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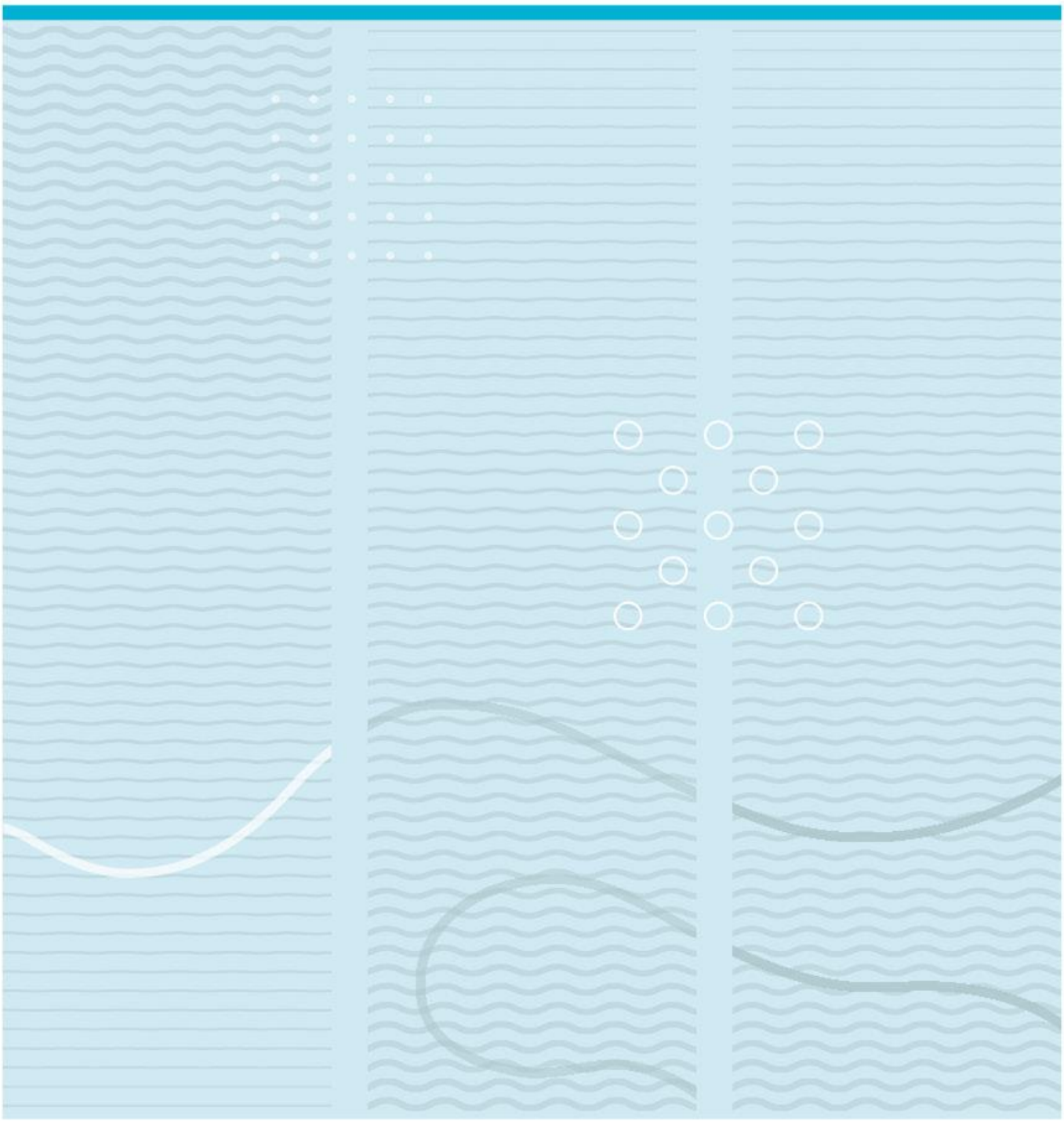
Microplastic in wastewater treatment plants in Telemark.



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This thesis is worth 60 study points

Abstract

Microplastics (defined as 5 mm to 1 µm in diameter) has been observed in zooplankton fish and human beings in various studies. The amount of microplastic is an increasing concern worldwide, and one of the sources that contributes to microplastic in the environment is wastewater treatment plants. The aim of this study was to analyse the presence of microplastics in inlet, effluent wastewater and sludge, and to develop an appropriate analysing procedure based on a thorough review of past literature. This study was executed in cooperation with SWECO located at Seljord, where a total of 8 wastewater treatment plants were chosen. Four of these plants carry out biological treatment and these plants were investigated in this thesis.

