 | 99.9 | FTIR1) |
| 7 WWTPs in UK | [108] | SF, BAFF, cloth filter, trickling filter | 360 | 2 | 25 | 0–22.3 | 99.6–100.0 | µ-FTIR2) |

Table 5.3: (continued)

| Studied WWTP | Ref. | Process | Volume of sampled final effluent (L) | No. of replicates per WWTP | Overall lower size limit (µm) | MP concentration of effluent (n/L) | Removal efficiency of MPs (%) | Identification method |
|---|---|---|---|---|---|---|---|---|
| WWTP in Australia | [74] | UF, RO | 200 | | 25 | 0.21 | | µ-FTIR1) |
| Nenäinniemi WWTP in Finland | [145] | DF | 10–1000 | 3 | 20 | 0.8 | 99 | µ-Raman1) |
| Pilots at Viikinmäki and Kenkäveronniemi WWTPs, Finland | [55] | MBR, DF | 2–6.1 | 3 | 20 | 0.01–0.03 | 98.5–99.9* | µ-FTIR1) |
| Kakolanmäki and Paroinen WWTPs, Finland | [55] | RSF, DAF | 16.1–25.5 | 3 | 20 | 0.02–0.10 | 95.0–97.1* | µ-FTIR1) |

1) Only representative particles and fibres or subsamples identified.
2) All particles and fibres identified.
* Efficiency is calculated only for the tertiary process, not for the whole WWTP.

According to this case study, the difference in the MP concentrations in influent (57.6 ± 12.4 MP/L) and effluent from the primary clarifier (0.6 ± 0.2 MP/L) confirms that the majority (99%) of MPs were removed from the wastewater and directed to the sludge already during the primary clarification. Likewise, previous studies have reported that the majority of MPs are removed during the mechanical and chemical pre-treatments, solids skimming and sludge settling processes at the very beginning of the wastewater treatment process [43, 45, 101].

Hence, the sludge fractions collected from primary and final clarification differ significantly in their microplastic concentrations. At least at Kenkäveronniemi WWTP, these sludges are combined and further treated by anaerobic digestion and dewatering before they are transported further and utilized for example in green construction. As the majority of the detected MPs are removed from the wastewater already in the primary clarification stage, the sludge collected from final clarification contains much lower concentrations of MPs than the sludge from primary clarification. If these sludges would be collected and treated separately, the sludge fraction from final clarification would be more suitable for use as fertilizer than the sludge that the studied WWTP is producing with the current combined sludge treatment. This approach could be part of the solution for the current situation, where the utilization of municipal sludges as fertilizers is limited due to the concerns related to their high concentrations of emerging pollutants, including microplastics.

During the sampling days of this case study, the average volumes of the incoming influent and discharged effluent were 10766 ± 501 $m^3$ and 10540 ± 425 $m^3$, respectively. According to the detected MP concentrations (>250 µm), approximately 1.0 x $10^7$ MPs were discharged daily with the effluent into the recipient lake. In addition, 87.3 ± 5.5 $m^3$ of digested sludge were produced daily for further dewatering, equalling a daily load of 4.5 x $10^8$ MP. If 20% of the MPs are recycled back into the process with the reject water [45], the actual daily load of discharged MPs from the WWTP with the treated sludge would be 3.6 x $10^8$ MPs.

Based on the assumption that the wastewater and sludge flows are stable in the studied WWTP, incoming (6.2 x $10^8$ MPs/d) and discharged MP loads via effluent and sludge (3.7 x $10^8$ MP/d) have a gap of 2.5 x $10^8$ MP/d. This difference is likely to be caused by the separation of MPs via other solid materials, which are removed during grit and grease separation at the beginning of the treatment [39]. In addition, because our influent samples were collected from the grit separation basin, but still after screening, the reported concentrations for the influent might underestimate the actual incoming MP load, and hence, the gap between the incoming and discharged MPs is likely to be even higher. In addition, the different pre-treatment and examination methods for water and sludge samples might cause uncertainties in the calculated MP loads.

In summary, to achieve the most accurate MPs removal rates and MP budgets for WWTPs, the initial influent should be collected before any screening or at least before any other waste streams are mixed with the influent. In addition, these locations should be described clearly along with the reported results. Likewise, all other samples, such as sludge and effluent, should be collected after all treatment steps to estimate the discharges from the WWTP as reliably as possible.

## 5.3 Environmental distribution and impacts of microplastic discharges from WWTPs

When the distribution or impacts of MPs are studied in water bodies receiving treated wastewater, there are multiple options for the included sample types. Different organisms live in different parts of the aquatic ecosystem—some live only in a water column, some live in the bottom sediment, while some prey on other organisms from the surface to the bottom. When this variation in the ecosystem is combined with the diverse environmental fates of different MPs, neither sediment nor water can be named as a more important matrix than another for estimating the impacts of MP discharges from WWTPs. Therefore, to assess the effects of MP pollution in a certain aquatic ecosystem, different environmental compartments, such as sediment and water, should be examined also in the studies assessing the effects of MP discharges from WWTPs.

However, the location and number of sampling sites together with the volume of collected samples should be selected so that they allow reliable discussion of results and offer data from differently exposed areas from WWTP effluents. Surface waters are flowing systems receiving MP discharges from both point and non-point sources, which means that MP concentrations in the water can fluctuate quite rapidly. Hence, water samples contain only those MPs that happened to be in the sampling location at the exact time that the sample was collected. Thus, the significance of larger sample volumes and the need for replicates is emphasized, when water samples are collected. In addition, the sampling depth should always be reported for water samples, because the abundance of MPs may vary from surface to bottom due to the different densities and, hence, varying environmental fate of different polymers [13, 93]. In contrast, MP concentrations in sediments are not expected to vary that much during a short period. Hence, sediments are usually sampled less frequently, but replicate samples would allow more reliable assessment of local and spatial variation.

Even though the volume of lake water samples (10.4 L) collected in this case study was in line with previous studies focusing on smaller sized MPs (>20–125 µm) [11, 152], the detected MP concentrations were very low. Hence, larger sampling volumes together with multiple sampling events would have increased the reliability of the results reported in this case study. This is also supported by a recent 12-month long sampling campaign, which revealed high variation in freshwater MP concentrations in the UK [153]. Likewise, increasing the amount of examined sediment samples from 29.6–74.1 g ww, together with replicate samples, would have improved the reliability of the collected data. On the other hand, these actions would have significantly increased the time needed for the identification of possible MPs in the environmental samples, and it would have been impossible if the same pre-treatment methods would have been used for larger volumes and higher numbers of samples.

Overall, the volume of collected samples should allow the detection of a sufficiently high number of MPs to represent the possible variation of MPs. Because MP concentrations are likely to be higher, when smaller particles and fibres are included, smaller volumes are acceptable for studies focusing on wider size range of MPs. Since different sample types can contain various MP concentrations, and different laboratories still focus on variously sized MPs, exact suggestions for suitable volumes cannot be provided.

In the case of lake or sea water studies conducted in Nordic conditions, the seasonal overturn of the water should be considered if MPs in a specific layer of the water column are examined. The lake water samples of this case study were collected in the autumn before overturn. Still, the temperature of the water samples was 12 °C both at the surface and the bottom layer at site 2, which was the closest sampling point to the WWTP discharge site. This might be explained by the fact that effluents from Kenkäveronniemi WWTP are discharged to the bottom layer of the lake, which can mix the thermocline in the surrounding area.

### 5.3.1 The effect of effluent discharges on the abundance of microplastics

In this case study, detected plastic (>100 µm) concentrations in the lake water were 0.4 ± 0.1 MPP/L, 0.3 ± 0.0 MPF/L and 0.7 ± 0.1 MP/L (Table 5.4), and the concentrations did not differ between the sampling sites (p=0.88–0.99, 1-way ANOVA). In the preliminary sampling in winter 2016–2017, 0.3 ± 0.1 MP/L (>250 µm) were detected in the lake water close to the shore of the discharge area.

Table 5.4: The detected concentrations of MPPs, MPFs and total MPs in lake water (n/L).

| Site | Sampling depth (m) | n | MPP/L ± SE | MPF/L ± SE | MP/L ± SE |
|---|---|---|---|---|---|
| 1 | 0.5 | 3 | 0.35 ± 0.14 | 0.26 ± 0.16 | 0.61 ± 0.22 |
| 2 | 0.5 | 3 | 0.51 ± 0.18 | 0.32 ± 0.03 | 0.83 ± 0.17 |
| 2 | 5.0 | 3 | 0.29 ± 0.10 | 0.29 ± 0.06 | 0.58 ± 0.06 |
| 3 | 0.5 | 3 | 0.58 ± 0.20 | 0.29 ± 0.10 | 0.86 ± 0.24 |
| All | | 12 | 0.43 ± 0.08 | 0.29 ± 0.04 | 0.72 ± 0.09 |

For the sediment layers representing the years 1990–2018, plastics (>100 µm) were detected in concentrations of 620 ± 110 MPP/kg dw, 1500 ± 340 MPF/kg dw and 2100 ± 410 MP/kg dw (Table 5.5). The MP concentrations in the sediment samples closest to the WWTP discharge site, i.e. site 2, were significantly higher than at other sampling sites for each target year between 1990 and 2018 (p=0.0002–0.007, 1-way ANOVA with Tukey test). The most significant differences were found in the concentrations of MPFs.

Although similar trends in MPs abundance were not observed for the water samples, there were some similarities in the MPs detected in the water and sediment close to the effluent discharge site, namely site 2 of this case study. PET was most detected polymer in both sediment (70%) and water samples (41%) at site 2. In addition, the majority (77%) of the detected MPs in the sediment were fibres, and 90% of them were confirmed to consist of PET. In the effluent of the Kenkäveronniemi WWTP, 52% of detected MPs (>250 µm) were PET, based on the identification of representative particles and fibres. According to the detected MP discharge rate (1 MP/L, >250 µm), more than 5 million MPs consisting of PET are daily discharged from the WWTP. Because the WWTP-related samples were collected using a 250-µm mesh, the load of smaller MPs and especially MPFs would probably be even higher. These observations together with the increased abundance of MPFs in the sediments from 1990 to 2010 in this case study are in line with the global trend of overall polyester production. During the target timeframe (1990–2018), global polyester production increased from 8 million tons to 55 million tons, now covering over 80% of the global production of synthetic fibres [1]. In addition to the increased use of plastics in textiles, other sources of MPs, such as mismanaged plastic waste is likely to have increased in the past decades due to the overall increase in the use of plastic packaging and wrappings. These sources of MPs can explain the MP pollution also in those parts of the studied area where the impact of WWTP effluents may be smaller.