Sampling was done in the period from June to October 2018. The samples were extracted during the monthly sampling for chemical analysis. The sampling was carried out by staff at the wastewater treatment plants where 38 wastewater samples and 19 sludge samples were taken during this period. Wastewater and sludge sample was sterilised with 70% ethanol, then the wastewater was vacuum filtrated with Whatman GF/C filters. After the sterilisation the sludge was dried before adding Fenton's reagent followed by a flotation with filtered tap water and vacuum filtration. GF/C filters from both samples was then stored in aluminium coated petri dishes after the vacuum filtration and visual observed with digital stereo microscope (Karl Zeiss Discovery V20). Microplastics was detected in all samples in this study with an average of 8544 particles kg^{-1} dry weight (d.w.) for sludge and 13644 microplastic particles/L for inlet and 6255 microplastic particles/L outlet wastewater samples. There seems not to be any relation between income of wastewater (m^3/d) and the amount of microplastic observed within the samples. Particles from wastewater consisted of fibres (11%), fragments (59%) and beads (30%) and most of the particles were white/transparent and blue. Particles from sludge consisted of fibres/filaments (72%), fragments (23%) and beads (5%), where most observed particles where black fibres. For further research a procedure that includes removal of cellulose would make it easier for visual detection of MP in the samples.

Keywords: Microplastic, Microplastic method, Microplastic in wastewater and sludge.

Contents

Foreword

This Master thesis is carried out from June 2018 to May 2019. This thesis is presented at University of Southeast Norway (USN) with collaboration with the Swedish consultants (SWECO) located at Seljord in Telemark.

Firstly, I would like to thank my supervisor Synne Kleiven for her support and guidance throughout this project and Anita C. Kirkevold, (Vannområdekordinator, Midtre Telemark) for economic support. Secondly, I would like to thank Paul Windt and the co-workers at SWECO for the assistance to enabling the execution of this thesis. Without their support, knowledge and time to carry out the sampling, the completion of this thesis would not be possible. I would also like to thank all laboratory members at the institute of nature health and environment at USN.

I would also like to thank my family for their support and service during my education at Bø in Telemark.

Bø in Telemark - May 2019
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1 Introduction

Microplastic (MP) is by Peez et al. (2019) defined as particles with a diameter from 5 mm to 1 µm and is a widespread pollutant and widely regarded contaminant of global concern (Coppock et al. 2017). MP is derived from synthetic made polymers and are categorised in two terms based of its origin, primary and secondary MP. Primary MP are industrial produced particles below 5 mm such as pellets, or beads. It is designed to fill different purposes, from personal care products to air blasting for various industries. Secondary MP derives from fragmentation of larger plastic items (above 5 mm) such as paint, tyres or textile (GESAMP, 2016). One of the most commonly used plastic types is polyethylene, often used for packaging since it is a low-cost and a high durable product (Orhan & Büyükgüngör, 2000; GESAMP, 2016). Some of the other often used plastic types are Polyvinyl chloride (PVC), polypropylene (PP) and Polyethylene terephthalate (PET) (Revel et al. 2018). Each type of plastic has different density which means some plastic fragments are floating or sinking dependent on density of the medium.

MPs have been observed within fish (Bråte et al. 2016) and have also been detected in zooplankton. Several researchers have found that zooplankton could have the possibility to ingest MP by mistaking this for food (Setälä et al. 2014; Canniff & Hoang, 2018). To understand the possible effects of the sources or pathways of MPs it is important to have an indication of how much MP enters the environment, and where it goes. Effluent wastewater from wastewater treatment plants (WWTPs) is suspected to be one of the contributors of MP to the environment. Since wastewater works as a transportation medium for MP through the discharged wastewater, it could enters rivers or lakes (Murphy et al. 2016). The WWTP has a lot of different sources from households, institutions, industries to rainwater run-off from urban places and are likely to receive thousands of litres of wastewater per day.

There are several studies of MP analysis in wastewater and sludge but further research is needed. One of the common problems in these studies is the comparability and the amount of cellulose. Different method has been used to find an optimal procedure to analyse MP. Where it has been used wet peroxide oxidation and use of cellulase enzymes

to remove the cellulose (Xiaowei et al. 2018). Another study by Lusher et al. (2017) investigated different protocols for organic matter removal in sludge. Where they combined different removal solution with temperature and exposure time. And showed that Fenton's reagent (30% H_2O_2 and Fe^{2+}) to be effective for the removal of organic matter and time-efficiency.

One of the first common descriptions of MP was put forward by Arthur et al. (2009) where they defined MP particles to be less than 5 mm. Since this study came out, several scientific studies have emerged. The main focus ten years ago was of practical matter and the potential ecological effects. A newer study by GESAMP (2016) considered MP size range to be 5 mm to 1 nm, and described what is commonly defined as MP particles. Since, a lower limit for MP size range has not been defined yet, these varying measurements appears confusing. A study by Peez et al. (2019) defines MP particle size to be 5-0.001 mm in diameter which are the definition in this thesis is based. Since the logical term microplastic should stop on the limit of 1 µm before it gets to nanometres (nanoplastic). In a study by Frias et al. (2018) the recommended lower size limit for identifying MP in sediments should be 100 µm. When analysing a filter or sample it is increasingly difficult to identify the particles when they are of that size. However, when taking into account the ecological effects it is important to not exclude the MPPs below 100 µm. A study by Canniff & Hoang (2018) on the environmental impact of microbeads below 100 µm showed that Daphnia magna ingested a significant amount of microbeads with a size from 63-75 µm. Setälä et al. (2014) demonstrated that microspheres with a size of 10 µm was ingested by copepods and larvae. By excluding the small MPPs the ecological impact is difficult to predict and the lack of a visual standard method makes it difficult to compare.

There are three different main treatments that are mostly used in the WWTPs; mechanical, chemical and biological treatments. Some of the biological facilities probably reduces the amount of particle matter in the effluent water. The amount of MP from WWTP has shown to be substantial. Due to very few quantitative studies (Magnusson & Norén, 2014) predicting the outcome of sources as households or industry is difficult to estimate. Even though the majority of MP seems to be stored in the sludge, it still

constitutes a possible source through biosolid in farming and forestry, and it could be transferred to agriculture as fertilizer (Leslie et al. 2017). It is a challenge to extract MP due to the large variation of content of the sludge. Most sample procedures are time consuming and requires some sort of reagents to degrade the organic material as well as a solution for density separation (Sujathan et al. 2017). Due to the lack of standardised methods this study combines available procedures (Murphy et al. 2016; Horton. et al. 2017; Lusher et al. 2017) with some modifications. This master thesis encompasses two aims:

1. To analyse the microplastic content of samples from three locations in the sewage treatment process, inlet, outlet and sludge.

2. To optimize a procedure enabling analysing the microplastic in the samples from these three locations.

2 Materials and Methods

Background

Pre-test were performed to ensure that the methods were functioning according to plan. One water sample and one sediment sample were taken in Borgjaevju located south-southeast from USN, Bø in Telemark. The water sample was vacuum filtered based on the procedure developed in Murphy et al. (2016) and the sediment was sieved with pore size of 5-2-1 mm and 63 µm before a flotation test with $ZnCl_2$ was used based on Horton. et al. (2017).

Second test: Sludge was dried at 60°C and used instead of sediment. In the sieving part 5-2-1 mm and 63 µm pore size sieves were used. Dry sludge was added into the sieves and the sieving part was left for 1 hour. The sludge was then crushed manually by hand into smaller pieces in each sieve with a metal spoon to be more easily sieved. Then it was transferred to an aluminium pan and each fraction size was transferred into 250 ml beakers for two $ZnCl_2$ flotation of 2 hours, then vacuum filtered with (Whatman) GF/C filters (Horton. et al. 2017).

Third test, was protocol 1a from Mapping microplastic in sludge described in Lusher et al. (2017) where sludge was dried in 250 ml beaker and added 30% hydrogen peroxide (H_2O_2) and left for 6 hours in a heat oven with a temperature of 60°C.

Fourth test, was protocol 2 from Mapping microplastic in sludge described in Lusher et al. (2017) where sludge was also dried in 250 ml beaker and added Fenton's reagent (30% H_2O_2 and $FeSo_4$) and left in a fume hood in room temperature for 2 hours.