Table 5.5: Detected concentrations of MPPs, MPFs and total MPs in bottom sediment (n/kg dw, >100 µm).

| Site | Distance from the WWTP (km) | n | Year | MPP/kg dw ± SE | MPF/kg dw ± SE | MP/kg dw ± SE |
|---|---|---|---|---|---|---|
| 1 | 2 | 4 | 1991–2018 | 920 ± 190 | 1200 ± 310 | 2100 ± 360 |
| | | 1 | 2018 | 850 | 850 | 1700 |
| | | 1 | 2009 | 1400 | 1800 | 3200 |
| | | 1 | 2000 | 450 | 1700 | 2100 |
| | | 1 | 1991 | 1000 | 510 | 1500 |
| | | 1 | 1927 | 0 | 0 | 0 |
| 2 | 0.6 | 4 | 1990–2018 | 980 ± 180 | 3400 ± 600 | 4400 ± 620 |
| | | 1 | 2018 | 1500 | 2800 | 4300 |
| | | 1 | 2010 | 930 | 5000 | 6000 |
| | | 1 | 2000 | 830 | 3600 | 4500 |
| | | 1 | 1990 | 670 | 2200 | 2900 |
| | | 1 | 1941 | 0 | 0 | 0 |
| 3 | 7 | 4 | 1991–2018 | 380 ± 150 | 510 ± 65 | 890 ± 200 |
| | | 1 | 2018 | 0 | 330 | 330 |
| | | 1 | 2010 | 300 | 600 | 900 |
| | | 1 | 2000 | 610 | 610 | 1200 |
| | | 1 | 1991 | 630 | 500 | 1100 |
| 4 | 3 | 4 | 1990–2018 | 200 ± 120 | 720 ± 220 | 920 ± 280 |
| | | 1 | 2018 | 340 | 340 | 670 |
| | | 1 | 2009 | 450 | 1200 | 1700 |
| | | 1 | 2001 | 0 | 990 | 990 |
| | | 1 | 1990 | 0 | 360 | 360 |
| | | 1 | 1864 | 230 | 0 | 230 |
| All | | 16 | 1990–2018 | 620 ± 110 | 1500 ± 340 | 2100 ± 410 |

Compared to very few other MP studies reported for Finnish lakes, the detected MP concentrations were much higher in both water and sediment in this case study, which highlights the possible effect of WWTP effluent on MP concentrations in the receiving water body. Uurasjärvi et al. sampled surface water from Lake Kallavesi with a Manta trawl (>333 µm) and with a pump with an on-site filtration setup (>20 µm), and reported average MP concentrations of 0.00027 and 0.169 MP/L, respectively [11]. In the case of lake sediments, Scopetani et al. reported average MP concentrations of 396 MPs/kg dw for Lake Vesijärvi and related Pikku-Vesijärvi pond in Lahti [154]. They identified MPs with an FPA-FTIR-microscope, which had resolution of 5.5 µm. Still, MPs were counted visually, which probably causes the exclusion of the smallest MPs.

As listed in Table 5.6, on a global scale average concentrations of detected MPs have varied at 0.00027–0.003 MP/L, when lake water has been sampled with a Manta trawl (>300 µm) [11, 12, 92]. When also smaller sized MPs have been included, the reported concentrations have been considerably higher, varying from 119 MP/L (>0.45 µm) in the Great Lakes [16] to 0.01379 MP/L (>100 µm) in Hungary [14]. The detected level of microplastics in this case study (0.72 MP/L) were similar or higher than the previously reported MP concentrations with similar size limits (>100–125 µm) [14], [152], even though comparison of results is only indicative due to the different size limitations and practices during sample processing. In this case study, high recovery of MPFs (85%) can explain the comparably high MP concentrations. For the methods used in other studies, no information was provided for the recovery of MPFs [14, 152].

Table 5.6: Previously reported concentrations of microplastics (n/L or n/km$^2$) in lake water with details of sampling. Only results, which were based on a spectroscopic confirmation of subsamples or all particles and fibres are included. The lower size limit is based on the physical limit via filtration or the limit caused by identification methods.

| **Location** | **Ref.** | **Sampling depth (m)** | **Average volume (L)** | **No. of sites** | **No. of replicates per site** | **Overall lower size limit (µm)** | **MP concentration** | **Identification method** |
|---|---|---|---|---|---|---|---|---|
| Lake Kallavesi, Finland | [11] | 0–0.16 | 58 000 | 8 | 1 | 333 | 0.00027 MP/L | µ-FTIR$^{2)}$ |
| Lake Taihu, China | [91] | 0–0.3 | | 11 | 1 | 333 | 10000–6800000 MP/km$^2$ | µ-FTIR, SEM/EDS$^{1)}$ |
| Lake Chiusi, Italy | [12] | 0–0.1 | | 2 | 1 | 300 | 0.00302 MP/L | UV-microscopy for Nile red stained MPs, SEM$^{1)}$ |
| Lake Bolsena, Italy | [12] | 0–0.1 | | 4 | 1 | 300 | 0.00222 MP/L | UV-microscopy for Nile red stained MPs, SEM$^{1)}$ |
| Lake Victoria, Uganda | [92] | Near-surface | 46228 | 8 | 3 | 300 | 0.00073 MP/L<br>120588 MP/km$^2$ | µ-FTIR$^{1)}$ |
| Lake Ontario, Canada | [152] | Near-surface | 10 | 4 | 3 | 125 | 0.8 MP/L | µ-Raman$^{1)}$ |
| *Lake Saimaa, Finland* | *This study* | *0.35–0.65* | *10.4* | *3* | *3* | *100* | *0.77 MP/L* | *µ-Raman, py-GC/MS*$^{1)}$ |
| *Lake Saimaa, Finland* | *This study* | *4.85–5.15* | *10.4* | *1* | *3* | *100* | *0.58 MP/L* | *µ-Raman, py-GC/MS*$^{1)}$ |
| Lakes, fishponds, and rivers, Hungary | [14] | 0.1–0.2 | 1492 | 13 | 1 | 100 | 0.01379 MP/L | µ-FTIR-ATR$^{2)}$ |

Table 5.6: (continued)

| Location | Ref. | Sampling depth (m) | Average volume (L) | No. of sites | No. of replicates per site | Overall lower size limit (μm) | MP concentration | Identification method |
|---|---|---|---|---|---|---|---|---|
| Lake Poyang, China | [14] | 0–1 | 20 | 21 | 2 | 50 | 5–34 MP/L | μ-Raman (SERS)[1] |
| 8 lakes in Changsha, China | [155] | Near-surface | 40 | 8 | 3 | 50 | 2.425–7.05 MP/L | μ-Raman, SEM[1] |
| Lake Kallavesi, Finland | [11] | 0–0.16 | 6.2–468* | 6 | 2 | >25 | 0.1688 MP/L | μ-FTIR[2] |
| Lake Taihu, China | [91] | Near-surface | 5 | 11 | 1 | 5 | 3.4–25.8 MP/L | μ-FTIR, SEM/EDS[1] |
| Great Lakes, USA | [16] | Near-surface | 1 | 8 | 1 | 0.45** | 119 MP/L | μ-FTIR[1] |

[1] Only representative particles and fibres or subsamples identified.
[2] All particles and fibres identified.
* Depending on the size fraction
** The lower size limit is likely higher due to manual examination with microscope.

Similarly, the detected MP concentrations in sediments (2100 ± 410 MP/kg dw, >100 µm) of this case study were higher than in many other recently studied lakes (Table 5.7). In Norway, MP concentrations (>36 µm) in the urbanized Lake Mjøsa and remote Lake Femunden were 40–7310 MP/kg dw and 0–690 MP/kg dw, respectively [17]. In China, 54–506 MP/kg dw (>50 µm) and 11–235 MP/kg dw (>5 µm) were detected in the sediments of Lake Poyang and Lake Taihu, respectively [15, 91]. In contrast, 7700 MP/kg dw (>100 µm) and 10476 MP/kg dw (>63 µm) were detected in the surface layer of sediments in Lake Donghu, China [125] and Lake Tollense, Germany [156], respectively.

The observations in this study are in line with other recent studies, where higher MP concentrations have been detected in both water and sediment close to WWTPs or other urban sources of MPs [8, 11, 17, 58, 152]. In Finland, higher MP concentrations (>20 µm) were detected in water close to WWTP discharge sites, harbours and snow dumping sites compared to other parts of Lake Kallavesi [11]. In Bergen, Norway, much higher MP concentrations (12 000–200 000 MP/kg dw, >11 µm) were reported for marine sediments close to WWTP discharge sites than what were detected in this case study [97]. The studied WWTPs in Bergen were operated with only primary treatment [97], and, hence, were likely to have discharged relatively more MPs into the recipient water body than the secondary WWTP studied in this case study. Still, since the majority of the MPs are usually removed in the primary treatments [48–51], the lower size limits used in this case study (100 µm) and the study by Haave et al. (11 µm) probably explain the large difference in the observed MP concentrations more than the differences in the related WWTPs.

Table 5.7: Previously reported concentrations of microplastics (n/kg dw) in bottom sediment with details of sampling. Only results, which were based on a spectroscopic confirmation of subsamples or all particles and fibres are included. The lower size limit is based on the physical limit via filtration or the limit caused by identification methods.

| **Location** | **Ref.** | **Presented years** | **Included depth of sediment (cm)** | **Sample size** | **No. of sites** | **No. of replicates per site** | **Overall lower size limit (µm)** | **MP concentrations (MP/kg dw)** | **Identification method** |
|---|---|---|---|---|---|---|---|---|---|
| Hampstead No.1 pond, UK | [130] | 1935–2009 | 0–50 | 20–90 g dw | 1 | 1 | 500 | 16–539 | µ-Raman$^{1)}$ |
| Lake Victoria, Uganda | [140] |  | 0–5 | 400 g ww | 12 | 5 | 300 | 0–15 | FTIR$^{1)}$ |
| *Lake Saimaa, Finland* | *This study* | *1990–2018* | *0–7 to 0–22* | *29.6–74.1 g ww* | *4* | *1* | *100* | *330–6000* | *µ-Raman*$^{1)}$ |
| Lake Donghu, China | [125] | 1959–2018 | 0–57 | 15–20 g ww | 1 | 1 | 100 | 741–7707 | Raman and SEM$^{1)}$ |
| Lake Ontario, Canada | [63] | 1995–2013 | 0–8 |  | 2 | 1 | n/a * | 87–616 | µ-ATR-FTIR$^{2)}$ |
| Lake Tollense, Germany | [156] |  |  | 100 mL (wet) | 8 | 4 | 63 | 2691–17504 | Fluorescent microscope$^{2)}$<br>µ-Raman$^{1)}$ |
| Lake Poyang, China | [15] |  |  |  | 21 | 1 | 50 | 54–506 | µ-Raman (SERS)$^{1)}$ |
| Lake Mjøsa, Norway | [17] | 1980s–2018 | 0–8 |  | 20 | 1 | 36 | 40–7310 | µ-FTIR, ATR-FTIR$^{2)}$<br>py-GC/MS$^{1)}$ |

Table 5.7: (continued)

| **Location** | **Ref.** | **Presented years** | **Included depth of sediment (cm)** | **Sample size** | **No. of sites** | **No. of replicates per site** | **Overall lower size limit (µm)** | **MP concentrations (MP/kg dw)** | **Identification method** |
|---|---|---|---|---|---|---|---|---|---|
| Lake Femunden, Norway | [17] | | 0–8 | | 10 | 1 | 36 | 0–690 | µ-FTIR, ATR-FTIR[2]<br>py-GC/MS[1] |
| Estuary in Bergen, Norway | [97] | | 0–1 | 1000 g ww | 5 | 3 | 11 | 71100–205085 | FPA-µ-FTIR (<500 µm),<br>ATR-FTIR |
| Lake Vesijärvi and Pikku Vesijärvi Pond, Finland | [154] | | 0–5 | | 2 | 1 | 5 * | 396 | µ-FTIR[1] |
| Lake Taihu, China | [91] | | | 1000 g ww | 11 | 3 | 5 * | 11–235 | µ-FTIR or SEM/EDS[1] |

[1] Only representative particles and fibres or subsamples identified.

[2] All particles and fibres identified.

* The lower size limit is likely >100 µm due to optical examination with stereo microscope.

Overall, when the effect of WWTP effluents in the recipient area are studied, other sources of MPs should also be considered. In case of the present study, the effect of urban discharges via stormwaters or direct emissions due to improperly sorted wastes cannot be distinguished from the MP discharges via effluents. This aspect could have been tackled by collecting samples upstream from the WWTP discharge site. In addition, atmospheric fallout can cause MP discharges in the studied environment [21]. However, according to the detected MP concentrations in the sediment samples (Table 5.5), the effect of the Kenkäveronniemi WWTP discharges on abundance of MPs seemed very probable. Regardless of the source, possible reasons for the quick sedimentation of MPs after discharge could be the discharge of effluents to the bottom layer of lake and the relatively small amount of dilution compared to sampling points further away from the discharge site. Other issues, which might have an influence on fast sedimentation can be biofouling or other attached material, which can increase the density of the MPs [59]. Hence, locating the discharge pipe at the bottom of the water body is likely to enhance the burial of WWTP-related MPs into the sediment and prevent the distribution of MPs further in the aquatic environment. After more detailed studies, this kind of setup could also be utilized to prevent the spreading of MPs in the aquatic environment.

### 5.3.2 The buried history of microplastic pollution

The formation of layered sediment allows bottom sediment samples collected from accumulation areas to reveal past temporal changes in the conditions of the water body. The estimation of the age of sediment layers requires additional dating of the sediment cores, which can be done for example according to Cs-137 and Pb-210 activities [125].

This phenomenon can also be utilized in MP research, as was demonstrated in this case study. The collected sediment cores were 32–42 cm long and their composition varied from fine clay to rocky sand between the sampling sites and within individual sediment cores. According to the Cs-137 activity profiles (Figure 5.3), the sedimentation rates varied between 0.3 cm/y (site 4) to 0.8 cm/y (site 3) (Table 5.8). The dating of the sediment cores revealed some mixing of the sediment over time, especially in sites 2 and 4, where the Cs-137 activity stayed at high levels towards the surface layers. Thus, the calculated years are only estimations of the formation time of each layer and the mixing of the sediment layers may have also affected the MP concentrations.

Table 5.8: Basic information about sediment samples.

| Site | Lake basin | Depth of collected sample (cm) | Years presented | Sedimentation rate (cm/y) | Water content (%) | Organic matter content of dry weight (LOI550) (%) |
|---|---|---|---|---|---|---|
| 1 | Launialanselkä | 38 | 1927–2018 | 0.4 | 68–92 | 7.2–15.4 |
| 2 | Pappilanselkä | 42 | 1941–2018 | 0.5 | 56–91 | 4.3–16.8 |
| 3 | Kyyhkylänselkä | 32 | 1981–2018 | 0.8 | 29–92 | 1.2–13.3 |
| 4 | Annilanselkä | 42 | 1864–2018 | 0.3 | 72–92 | 7.0–14.2 |

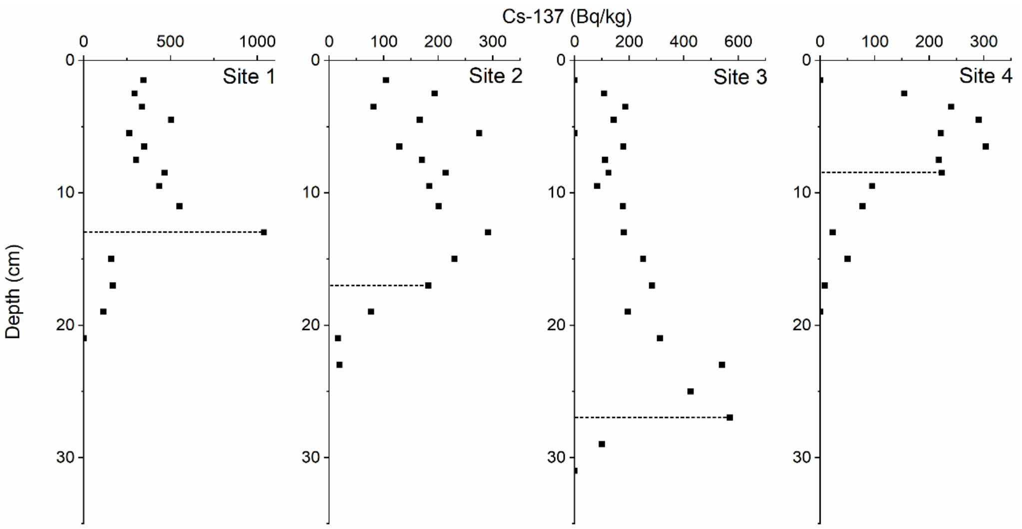

Figure 5.3: Cesium-137 activity in the sediment samples. The peak or clear increase in Cs-137 activity was caused by the Chernobyl accident (1986) and is marked with dash line. The dating of the analysed sediment samples was based on these results.

In this case study, the highest concentrations of MPPs, MPFs and MPs in general were detected in sediment samples representing the year 2010 (Table 5.5), even though the differences between layers were not significant (p=0.58–0.86, 1-way ANOVA). Nevertheless, the observed MP concentrations indicated that MP pollution has slightly increased in the studied lake area in recent decades. The same trends have been previously observed in the sediments of lakes [17, 63, 125, 130], seas [8], beaches [58] and salt marshes [157].

In addition, increased MP concentrations detected in sediments are in line with the increased production rates of plastics [2]. In Figure 5.4, the MP concentrations in each sediment layer of this case study are combined with data by Turner et al. [130] and Dong et al. [125], presenting variation of MPs in the sediment cores of a British pond and a Chinese lake, respectively. When this data is related with the annual production of non-fibrous plastics and polyester fibres, it seems that the increasing production rates of plastics also cause an increase in the rates of microplastic discharges into the aquatic environment.

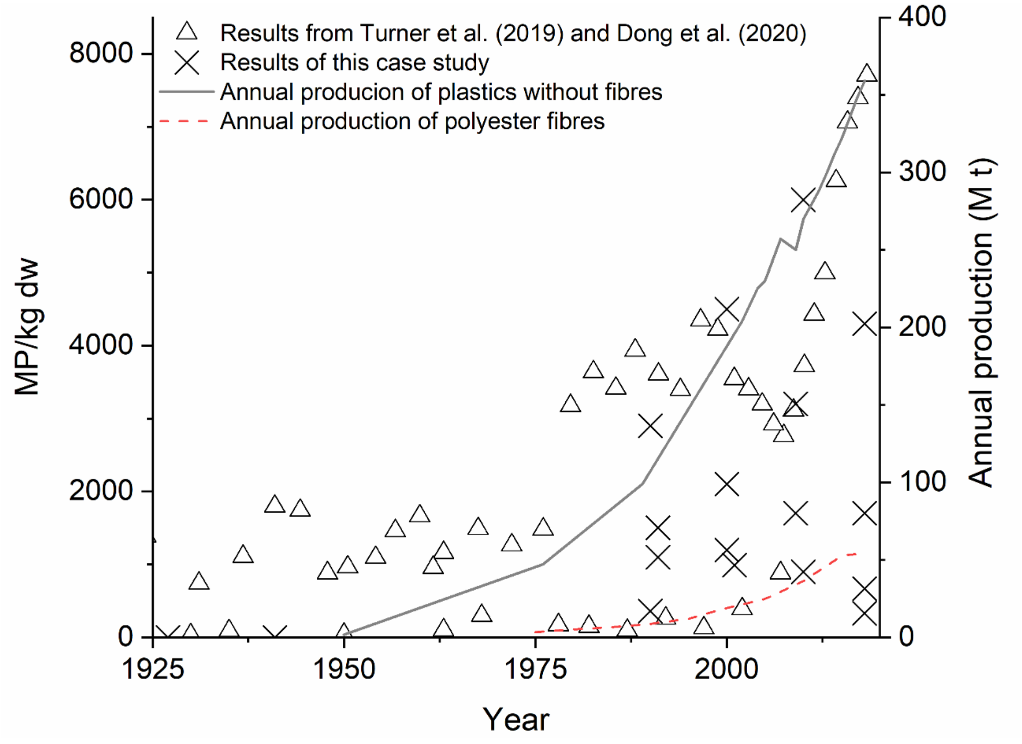

Figure 5.4: Detected concentrations of MPs in sediment cores in relation to the global production level of plastics [158–163]. Microplastics data include raw data by Turner et al. (>500 µm) [130], Dong et al. (>100 µm) [125] and this case study (>100 µm).

In studies focusing on the layered structure of the sediment, samples are usually collected with different kinds of corers, including Limnos samplers. Unfortunately, sediment sampling with Limnos also revealed some drawbacks, which should be considered in future sampling. In the topmost sediment layer, MP concentrations (1800 ± 900 MP/kg dw) were lower than in the samples representing the year 2010 (2900 ± 1100 MP/kg dw) or even all samples representing the last 30 years (2200 ± 480 MP/kg dw). The most recently formed sediment layers (depth 0–1 cm) had a higher water content (92 ± 0.3%) than the other included layers (79 ± 2.1%) and, thus, is looser than the other layers. Therefore, part of the MPs might have been flushed away from the loose top layer due to the movement of water during the sampling.

Because no increase in MP concentrations in relation to the age of the sediment was found at site 3 (Table 5.5), which was located the furthest away from the WWTP, at least part of the detected MPs may have originated from other sources than the WWTP. This highlights the need for focusing also on other sources than wastewater in the future monitoring studies.

Some MPPs were detected for the oldest sediment layer at site 4, corresponding to a concentration of 230 MP/kg dw. The observed MPPs were likely to be caused either by contamination or by the mixing of sediment because no plastics should have been present according to the calculated formation year of 1864. No MPs were detected in other sediment samples representing the time before the mass production of plastics in this case study. Generally, water currents and bioturbation can cause MPs to be transferred in sediment cores [164], which in turn may reduce the reliability of relating MP concentrations to the age of sediment layers.

According to this case study and other previous studies [8, 17, 58, 63, 125, 130, 157], sediment cores could still be utilized more in future environmental monitoring of microplastics. They could be helpful in assessing the efficiency of past improvements in wastewater treatment processes in the light of MP discharges. In that case, replicate samples should be collected in the discharge area to represent the possible variation in the MP concentrations.

## 5.4 Ecotoxicity and environmental effects

To assess the possible ecotoxicological and ecological effects of WWTP-related MPs, different compartments should be discussed separately [17, 146]. For example, if discharged MPs sink to the bottom rapidly due to attached material, bottom dwelling organisms may be exposed to high concentrations of MPs in proximity to the discharge site, while lake water remains relatively free of MPs. In the light of this case study, the most relevant discussion should be pointed to possible effects of PET fibres and PE fragments, which were common in the wastewater and environmental samples.

To assess the ecotoxicological effects, water fleas are commonly used as model species representing the organisms living in the water phase. Even though PET fibres are not likely to be ingested by water fleas, they can cause deformities in carapaces and antennas [165]. In addition, PET fibre concentrations above 4300 MPF/L (500 µg/L, mostly 100–400 µm) have been found to reduce the reproductivity and body size of *Ceriodaphnia dubia* in 8-d chronic exposure, which is likely to be due to entanglement [165]. In the same study, PE beads, in sizes of 1–4 µm, had an EC50 value of 958 µg/L for the reproduction of *C. dubia* compared to 429 µg/L for PET fibres [165]. In the lake water samples of this case study, even the highest detected PE (39.8 µg/L) and PET fibre (0.4 MPF/L) concentrations were much lower than the effective values reported by Ziajahromi et al. [165]. Still, the detected MPs were much larger than the model MPs used in the study by Ziajahromi et al. [165], and the effects of smaller MPs cannot be generalized for other size classes.