The final methods utilized were based on these three tests mentioned above (including some modifications), and the following three articles; for the wastewater method Murphy et al. (2016), Horton. et al. (2017) and sludge method Lusher et al. (2017). The first modification in the wastewater method was to sterilise sample with 70 % ethanol, and the second modification was adding 7 drops of 30 % Hydrogen peroxide (H_2O_2) on the filter prior to drying. The first modification in the sludge method was also to sterilise the sample with 70 % ethanol and then evaporate at 60°C before drying. Zinc chloride

flotation was skipped, and GF/C filtered tap water flotation was done. It is important to convey the possibilities of pollution or bias in these methods. Most of today's equipment consist of plastic because of its durability and better performing characteristics. Therefore, some equipment containing plastic has been used during this research, as storing bottles, vinyl gloves and wash bottle. Vinyl gloves was only used during the wastewater analysis. One issue in this research is the air contamination, and a study by Dris et al. (2015) estimated the total atmospheric MP fallout to be from 29 to 280 particles m^{-2} day^{-1}. Therefore, a possibility for contamination during different steps is present. The wastewater samples gathered from location 2 in June, and of relevance to the procedure two of the wastewater samples were analysed in a different way, the total volume of each sample was filtered. A formula was made to calculate the percentage of MP in 100ml of wastewater (annex 4).

2.1 Study Area

A total of eight WWTPs are involved in this research and the treatment facilities were divided in two groups based on treatment technologies, biological and mechanical treatment respectively. A fellow student was given the mechanical treatment, and this thesis presents the data from 4 plants with biological treatment. The selection of WWTP for this investigation was performed by Paul Windt at the Swedish Consultants (SWECO) located in Seljord. Four treatment plants were selected; Bø, Søvitt, Seljord and Rauland wastewater treatment facilities. The situation of each location is shown in figure 1.

For the WWTPs involved in this study, at the first step there is located an inlet-sieve from 6 to 3 mm in diameter for the facilities to remove large objects and to prevent large non-organic material to enter the WWTP. There are some differences between the facilities, Bø has an integrated washing and dewatering step where most of the sand is removed, then it goes through a biological moving bed biofilm reactor (MBBR) treatment followed by a flocculation (binding matter) where sludge and particles are bonded together for a flotation where sludge get separated and stored. Ulefoss and Seljord has a settling stage after the inlet sieve followed by MBBR with a final chemical treatment with sedimentation. And Rauland has a sand trap as a first step where most of the sand is

removed followed by a sedimentation stage where it goes to the MBBR treatment with an Actiflo pool. Actiflo pool is a system where micro-sand is used in the flocculation clarification process that performs at a high rate (Benson et al. 2007). MBBR is an advanced technique in the biological process with two separated growth systems of microorganisms to reduce biochemical oxygen demand (BOD), chemical oxygen demand (COD) and nutrients as nitrogen and phosphate (Kawan et al. 2016).