In the case of sediments, long-term exposure to environmentally relevant MP concentrations can potentially affect benthic biota [166]. Bour et al. noticed a dose-dependent reduction in the total energy of bivalves exposed to PE particles (125–500 µm) in concentrations of 1–25 mg/kg [166]. Smaller PE particles have also been found to reduce survival, growth and emergence of *Chironomus tepperi* in concentration of 500 MPP/kg dw of sediment, with the effects being most severe with particles in the size range of 10–27 µm [167]. Even though larger PE particles (100–126 µm) did not affect the growth or survival of *C. tepperi* in similar concentrations, they reduced the number of emerged adults [167]. As chironomids are common in Finnish aquatic environments, especially in eutrophic lakes [168], similar kinds of effects are likely to happen also in the Finnish environment, if concentrations of PE containing MPs increase to those levels. In this case study, the total MP concentrations varied between 330 and 6000 MP/kg dw in the surface layers of the bottom sediment and were highest closer to the WWTP discharge site. On average, 11% of detected MPs in all sediment layers were PE, but the studied MPs were overall larger than the ones used in most of the exposure studies [167] and their weight was not studied in this case study.

Surprisingly, very few ecotoxicity studies have focused on the impacts of PET fibres on organisms living in sediments. Setyorini et al. tested chronic toxicity of PET fibres (50 µm) with larval *Chironomus riparius* in a 28-day long exposure [169]. They reported both ingestion of PET fibres and the transfer of fibres to the next life stages. Nevertheless, no effects on survival, general stress response or growth were observed for the studied PET concentrations (500–50000 n/kg dw). Similarly, snails living in the soil have been found to eat PET fibres, which can damage their intestines with negative impacts on feeding and excretion [170].

Overall, in this study, PE (MPPs, >100 µm) and PET (MPFs, >100 µm) were detected in concentrations which should not cause effects in the environment according to current knowledge. Nevertheless, the possibility of ecotoxicological effects caused by the current PE and PET concentrations cannot be totally excluded, because our examinations excluded MPs smaller than 20 and 100 µm based on py-GC/MS and Raman micro-spectroscopy analysis, respectively. According to previous studies, the number of MPs increases rapidly when smaller MPs are included in the study [11, 13, 93–98]. Thus, the total concentration of MPs is likely to be much higher than the MP concentrations detected in this case study, and those concentrations might affect organisms living in the study area. For example small perches and vendaces in Lake Kallavesi, Finland, were reported to have especially MPs in sizes between 20–100 µm in their gastrointestinal tracts [27]. This further highlights the importance of focusing on the smallest MPs in future studies. In addition, it needs to be recalled that the limitations in the sample processing and identification of MPs might have caused an underestimation of the MP concentrations in this case study.

Hence, further investigations are needed to assess the abundance of MPs smaller than 100 µm together with nanoplastics and the potential risks related to them. In addition, different ways of MPPs and MPFs to attack organisms highlight the need of reporting separate results for differently shaped MPs also in the future. Furthermore, this study did not focus on other possible pollutants, whose ecotoxicity might be enhanced by their attachment to the surface of MPs. Because part of the MPs in the studied area are assumed to be discharged from the WWTP, they may have a higher possibility to act as a vector for other pollutants present in wastewater.

# 6 Conclusions and recommendations for future research

The microplastics research field has developed rapidly in the past decade and various methodologies are currently used to assess the environmental concentrations of MPs. This study discussed the variation of methods in the sampling and the pre-treatment of environmental samples together with the actual identification of MPs. Furthermore, the impacts of the variable methodology on the interpretation of the reported MP concentrations were discussed. In addition, this study offered recommendations on how the reporting of MP research could be improved and how the reported results should be interpreted in the light of the heterogeneous nature of MPs. To evaluate these issues, a case study was conducted at a local WWTP and its recipient lake area. The observations from the case study were considered along with the literature.

The most significant factor influencing the reported MP concentrations is the lower size limit that is set by the selected sampling, pre-treatment, and identification methods. Currently, these limits vary widely from the micrometre level to hundreds of micrometres. Generally, it has been demonstrated that the number of MPs increases exponentially, when the lower size limit is decreased. Overall, different methods and the following size limits support the collection of different kinds of data. This needs to be considered whenever MP studies are planned, the reported MP concentrations are compared to each other, or if the results are interpreted to assess the possible effects caused by MPs. For example, MP studies focusing on large areas with Manta trawls can provide a good insight into the general state of plastic pollution (>300 µm) in aquatic environments. On the other hand, if the aim is to assess the ecotoxicological risks of MPs, the sample processing techniques should allow the collection and identification of smaller MPs (>0–100 µm). In addition, the target unit, such as n/L or µg/L, and the required resolution affect the selection of identification methods. As a result, the lower size limit should always be provided with the reported microplastics results. Moreover, the provided size limit should consider all size-related limitations from the sampling to the final analysis of MPs.

Basically, microplastic research is currently conducted either to estimate the environmental concentrations of MPs or to evaluate the possible risks that MPs may cause to organisms. Due to the practical challenges in the sampling, pre-treatment, and identification, studies tackling these issues often target differently sized MPs. From the ecotoxicological perspective, the current way of monitoring microplastics does not provide suitable data for assessing the possible environmental effects caused by MPs. Most of the environmental monitoring studies exclude the smallest MPs from the results either by filtering them off during the sampling or pre-treatment, or by using identification methods that do not allow the detection of the smallest particles and fibres. In turn, ecotoxicological studies often provide information of the toxicity caused by the MPs in micrometres of size. In addition to the need for filling the size-related gap between these different research directions, the detected heterogeneity of MPs in the environmental samples should be considered also in ecotoxicological studies. This situation could be improved by including varying polymers and shapes, especially fibres, in ecotoxicological testing.

On the other hand, the sample processing methods still need to be optimized to allow focusing on the smallest MPs, which are likely to cause more (eco)toxicological effects. This will also help the research field in its next challenge, which is the examination of nanoplastics in different compartments of the environment. The size of nanoplastics (<100–1000 nm) allow them to pass through intestinal barriers or even to enter cells more than MPs. Thus, they can cause more severe (eco)toxicological effects, which need to be recognized and resolved. Hence, sampling, pre-treatment, and identification methods must be further improved to respond to the more demanding needs of NP studies.

Still, it is likely that the methodologies utilized in the sampling and pre-treatment of MP samples will not be standardized in the near future by the research community. Different samples need different pre-treatment steps to allow a reliable analysis of MPs in the targeted size range. Thus, different chemical and physical separation and purification methods are utilized, which not only affect the purity of the analysed sample, but also may cause the exclusion of some MPs due to their heterogenic properties. For example, oxidation with hydrogen peroxide has been widely used to remove organic material, but the aggressive oxidation process can affect the most sensitive MPs such as polyamides. Hence, more gentle enzymatic treatments have been developed, but their drawbacks lie in their multistep procedures with long processing times. Different variations of density separation, in turn, have been successfully utilized to separate MPs from denser, inorganic material.

Nevertheless, some practices in the MP research field need to be improved to allow the comparison and interpretation of differently collected MP results. It would be crucial to assess the representativeness of the resulting MP concentrations with the selected set of methods. One option would be the use of standardized test materials in recovery rate testing. These materials should represent the heterogeneity of MPs in terms of polymers, sizes, shapes, and colours. According to this study, the detection rates of MPFs were clearly lower than for larger MPPs, even if the lower size limit was reduced from 250 to 20 µm. Still, MPFs covered 40–92% of the MPs (>100–250 µm) detected in this case study, depending on the sample type and selected methods. Hence, it would be essential to also include fibres in the standardized model MPs. This would need to be supported by a wider variety of commercial MPs, including MPFs along with various MPPs.

However, some limitations are difficult to tackle even with improved recovery rate testing. For example, the use of Raman microscopy in this case study most probably caused the exclusion of MPs that contained dyes which overwrote the material spectrum or fluorescent MPs that could not be identified as plastics with the available high energy laser. Hence, these kinds of limitations should be highlighted openly in the reported results along with the recovery rates of the standardized materials with the selected methods. In addition, the contamination aspect should be emphasized and once there is a reliable way to process contamination control samples and a way to exclude contamination consistently and reliably from the detected MP levels it should be followed.

Thus, the reported MP concentrations should never be considered as accurate values, and the details behind the reported concentrations related to the sampling, pre-treatment, and identification should be discussed openly and considered during interpretation. These issues should be considered not only in the scientific discussion, but also in the public debate.

Overall, it can be also questioned whether it is reasonable to group this wide range of synthetic, particulate pollutants under an artificial term “microplastics”. As discussed in this study, the reported results for MPs can represent very different shots of the real situation of MP pollution. When the methods develop further, this wide research field might start to differentiate into smaller branches focusing on certain types of MPs. This would allow the usage of more specific pre-treatment and identification methods, when targeted polymers could be separated from the environmental matrix, for example according to their specific density.

In case of the studied CAS-based WWTP and its recipient area, MPs were removed effectively (98.3%) from the wastewater and directed into other waste streams when the lower size limit was set at 250 µm. Still, a continuous flow of MPs (1.0 MP/L) was discharged into the recipient lake area, which equals a daily dose of over 10 million MPs. If also MPs smaller than 250 µm would have been included in this case study, the MP concentrations in the effluent would have most likely been higher. Anyway, even if the MP concentrations in the treated effluents would be low, their continuous flow into the environment together with the high volumes of treated wastewater can cause significant cumulative pollution in the recipient area. This was further demonstrated in this study, as the effects of MP discharges from the WWTP or nearby city centre were obvious for the target years of 1990–2018. According to the detected MP concentrations (>100 µm), microplastic pollution has also slightly increased in the past 30 years in the studied area, which is most likely to have been caused by the increased consumption of plastics.

Overall, PET was found to be the most abundant polymer in all WWTP-related samples as well as in all sediment samples of this case study. The majority of the PET was detected as fibres. These findings were in line with the massive and increasing use of PET in the textile industry. The production of PET fibres increased from roughly 8 million tons to 58 million tons during 1990–2019, now covering over 50% of the global fibre production [1]. Together with previous studies, this further highlights the importance of including MPFs, especially PET fibres, not only in the monitoring studies, but also in the validation of the used methods as well as in the ecotoxicity testing in the future. They should also be considered in restrictions which target unintentionally discharged MPs.

Some recommendations for limiting the discharge and distribution of MPs from the WWTPs into the environment were made according to the observations from this case study. First, most of the MPs (>250 µm) were removed from the wastewater during the primary treatments at the studied WWTP. As an alternative approach to the current way of combining all sludge fractions before further treatments, sludges from primary and final clarifiers could be collected separately. Because sludge from final clarifiers should contain much lower concentrations of MPs, it could then be utilized more safely as a fertilizer. In addition, significantly higher levels of MPs (>100 µm) were detected in the bottom sediment near the discharge site of the WWTP compared to other parts of the recipient lake. Hence, at least part of the discharged MPs (>100 µm) are buried in the sediment near the WWTP. Therefore, locating discharge pipes for treated municipal effluents in the bottom of the water body could prevent the distribution of MPs further into the aquatic environment.

# References

[1] Opperskalski, S., Siew, S., Tan, E., & Truscott, L. (2020). Preferred Fiber & Materials: Market Report 2020 [Retrieved 30.5.2021], url: https://store.textileexchange.org/product-category/corporate-fiber-materials-reports/

[2] PlasticsEurope (2020). Plastics - the Facts 2020 [Retrieved 30.5.2021], url: https://www.plasticseurope.org/en/resources/publications/4312-plastics-facts-2020

[3] Carpenter, E. J., Anderson, S. J., Harvey, G. R., Miklas, H. P., & Peck, B. B. (1972). Polystyrene spherules in coastal waters. Science, 178(4062), 749–750. doi:10.1126/science.178.4062.749.

[4] Colton, J. B., Burns, B. R., & Knapp, F. D. (1974). Plastic Particles in Surface Waters of the Northwestern Atlantic. Science, 185(4150), 491–497. doi:10.1126/science.185.4150.491.

[5] Eriksen, M., Lebreton, L. C. M., Carson, H. S., Thiel, M., Moore, C. J., Borerro, J. C., Galgani, F., Ryan, P. G., & Reisser, J. (2014). Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLOS ONE, 9(12), e111913. doi:10.1371/journal.pone.0111913.

[6] Arthur, C., Baker, J., & Bamford, H. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. Sept 9-11, 2008 [Retrieved 1.6.2021], url: https://marinedebris.noaa.gov/sites/default/files/publications-files/TM_NOS-ORR_30.pdf

[7] Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environmental Science & Technology, 46(6), 3060–3075. doi.org:10.1021/es2031505.

[8] Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., Sakurai, S., Suzuki, T., Itoh, M., Okazaki, Y., Boonyatumanond, R., Zakaria, M. P., Weerts, S., & Newman, B. (2017). Microplastics in Sediment Cores from Asia and Africa as Indicators of Temporal Trends in Plastic Pollution. Archives of Environmental Contamination and Toxicology, 73(2), 230–239. doi:10.1007/s00244-017-0414-9.

[9] Morét-Ferguson, S., Law, K. L., Proskurowski, G., Murphy, E. K., Peacock, E. E., & Reddy, C. M. (2010). The size, mass, and composition of plastic debris in the western North Atlantic Ocean. Marine Pollution Bulletin, 60(10), 1873–1878. doi:10.1016/j.marpolbul.2010.07.020.

[10] Zobkov, M. B., Esiukova, E. E., Zyubin, A. Y., & Samusev, I. G. (2019). Microplastic content variation in water column: The observations employing a novel sampling tool in stratified Baltic Sea. Marine Pollution Bulletin, 138, 193–205. doi:10.1016/j.marpolbul.2018.11.047.

[11] Uurasjärvi, E., Hartikainen, S., Setälä, O., Lehtiniemi, M., & Koistinen, A. (2019). Microplastic concentrations, size distribution, and polymer types in the surface waters of a northern European lake. Water Environment Research, 92, 149–156. doi:10.1002/wer.1229.

[12] Fischer, E. K., Paglialonga, L., Czech, E., & Tamminga, M. (2016). Microplastic pollution in lakes and lake shoreline sediments – A case study on Lake Bolsena and Lake Chiusi (central Italy). Environmental Pollution, 213, 648–657. doi:10.1016/j.envpol.2016.03.012.

[13] Koelmans, A. A., Mohamed Nor, N. H., Hermsen, E., Kooi, M., Mintenig, S. M., & De France, J. (2019). Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. Water Research, 155, 410–422. doi:10.1016/j.watres.2019.02.054.

[14] Bordós, G., Urbányi, B., Micsinai, A., Kriszt, B., Palotai, Z., Szabó, I., Hantosi, Z., & Szoboszlay, S. (2019). Identification of microplastics in fish ponds and natural freshwater environments of the Carpathian basin, Europe. Chemosphere, 216, 110–116. doi:10.1016/j.chemosphere.2018.10.110.

[15] Yuan, W., Liu, X., Wang, W., Di, M., & Wang, J. (2019). Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. Ecotoxicology and Environmental Safety, 170, 180–187. doi:10.1016/j.ecoenv.2018.11.126.

[16] Whitaker, J. M., Garza, T. N., & Janosik, A. M. (2019). Sampling with Niskin bottles and microfiltration reveals a high prevalence of microfibers. Limnologica, 78, 125711. doi:10.1016/j.limno.2019.125711.

[17] Lusher, A., Buenaventura, N. T., Eidsvoll, D., Thrane, J.-E., Økelsrud, A., & Jartun, M. (2018). Freshwater microplastics in Norway – A first look at sediment, biota and historical plankton samples from Lake Mjøsa and Lake Femunden [Retrieved 1.6.2021], url: https://niva.brage.unit.no/niva-xmlui/handle/11250/2588713

[18] Leslie, H. A., Brandsma, S. H., van Velzen, M. J. M., & Vethaak, A. D. (2017). Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environment International, 101, 133–142. doi:10.1016/j.envint.2017.01.018.

[19] Van Cauwenberghe, L., Vanreusel, A., Mees, J., & Janssen, C. R. (2013).

Microplastic pollution in deep-sea sediments. Environmental Pollution, 182, 495–499. doi:10.1016/j.envpol.2013.08.013.

[20] Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L., & Gerdts, G. (2018). Arctic sea ice is an important temporal sink and means of transport for microplastic. Nature Communications, 9(1), 1505. doi:10.1038/s41467-018-03825-5.

[21] Dris, R., Gasperi, J., Saad, M., Mirande, C., & Tassin, B. (2016). Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Marine Pollution Bulletin, 104(1–2), 290–293. doi:10.1016/j.marpolbul.2016.01.006.

[22] Vianello, A., Jensen, R. L., Liu, L., & Vollertsen, J. (2019). Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Scientific Reports, 9(9), 8670. doi: 10.1038/s41598-019-45054-w.

[23] Rochman, C. M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S. B., Wu, T., Santoro, S., Werbowski, L. M., Zhu, X., Giles, R. K., Hamilton, B. M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., & Hung, C. (2019). Rethinking microplastics as a diverse contaminant suite. Environmental Toxicology and Chemistry, 38(4), 703–711. doi:10.1002/etc.4371.

[24] Talvitie, J., Heinonen, M., Pääkkönen, J.-P., Vahtera, E., Mikola, A., Setälä, O., & Vahala, R. (2015). Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. Water Science and Technology : A Journal of the International Association on Water Pollution Research, 72(9), 1495–1504. doi:10.2166/wst.2015.360.

[25] Vaughan, R., Turner, S. D., & Rose, N. L. (2017). Microplastics in the sediments of a UK urban lake. Environmental Pollution, 229, 10–18. doi:10.1016/j.envpol.2017.05.057.

[26] Horton, A. A., Svendsen, C., Williams, R. J., Spurgeon, D. J., & Lahive, E. (2017). Large microplastic particles in sediments of tributaries of the River Thames, UK – Abundance, sources and methods for effective quantification. Marine Pollution Bulletin, 114(1), 218–226. doi:10.1016/j.marpolbul.2016.09.004.

[27] Uurasjärvi, E., Sainio, E., Setälä, O., Lehtiniemi, M., & Koistinen, A. (2021) Validation of an imaging FTIR spectroscopic method for analyzing microplastics ingestion by Finnish lake fish (*Perca fluviatilis* and *Coregonus albula*). Environmental Pollution, 288, 117780. doi:10.1016/j.envpol.2021.117780.

[28] ECHA (2020). Background Document to the Opinion on the Annex XV report proposing restrictions onintentionally added microplastics. Helsinki, p. 207

[Retrieved 30.7.2021], url: https://echa.europa.eu/documents/10162/b56c6c7e-02fb-68a4-da69-0bcbd504212b

[29] Besseling, E., Redondo-Hasselerharm, P., Foekema, E. M., & Koelmans, A. A. (2019). Quantifying ecological risks of aquatic micro- and nanoplastic. Critical Reviews in Environmental Science and Technology, 49(1), 32–80. doi:10.1080/10643389.2018.1531688.

[30] Kirstein, I. V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M., & Gerdts, G. (2016). Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. Marine Environmental Research, 120, 1–8. doi:0.1016/j.marenvres.2016.07.004.

[31] Leiser, R., Wu, G.-M., Neu, T. R., & Wendt-Potthoff, K. (2020). Biofouling, metal sorption and aggregation are related to sinking of microplastics in a stratified reservoir. Water Research, 176, 115748. doi:10.1016/j.watres.2020.115748.

[32] Teuten, E. L., Saquing, J. M., Knappe, D. R. U., Barlaz, M. A., Jonsson, S., Björn, A., Rowland, S. J., Thompson, R. C., Galloway, T. S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P. H., Tana, T. S., Prudente, M., Boonyatumanond, R., Zakaria, M. P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., & Takada, H. (2009). Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), 2027–2045. doi:10.1098/rstb.2008.0284.

[33] Fendall, L. S., & Sewell, M. A. (2009). Contributing to marine pollution by washing your face: Microplastics in facial cleansers. Marine Pollution Bulletin, 58(8), 1225–1228. doi:10.1016/j.marpolbul.2009.04.025.

[34] Mateos-Cárdenas, A., O’Halloran, J., van Pelt, F. N. A. M., & Jansen, M. A. K. (2020). Rapid fragmentation of microplastics by the freshwater amphipod *Gammarus duebeni* (Lillj.). Scientific Reports, 10(1), 12799. doi:10.1038/s41598-020-69635-2.

[35] Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T., & Reemtsma, T. (2018). Tire wear particles in the aquatic environment - A review on generation, analysis, occurrence, fate and effects. Water Research, 139, 83–100. doi:10.1016/j.watres.2018.03.051.

[36] Liu, P., Zhan, X., Wu, X., Li, J., Wang, H., & Gao, S. (2020). Effect of weathering on environmental behavior of microplastics: Properties, sorption and potential risks. Chemosphere, 242, 125193. doi:10.1016/j.chemosphere.2019.125193.

[37] Song, Y. K., Hong, S. H., Jang, M., Han, G. M., Jung, S. W., & Shim, W. J. (2017). Combined Effects of UV Exposure Duration and Mechanical Abrasion on

Microplastic Fragmentation by Polymer Type. Environmental Science & Technology, 51(8), 4368–4376. doi:10.1021/acs.est.6b06155.

[38] Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. Environmental Science & Technology, 45(21), 9175–9179. doi:10.1021/es201811s.

[39] Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. Environmental Science & Technology, 50(11), 5800–5808. doi:10.1021/acs.est.5b05416.

[40] De Falco, F., Di Pace, E., Cocca, M., & Avella, M. (2019). The contribution of washing processes of synthetic clothes to microplastic pollution. Scientific Reports, 9(1), 6633. doi:10.1038/s41598-019-43023-x.

[41] Carney Almroth, B. M., Åström, L., Roslund, S., Petersson, H., Johansson, M., & Persson, N.-K. (2018). Quantifying shedding of synthetic fibers from textiles; a source of microplastics released into the environment. Environmental Science and Pollution Research, 25(2), 1191–1199. doi:10.1007/s11356-017-0528-7.

[42] Sillanpää, M., & Sainio, P. (2017). Release of polyester and cotton fibers from textiles in machine washings. Environmental Science and Pollution Research International, 24(23), 19313–19321. doi:10.1007/s11356-017-9621-1.

[43] Carr, S. A., Liu, J., & Tesoro, A. G. (2016). Transport and fate of microplastic particles in wastewater treatment plants. Water Research, 91, 174–182. doi:10.1016/j.watres.2016.01.002.

[44] Mintenig, S. M., Int-Veen, I., Löder, M. G. J., Primpke, S., & Gerdts, G. (2017). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Research, 108, 365–372. doi:10.1016/j.watres.2016.11.015.

[45] Talvitie, J., Mikola, A., Setälä, O., Heinonen, M., & Koistinen, A. (2017). How well is microlitter purified from wastewater? – A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant. Water Research, 109, 164–172. doi:10.1016/j.watres.2016.11.046.

[46] Park, H.-J., Oh, M.-J., Kim, P.-G., Kim, G., Jeong, D.-H., Ju, B.-K., Lee, W.-S., Chung, H.-M., Kang, H.-J., & Kwon, J.-H. (2020). National Reconnaissance Survey of Microplastics in Municipal Wastewater Treatment Plants in Korea. Environmental Science & Technology, 54(3), 1503–1512. doi:10.1021/acs.est.9b04929.

[47] Magnusson, K., & Norén, F. (2014). Screening of Microplastic Particles in and Down-stream a Wastewater Treatment Plant. IVL Swedish Environmental Research Institute.

[48] Gies, E. A., LeNoble, J. L., Noël, M., Etemadifar, A., Bishay, F., Hall, E. R., & Ross, P. S. (2018). Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. Marine Pollution Bulletin, 133, 553–561. doi:10.1016/j.marpolbul.2018.06.006.

[49] Blair, R. M., Waldron, S., & Gauchotte-Lindsay, C. (2019). Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period. Water Research, 163, 114909. doi:10.1016/j.watres.2019.114909.

[50] Ziajahromi, S., Neale, P. A., Telles Silveira, I., Chua, A., & Leusch, F. D. L. (2021). An audit of microplastic abundance throughout three Australian wastewater treatment plants. Chemosphere, 263, 128294. doi:10.1016/j.chemosphere.2020.128294.

[51] Michielssen, M. R., Michielssen, E. R., Ni, J., & Duhaime, M. B. (2016). Fate of microplastics and other small anthropogenic litter (SAL) in wastewater treatment plants depends on unit processes employed. Environ. Sci.: Water Res. Technol., 2(6), 1064–1073. doi:10.1039/c6ew00207b.

[52] Magni, S., Binelli, A., Pittura, L., Avio, C. G., Della Torre, C., Parenti, C. C., Gorbi, S., & Regoli, F. (2019). The fate of microplastics in an Italian Wastewater Treatment Plant. Science of The Total Environment, 652, 602–610. doi:10.1016/j.scitotenv.2018.10.269.

[53] Tagg, A. S., Sapp, M., Harrison, J. P., Sinclair, C. J., Bradley, E., Ju-Nam, Y., & Ojeda, J. J. (2020). Microplastic Monitoring at Different Stages in a Wastewater Treatment Plant Using Reflectance Micro-FTIR Imaging. Frontiers in Environmental Science, 8, 145. doi:10.3389/fenvs.2020.00145.

[54] Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F., & Rosal, R. (2020). Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge. Environmental Pollution, 259, 113837. doi:10.1016/j.envpol.2019.113837.

[55] Talvitie, J., Mikola, A., Koistinen, A., & Setälä, O. (2017). Solutions to microplastic pollution - Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Research, 123, 401–407. doi:10.1016/j.watres.2017.07.005.

[56] Vilpanen, M., & Toivikko, S. (2017). Yhdyskuntalietteen käsittelyn ja hyödyntämisen nykytilannekatsaus. Suomen Vesilaitosyhdistys ry.

[57] Van den Berg, P., Huerta-Lwanga, E., Corradini, F., & Geissen, V. (2020). Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils. Environmental Pollution, 261, 114198. doi:10.1016/j.envpol.2020.114198.

[58] Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K., & Janssen, C. R. (2011). Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Marine Pollution Bulletin, 62(10), 2199–2204. https://doi.org/10.1016/j.marpolbul.2011.06.030.

[59] Wu, N., Zhang, Y., Li, W., Wang, J., Zhang, X., He, J., Li, J., Ma, Y., & Niu, Z. (2020). Co-effects of biofouling and inorganic matters increased the density of environmental microplastics in the sediments of Bohai Bay coast. Science of The Total Environment, 717, 134431. doi:10.1016/j.scitotenv.2019.134431.

[60] Khatmullina, L., & Isachenko, I. (2017). Settling velocity of microplastic particles of regular shapes. Marine Pollution Bulletin, 114(2), 871–880. doi:10.1016/j.marpolbul.2016.11.024.

[61] Prata, J. C., da Costa, J. P., Duarte, A. C., & Rocha-Santos, T. (2019). Methods for sampling and detection of microplastics in water and sediment: A critical review. TrAC Trends in Analytical Chemistry, 110, 150–159. doi:10.1016/j.trac.2018.10.029.

[62] Zobkov, M., & Esiukova, E. (2017). Microplastics in Baltic bottom sediments: Quantification procedures and first results. Marine Pollution Bulletin, 114(2), 724–732. doi:10.1016/j.marpolbul.2016.10.060.

[63] Corcoran, P. L., Norris, T., Ceccanese, T., Walzak, M. J., Helm, P. A., & Marvin, C. H. (2015). Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record. Environmental Pollution, 204, 17–25. doi:10.1016/j.envpol.2015.04.009.

[64] Directive 2000/60/EC of 23 October 2000 establishing a framework for Community action in the field of water policy. (2000). Council of the European Union.

[65] Mäkynen, A. (2010). Pappilanselkä tehokkaasti käsiteltyjen jätevesien purkupaikkana. Ramboll Finland Oy.

[66] Lares, M., Ncibi, M. C., Sillanpää, M., & Sillanpää, M. (2019). Intercomparison study on commonly used methods to determine microplastics in wastewater and sludge samples. Environmental Science and Pollution Research, 26(12), 12109–12122. doi:10.1007/s11356-019-04584-6.

[67] Lares, M., Ncibi, M. C., Sillanpää, M., & Sillanpää, M. (2018). Occurrence, identification and removal of microplastic particles and fibers in conventional

activated sludge process and advanced MBR technology. Water Research, 133, 236–246. doi:10.1016/j.watres.2018.01.049.

[68] Masura, J., Baker, J., Foster, G., & Arthur, C. (2015). Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48 [Retrieved 4.6.2021], url: https://marinedebris.noaa.gov/sites/default/files/publications-files/noaa_microplastics_methods_manual.pdf

[69] Champagne, P., & Li, C. (2009). Enzymatic hydrolysis of cellulosic municipal wastewater treatment process residuals as feedstocks for the recovery of simple sugars. Bioresource Technology, 100(23), 5700–5706. doi:10.1016/j.biortech.2009.06.051.

[70] Löder, M. G. J., Imhof, H. K., Ladehoff, M., Löschel, L. A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I., Laforsch, C., & Gerdts, G. (2017). Enzymatic Purification of Microplastics in Environmental Samples. Environmental Science & Technology, 51(24), 14283–14292. doi:10.1021/acs.est.7b03055.

[71] Frias, J., Pagter, E., Nash, R., O’Connor, I., Carretero, O., Filgueiras, A., Viñas, L., Gago, J., Antunes, J., Bessa, F., Sobral, P., Goruppi, A., Tirelli, V., Pedrotti, M. L., Suaria, G., Aliani, S., Lopes, C., Raimundo, J., Caetano, M., Palazzo, L., de Lucia, G. A., Camedda, A., Muniategui, S., Grueiro, G., Fernandez, V., Andrade, J., Dris, R., Laforsch C., Scholz-Böttcher, B., & Gerdts, G. (2018). Standardised protocol for monitoring microplastics in sediments. doi:10.13140/rg.2.2.36256.89601/1.

[72] Karami, A., Golieskardi, A., Choo, C. K., Romano, N., Ho, Y. Bin, & Salamatinia, B. (2017). A high-performance protocol for extraction of microplastics in fish. Science of The Total Environment, 578, 485–494. doi:10.1016/j.scitotenv.2016.10.213.

[73] Crichton, E. M., Noël, M., Gies, E. A., & Ross, P. S. (2017). A novel, density-independent and FTIR-compatible approach for the rapid extraction of microplastics from aquatic sediments. Analytical Methods, 9(9), 1419–1428. doi:10.1039/c6ay02733d.

[74] Ziajahromi, S., Neale, P. A., Rintoul, L., & Leusch, F. D. L. (2017). Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. Water Research, 112, 93–99. doi:10.1016/j.watres.2017.01.042.

[75] Renner, G., Schmidt, T. C., & Schram, J. (2018). Analytical methodologies for monitoring micro(nano)plastics: Which are fit for purpose? Current Opinion in Environmental Science & Health, 1, 55–61. doi:10.1016/j.coesh.2017.11.001.

[76] Crawford, C. B., & Quinn, B. (2017). 10 - Microplastic identification techniques. In C. B. Crawford & B. Quinn (Eds.), Microplastic Pollutants, pp. 219–267. Elsevier. doi:10.1016/b978-0-12-809406-8.00010-4.

[77] Norén, F. (2007). Small plastic particles in coastal Swedish waters. N-research.

[78] Raman, C. V., & Krishnan, K. S. (1928). A New Type of Secondary Radiation. Nature, 121(3048), 501–502. doi:10.1038/121501c0.

[79] Lenz, R., Enders, K., Stedmon, C. A., Mackenzie, D. M. A., & Nielsen, T. G. (2015). A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement. Marine Pollution Bulletin, 100(1), 82–91. doi:10.1016/j.marpolbul.2015.09.026.

[80] Zhang, K., Xiong, X., Hu, H., Wu, C., Bi, Y., Wu, Y., Zhou, B., Lam, P. K. S., & Liu, J. (2017). Occurrence and Characteristics of Microplastic Pollution in Xiangxi Bay of Three Gorges Reservoir, China. Environmental Science & Technology, 51(7), 3794–3801. doi:10.1021/acs.est.7b00369.

[81] Frère, L., Paul-Pont, I., Moreau, J., Soudant, P., Lambert, C., Huvet, A., & Rinnert, E. (2016). A semi-automated Raman micro-spectroscopy method for morphological and chemical characterizations of microplastic litter. Marine Pollution Bulletin, 113(1), 461–468. doi:10.1016/j.marpolbul.2016.10.051.

[82] Käppler, A., Fischer, M., Scholz-Böttcher, B. M., Oberbeckmann, S., Labrenz, M., Fischer, D., Eichhorn, K. J., & Voit, B. (2018). Comparison of μ-ATR-FTIR spectroscopy and py-GCMS as identification tools for microplastic particles and fibers isolated from river sediments. Analytical and Bioanalytical Chemistry, 410(21), 5313–5327. doi:10.1007/s00216-018-1185-5.

[83] Steinmetz, Z., Kintzi, A., Muñoz, K., & Schaumann, G. E. (2020). A simple method for the selective quantification of polyethylene, polypropylene, and polystyrene plastic debris in soil by pyrolysis-gas chromatography/mass spectrometry. Journal of Analytical and Applied Pyrolysis, 147, 104803. doi:10.1016/j.jaap.2020.104803.

[84] Scopetani, C., Esterhuizen-Londt, M., Chelazzi, D., Cincinelli, A., Setälä, H., & Pflugmacher, S. (2020). Self-contamination from clothing in microplastics research. Ecotoxicology and Environmental Safety, 189, 110036. doi:10.1016/j.ecoenv.2019.110036.

[85] Suomen standardisoimisliitto SFS (1990). Veden, lietteen ja sedimentin kuiva-aineen ja hehkutusjäännöksen määritys (SFS 3008).

[86] Klaminder, J., Appleby, P., Crook, P., & Renberg, I. (2012). Post-deposition diffusion of 137Cs in lake sediment: Implications for radiocaesium dating.

Sedimentology, 59(7), 2259–2267. doi:10.1111/j.1365-3091.2012.01343.x.

[87] Callaway, J. C., DeLaune, R. D., & Patrick Jr., W. H. (1996). Chernobyl 137Cs used to determine sediment accretion rates at selected northern European coastal wetlands. Limnology and Oceanography, 41(3), 444–450. doi:10.4319/lo.1996.41.3.0444.

[88] Cadiou, J.-F., Gerigny, O., Koren, Š., Zeri, C., Kaberi, H., Alomar, C., Panti, C., Fossi, M. C., Adamopoulou, A., Digka, N., Deudero, S., Concato, M., Carbonell, A., Baini, M., Galli, M., & Galgani, F. (2020). Lessons learned from an intercalibration exercise on the quantification and characterisation of microplastic particles in sediment and water samples. Marine Pollution Bulletin, 154, 111097. doi:10.1016/j.marpolbul.2020.111097.

[89] Pico, Y., Alfarhan, A., & Barcelo, D. (2019). Nano- and microplastic analysis: Focus on their occurrence in freshwater ecosystems and remediation technologies. TrAC Trends in Analytical Chemistry, 113, 409–425. doi:10.1016/j.trac.2018.08.022.

[90] Magnusson, K. (2014). Microlitter and other microscopic anthropogenic particles in the sea area of Rauma and Turku, Finland. IVL Swedish Environmental Research Institute.

[91] Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., & Shi, H. (2016). Microplastics in Taihu Lake, China. Environmental Pollution, 216, 711–719. doi:10.1016/j.envpol.2016.06.036.

[92] Egessa, R., Nankabirwa, A., Ocaya, H., & Pabire, W. G. (2020). Microplastic pollution in surface water of Lake Victoria. Science of The Total Environment, 741, 140201. doi:10.1016/j.scitotenv.2020.140201.

[93] Covernton, G. A., Pearce, C. M., Gurney-Smith, H. J., Chastain, S. G., Ross, P. S., Dower, J. F., & Dudas, S. E. (2019). Size and shape matter: A preliminary analysis of microplastic sampling technique in seawater studies with implications for ecological risk assessment. Science of The Total Environment, 667, 124–132. doi:10.1016/j.scitotenv.2019.02.346.

[94] Ben-David, E. A., Habibi, M., Haddad, E., Hasanin, M., Angel, D. L., Booth, A. M., & Sabbah, I. (2021). Microplastic distributions in a domestic wastewater treatment plant: Removal efficiency, seasonal variation and influence of sampling technique. Science of The Total Environment, 752, 141880. doi:10.1016/j.scitotenv.2020.141880.

[95] Uurasjärvi, E., Pääkkönen, M., Setälä, O., Koistinen, A., & Lehtiniemi, M. (2021). Microplastics accumulate to thin layers in the stratified Baltic Sea. Environmental Pollution, 268, 115700. doi:10.1016/j.envpol.2020.115700.

[96] Lindeque, P. K., Cole, M., Coppock, R. L., Lewis, C. N., Miller, R. Z., Watts, A. J. R., Wilson-McNeal, A., Wright, S. L., & Galloway, T. S. (2020). Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. Environmental Pollution, 265, 114721. doi:10.1016/j.envpol.2020.114721.

[97] Haave, M., Lorenz, C., Primpke, S., & Gerdts, G. (2019). Different stories told by small and large microplastics in sediment - first report of microplastic concentrations in an urban recipient in Norway. Marine Pollution Bulletin, 141, 501–513. doi:10.1016/j.marpolbul.2019.02.015.

[98] Cabernard, L., Roscher, L., Lorenz, C., Gerdts, G., & Primpke, S. (2018). Comparison of Raman and Fourier Transform Infrared Spectroscopy for the Quantification of Microplastics in the Aquatic Environment. Environmental Science & Technology, 52(22), 13279–13288. doi:10.1021/acs.est.8b03438.

[99] Dyachenko, A., Mitchell, J., & Arsem, N. (2017). Extraction and identification of microplastic particles from secondary wastewater treatment plant (WWTP) effluent. Analytical Methods, 9(9), 1412–1418. doi:10.1039/c6ay02397e.

[100] Mason, S. A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., & Rogers, D. L. (2016). Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environmental Pollution, 218, 1045–1054. doi:10.1016/j.envpol.2016.08.056.

[101] Conley, K., Clum, A., Deepe, J., Lane, H., & Beckingham, B. (2019). Wastewater treatment plants as a source of microplastics to an urban estuary: Removal efficiencies and loading per capita over one year. Water Research X, 3, 100030. doi:10.1016/j.wroa.2019.100030.

[102] Gündoğdu, S., Çevik, C., Güzel, E., & Kilercioğlu, S. (2018). Microplastics in municipal wastewater treatment plants in Turkey: a comparison of the influent and secondary effluent concentrations. Environmental Monitoring and Assessment, 190(11), 626. doi:10.1007/s10661-018-7010-y.

[103] Bayo, J., Olmos, S., & López-Castellanos, J. (2020). Microplastics in an urban wastewater treatment plant: The influence of physicochemical parameters and environmental factors. Chemosphere, 238, 124593. doi:10.1016/j.chemosphere.2019.124593.

[104] Raju, S., Carbery, M., Kuttykattil, A., Senthirajah, K., Lundmark, A., Rogers, Z., SCB, S., Evans, G., & Palanisami, T. (2020). Improved methodology to determine the fate and transport of microplastics in a secondary wastewater treatment plant. Water Research, 173, 115549. doi:10.1016/j.watres.2020.115549.

[105] Bretas Alvim, C., Bes-Piá, M. A., & Mendoza-Roca, J. A. (2020). Separation and

identification of microplastics from primary and secondary effluents and activated sludge from wastewater treatment plants. Chemical Engineering Journal, 402, 126293. doi:10.1016/j.cej.2020.126293.

[106] Sutton, R., Mason, S. A., Stanek, S. K., Willis-Norton, E., Wren, I. F., & Box, C. (2016). Microplastic contamination in the San Francisco Bay, California, USA. Marine Pollution Bulletin, 109(1), 230–235. doi:10.1016/j.marpolbul.2016.05.077.

[107] Jiang, J., Wang, X., Ren, H., Cao, G., Xie, G., Xing, D., & Liu, B. (2020). Investigation and fate of microplastics in wastewater and sludge filter cake from a wastewater treatment plant in China. Science of The Total Environment, 746, 141378. doi:10.1016/j.scitotenv.2020.141378.

[108] Horton, A. A., Cross, R. K., Read, D. S., Jürgens, M. D., Ball, H. L., Svendsen, C., Vollertsen, J., & Johnson, A. C. (2021). Semi-automated analysis of microplastics in complex wastewater samples. Environmental Pollution, 268, 115841. doi:10.1016/j.envpol.2020.115841.

[109] Wang, F., Wang, B., Duan, L., Zhang, Y., Zhou, Y., Sui, Q., Xu, D., Qu, H., & Yu, G. (2020). Occurrence and distribution of microplastics in domestic, industrial, agricultural and aquacultural wastewater sources: A case study in Changzhou, China. Water Research, 182, 115956. doi:10.1016/j.watres.2020.115956.

[110] Wei, S., Luo, H., Zou, J., Chen, J., Pan, X., Rousseau, D. P. L., & Li, J. (2020). Characteristics and removal of microplastics in rural domestic wastewater treatment facilities of China. Science of The Total Environment, 739, 139935. doi:10.1016/j.scitotenv.2020.139935.

[111] Tang, N., Liu, X., & Xing, W. (2020). Microplastics in wastewater treatment plants of Wuhan, Central China: Abundance, removal, and potential source in household wastewater. Science of The Total Environment, 745, 141026. doi:10.1016/j.scitotenv.2020.141026.

[112] Franco, A. A., Arellano, J. M., Albendín, G., Rodríguez-Barroso, R., Quiroga, J. M., & Coello, M. D. (2021). Microplastic pollution in wastewater treatment plants in the city of Cádiz: Abundance, removal efficiency and presence in receiving water body. Science of The Total Environment, 776, 145795. doi:10.1016/j.scitotenv.2021.145795.

[113] Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., & Zeng, E. Y. (2018). Microplastics in sewage sludge from the wastewater treatment plants in China. Water Research, 142, 75–85. doi:10.1016/j.watres.2018.05.034.

[114] Ren, P., Dou, M., Wang, C., Li, G., & Jia, R. (2020). Abundance and removal characteristics of microplastics at a wastewater treatment plant in Zhengzhou.

Environmental Science and Pollution Research. doi:10.1007/s11356-020-09611-5.

[115] Franco, A. A., Arellano, J. M., Albendín, G., Rodríguez-Barroso, R., Zahedi, S., Quiroga, J. M$^{a}$., Coello, M$^{a}$. D. (2020). Mapping microplastics in Cadiz (Spain): Occurrence of microplastics in municipal and industrial wastewaters. Journal of Water Process Engineering, 38, 101596. doi:10.1016/j.jwpe.2020.101596.

[116] Castro, R. O., Silva, M. L., Marques, M. R. C., & de Araújo, F. V. (2016). Evaluation of microplastics in Jurujuba Cove, Niterói, RJ, Brazil, an area of mussels farming. Marine Pollution Bulletin, 110(1), 555–558. doi:10.1016/j.marpolbul.2016.05.037.

[117] Piribauer, B., Laminger, T., Ipsmiller, W., Koch, D., & Bartl, A. (2020). Assessment of microplastics in the environment - Fibres: The disregarded twin? Detritus, 9, 201–212. doi:10.31025/2611-4135/2019.13873.

[118] Klein, S., Worch, E., & Knepper, T. P. (2015). Occurrence and Spatial Distribution of Microplastics in River Shore Sediments of the Rhine-Main Area in Germany. Environmental Science & Technology, 49(10), 6070–6076. doi:10.1021/acs.est.5b00492.

[119] Bannick, C. G., Szewzyk, R., Ricking, M., Schniegler, S., Obermaier, N., Barthel, A. K., Altmann, K., Eisentraut, P., & Braun, U. (2019). Development and testing of a fractionated filtration for sampling of microplastics in water. Water Research, 149, 650–658. doi:10.1016/j.watres.2018.10.045.

[120] Hanvey, J. S., Lewis, P. J., Lavers, J. L., Crosbie, N. D., Pozo, K., & Clarke, B. O. (2017). A review of analytical techniques for quantifying microplastics in sediments. Analytical Methods, 9(9), 1369–1383. doi:10.1039/c6ay02707e.

[121] Li, J., Liu, H., & Paul Chen, J. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. Water Research, 137, 362–374. doi:10.1016/j.watres.2017.12.056.

[122] Lusher, A. L., Welden, N. A., Sobral, P., & Cole, M. (2017). Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Analytical Methods, 9(9), 1346–1360. doi:10.1039/c6ay02415g.

[123] Lessa Belone, M. C., Kokko, M., & Sarlin, E. (2021). Degradation of common polymers in sewage sludge purification process developed for microplastic analysis. Environmental Pollution, 269, 116235. doi:10.1016/j.envpol.2020.116235.

[124] Nguyen, N. B., Kim, M.-K., Le, Q. T., Ngo, D. N., Zoh, K.-D., & Joo, S.-W. (2021). Spectroscopic analysis of microplastic contaminants in an urban

wastewater treatment plant from Seoul, South Korea. Chemosphere, 263, 127812. doi:10.1016/j.chemosphere.2020.127812.

[125] Dong, M., Luo, Z., Jiang, Q., Xing, X., Zhang, Q., & Sun, Y. (2020). The rapid increases in microplastics in urban lake sediments. Scientific Reports, 10(1), 848. doi:10.1038/s41598-020-57933-8.

[126] Underwood, A. J., Chapman, M. G., & Browne, M. A. (2017). Some problems and practicalities in design and interpretation of samples of microplastic waste. Analytical Methods, 9(9), 1332–1345. doi:10.1039/c6ay02641a.

[127] Hale, R. (2017). Analytical challenges associated with the determination of microplastics in the environment. Analytical Methods, 9, 1326–1327. doi:10.1039/c7ay90015e.

[128] Munno, K., Helm, P. A., Jackson, D. A., Rochman, C., & Sims, A. (2018). Impacts of temperature and selected chemical digestion methods on microplastic particles. Environmental Toxicology and Chemistry, 37(1), 91–98. doi:10.1002/etc.3935.

[129] Pagter, E., Frias, J., & Nash, R. (2018). Microplastics in Galway Bay: A comparison of sampling and separation methods. Marine Pollution Bulletin, 135, 932–940. doi:10.1016/j.marpolbul.2018.08.013.

[130] Turner, S., Horton, A. A., Rose, N. L., & Hall, C. (2019). A temporal sediment record of microplastics in an urban lake, London, UK. Journal of Paleolimnology, 61(4), 449–462. doi:10.1007/s10933-019-00071-7.

[131] Zobkov, M., Zobkova, M., Galakhina, N., & Efremova, T. (2020). Method for microplastics extraction from Lake sediments. MethodsX, 7, 101140. doi:10.1016/j.mex.2020.101140.

[132] Woodall, L. C., Gwinnett, C., Packer, M., Thompson, R. C., Robinson, L. F., & Paterson, G. L. J. (2015). Using a forensic science approach to minimize environmental contamination and to identify microfibres in marine sediments. Marine Pollution Bulletin, 95(1), 40–46. doi:10.1016/j.marpolbul.2015.04.044.

[133] Coppock, R. L., Cole, M., Lindeque, P. K., Queirós, A. M., & Galloway, T. S. (2017). A small-scale, portable method for extracting microplastics from marine sediments. Environmental Pollution, 230, 829–837. doi:10.1016/j.envpol.2017.07.017.

[134] Scopetani, C., Chelazzi, D., Mikola, J., Leiniö, V., Heikkinen, R., Cincinelli, A., & Pellinen, J. (2020). Olive oil-based method for the extraction, quantification and identification of microplastics in soil and compost samples. Science of The Total Environment, 733, 139338. doi:10.1016/j.scitotenv.2020.139338.

[135] Ribeiro-Claro, P., Nolasco, M. M., & Araújo, C. (2017). Chapter 5 - Characterization of Microplastics by Raman Spectroscopy. In T. A. P. Rocha-Santos & A. C. B. T.-C. A. C. Duarte (Eds.), Characterization and Analysis of Microplastics (Vol. 75, pp. 119–151). Elsevier. doi:10.1016/bs.coac.2016.10.001.

[136] Primpke, S., A. Dias, P., & Gerdts, G. (2019). Automated identification and quantification of microfibres and microplastics. Analytical Methods, 11(16), 2138–2147. doi:10.1039/c9ay00126c.

[137] Löder, M. G. J., & Gerdts, G. (2015). Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal BT - Marine Anthropogenic Litter (M. Bergmann, L. Gutow, & M. Klages (Eds.); pp. 201–227). Springer International Publishing. doi:10.1007/978-3-319-16510-3_8.

[138] Fischer, M., & Scholz-Böttcher, B. M. (2017). Simultaneous Trace Identification and Quantification of Common Types of Microplastics in Environmental Samples by Pyrolysis-Gas Chromatography–Mass Spectrometry. Environmental Science & Technology, 51(9), 5052–5060. doi:10.1021/acs.est.6b06362.

[139] de Sá, L. C., Luís, L. G., & Guilhermino, L. (2015). Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environmental Pollution, 196, 359–362. doi:10.1016/j.envpol.2014.10.026.

[140] Egessa, R., Nankabirwa, A., Basooma, R., & Nabwire, R. (2020). Occurrence, distribution and size relationships of plastic debris along shores and sediment of northern Lake Victoria. Environmental Pollution, 257, 113442. doi:10.1016/j.envpol.2019.113442.

[141] Vandermeersch, G., Van Cauwenberghe, L., Janssen, C. R., Marques, A., Granby, K., Fait, G., Kotterman, M. J. J., Diogène, J., Bekaert, K., Robbens, J., & Devriese, L. (2015). A critical view on microplastic quantification in aquatic organisms. Environmental Research, 143, 46–55. doi:10.1016/j.envres.2015.07.016.

[142] Bayo, J., López-Castellanos, J., & Olmos, S. (2020). Membrane bioreactor and rapid sand filtration for the removal of microplastics in an urban wastewater treatment plant. Marine Pollution Bulletin, 156, 111211. doi:10.1016/j.marpolbul.2020.111211.

[143] Araujo, C. F., Nolasco, M. M., Ribeiro, A. M. P., & Ribeiro-Claro, P. J. A. (2018). Identification of microplastics using Raman spectroscopy: Latest developments and future prospects. Water Research, 142, 426–440. doi:10.1016/j.watres.2018.05.060.

[144] Majewsky, M., Bitter, H., Eiche, E., & Horn, H. (2016). Determination of

microplastic polyethylene (PE) and polypropylene (PP) in environmental samples using thermal analysis (TGA-DSC). Science of The Total Environment, 568, 507–511. doi:10.1016/j.scitotenv.2016.06.017.

[145] Salmi, P., Ryymin, K., Karjalainen, A. K., Mikola, A., Uurasjärvi, E., & Talvitie, J. (2021) Particle balance and return loops for microplastics in a tertiary-level wastewater treatment plant. Water Science & Technology, 84(1), 89–100. doi:10.2166/wst.2021.209.

[146] Kögel, T., Bjorøy, Ø., Toto, B., Bienfait, A. M., & Sanden, M. (2020). Micro- and nanoplastic toxicity on aquatic life: Determining factors. Science of The Total Environment, 709, 136050. doi:10.1016/j.scitotenv.2019.136050.

[147] Setälä, O., Fleming-Lehtinen, V., & Lehtiniemi, M. (2014). Ingestion and transfer of microplastics in the planktonic food web. Environmental Pollution, 185, 77–83. doi:10.1016/j.envpol.2013.10.013.

[148] Ha, J., & Yeo, M.-K. (2018). The environmental effects of microplastics on aquatic ecosystems. Molecular & Cellular Toxicology, 14(4), 353–359. doi:10.1007/s13273-018-0039-8.

[149] Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., & Xing, B. (2020). Microplastics in aquatic environments: Toxicity to trigger ecological consequences. Environmental Pollution, 261, 114089. doi:10.1016/j.envpol.2020.114089.

[150] Mahon, A. M., O’Connell, B., Healy, M. G., O’Connor, I., Officer, R., Nash, R., & Morrison, L. (2017). Microplastics in Sewage Sludge: Effects of Treatment. Environmental Science & Technology, 51(2), 810–818. doi:10.1021/acs.est.6b04048.

[151] Mak, C. W., Tsang, Y. Y., Leung, M. M.-L., Fang, J. K.-H., & Chan, K. M. (2020). Microplastics from effluents of sewage treatment works and stormwater discharging into the Victoria Harbor, Hong Kong. Marine Pollution Bulletin, 157, 111181. doi:10.1016/j.marpolbul.2020.111181.

[152] Grbić, J., Helm, P., Athey, S., & Rochman, C. M. (2020). Microplastics entering northwestern Lake Ontario are diverse and linked to urban sources. Water Research, 174, 115623. doi:10.1016/j.watres.2020.115623.

[153] Stanton, T., Johnson, M., Nathanail, P., MacNaughtan, W., & Gomes, R. L. (2020). Freshwater microplastic concentrations vary through both space and time. Environmental Pollution, 263, 114481. doi:10.1016/j.envpol.2020.114481.

[154] Scopetani, C., Chelazzi, D., Cincinelli, A., & Esterhuizen-Londt, M. (2019). Assessment of microplastic pollution: occurrence and characterisation in Vesijärvi lake and Pikku Vesijärvi pond, Finland. Environmental Monitoring and

Assessment, 191(11), 652. doi:10.1007/s10661-019-7843-z.

[155] Yin, L., Jiang, C., Wen, X., Du, C., Zhong, W., Feng, Z., Long, Y., & Ma, Y. (2019). Microplastic Pollution in Surface Water of Urban Lakes in Changsha, China. International Journal of Environmental Research and Public Health, 16(9), 1650. doi:10.3390/ijerph16091650.

[156] Hengstmann, E., Weil, E., Wallbott, P. C., Tamminga, M., & Fischer, E. K. (2021). Microplastics in lakeshore and lakebed sediments – External influences and temporal and spatial variabilities of concentrations. Environmental Research, 197, 111141. doi:10.1016/j.envres.2021.111141.

[157] Li, Jingjing, Huang, W., Xu, Y., Jin, A., Zhang, D., & Zhang, C. (2020). Microplastics in sediment cores as indicators of temporal trends in microplastic pollution in Andong salt marsh, Hangzhou Bay, China. Regional Studies in Marine Science, 35, 101149. doi:10.1016/j.rsma.2020.101149.

[158] Plastics Europe (2019). Plastics - the Facts 2019 [Retrieved 18.4.2021], url: https://www.plasticseurope.org/application/files/9715/7129/9584/FINAL_web_version_Plastics_the_facts2019_14102019.pdf.

[159] PlasticsEurope. (2013). Plastics - the Facts 2013 [Retrieved 18.4.2021], url: https://www.plasticseurope.org/application/files/7815/1689/9295/2013plastics_the_facts_PubOct2013.pdf.

[160] PlasticsEurope. (2015). Plastics - the Facts 2015 [Retrieved 18.4.2021], url: https://www.plasticseurope.org/application/files/3715/1689/8308/2015plastics_the_facts_14122015.pdf.

[161] PlasticsEurope. (2016). Plastics - the Facts 2016 [Retrieved 18.4.2021], url: https://www.plasticseurope.org/application/files/4315/1310/4805/plastic-the-fact-2016.pdf.

[162] PlasticsEurope. (2017). Plastics - the Facts 2017 [Retrieved 18.4.2021], url: https://www.plasticseurope.org/application/files/5715/1717/4180/Plastics_the_facts_2017_FINAL_for_website_one_page.pdf.

[163] Chemiefaser, I. (2018). Polyester fiber production globally 1975-2017 (in 1,000 metric tons). Statista [Retrieved 27.4.2021], url: https://www-statista-com.ezproxy.cc.lut.fi/statistics/912301/polyester-fiber-production-worldwide/.

[164] Näkki, P., Setälä, O., & Lehtiniemi, M. (2017). Bioturbation transports secondary microplastics to deeper layers in soft marine sediments of the northern Baltic Sea. Marine Pollution Bulletin, 119(1), 255–261. doi:10.1016/j.marpolbul.2017.03.065.

[165] Ziajahromi, S., Kumar, A., Neale, P. A., & Leusch, F. D. L. (2017). Impact of Microplastic Beads and Fibers on Waterflea (*Ceriodaphnia dubia*) Survival, Growth, and Reproduction: Implications of Single and Mixture Exposures. Environmental Science & Technology, 51(22), 13397–13406. doi:10.1021/acs.est.7b03574.

[166] Bour, A., Haarr, A., Keiter, S., & Hylland, K. (2018). Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. Environmental Pollution, 236, 652–660. doi:10.1016/j.envpol.2018.02.006.

[167] Ziajahromi, S., Kumar, A., Neale, P. A., & Leusch, F. D. L. (2018). Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. Environmental Pollution, 236, 425–431. doi:10.1016/j.envpol.2018.01.094.

[168] Luoto, T. P. (2011). The relationship between water quality and chironomid distribution in Finland—A new assemblage-based tool for assessments of long-term nutrient dynamics. Ecological Indicators, 11(2), 255–262. doi:10.1016/j.ecolind.2010.05.002.

[169] Setyorini, L., Michler-Kozma, D., Sures, B., & Gabel, F. (2021) Transfer and effects of PET microfibers in *Chironomus riparius*. Science of The Total Environment, 757, 143735. doi:10.1016/j.scitotenv.2020.143735.

[170] Song, Y., Cao, C., Qiu, R., Hu, J., Liu, M., Lu, S., Shi, H., Raley-Susman, K. M., & He, D. (2019). Uptake and adverse effects of polyethylene terephthalate microplastics fibers on terrestrial snails (*Achatina fulica*) after soil exposure. Environmental Pollution, 250, 447–455. doi:10.1016/j.envpol.2019.04.066.

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