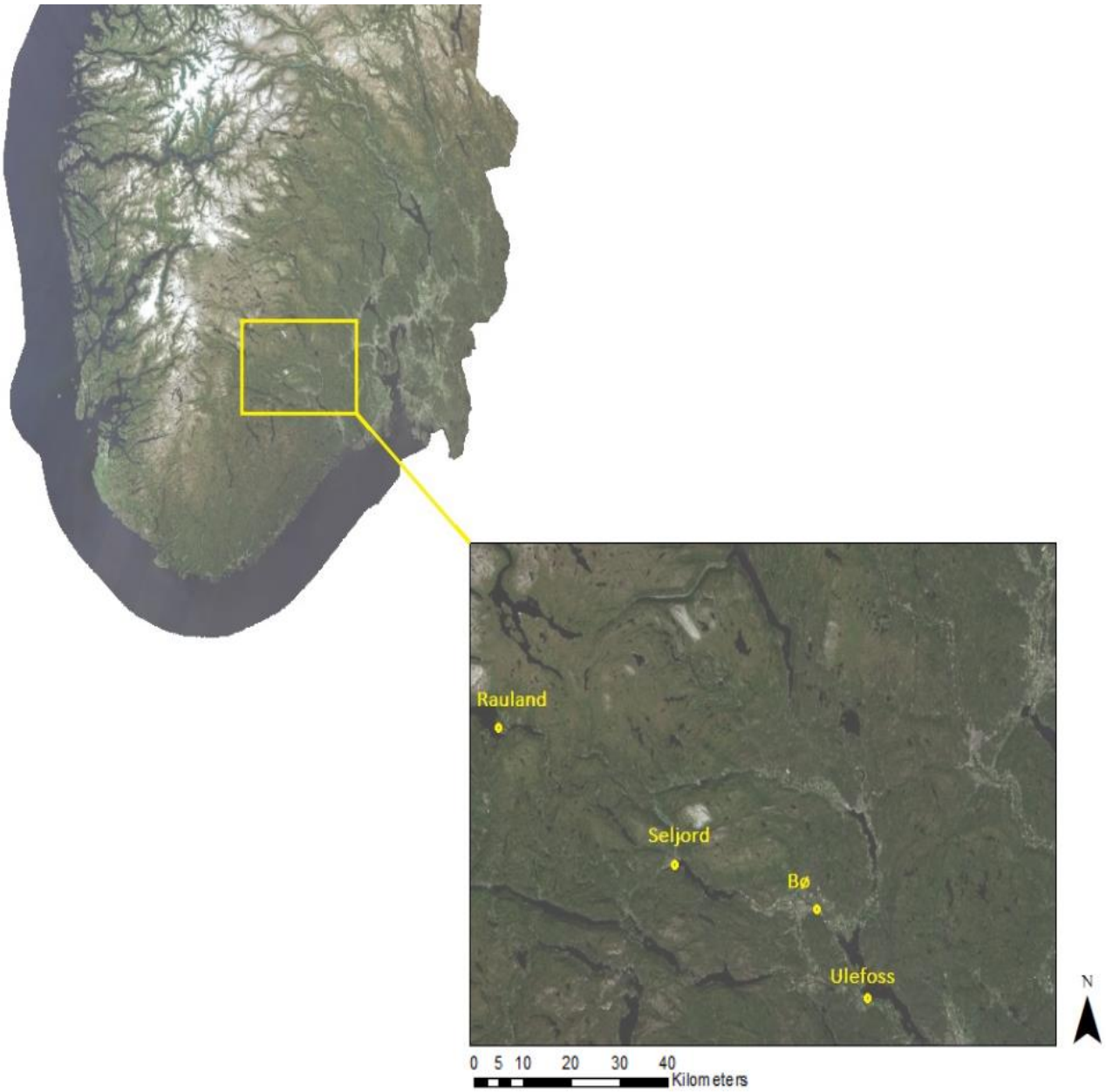

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Figure 1. The geographical position of the four treatment plants located in southeast Norway. The yellow circles from left to right shows location of Rauland, Seljord, Bø and Ulefoss (Søvitt) respectively.

2.2 Sampling

Sampling took place in the five month period from June to October 2018. Staff from each WWTP collected the wastewater samples simultaneously with the monthly sampling for chemical analyses. Collecting the sample was done according to ``*Instruks for prøvetaking på renseanlegg med akkreditert prøvetaking*`` *(SWECO, 2017).* Sampling stations for the wastewater was in inlet and outlet of the WWTP. In the sampling station representative samples were collected and stored in 15L waterproof drums. Subsamples were taken and poured in metal cans, and stored in a freezer containing a temperature of -18°C. On the same day as the wastewater sampling took place, sludge samples were collected from the sludge storage and frozen. In the sampling period the weather varied from very dry during June to July, with an increase in rainfall mid of July to August, and dry again from August to October. The process of defrosting of the wastewater and the sludge samples began one day prior to the initiation of the laboratory work.

2.3 Preparatory work

Fenton`s reagent solution

30 ml of Fenton's reagent was made by weighing out 0.0667 g of ferrous sulphate ($FeSO_4$) which was mixed with 10 ml distilled water in a 50 ml graduated beaker. The concentration of the solution was 6.67 mg /ml^{-1} Tagg et al. (2017). 20 ml of 30 % Hydrogen peroxide (H_2O_2) was taken out by a glass pipette (20 ml) and poured into the sample with the ferrous sulphate solution in accordance to Lusher et al. (2017).

Glass Petri dish with cotton line

A glass petri dish was prepared with 2 lines of 100 % cotton where the cotton ends was taped to hold the line stretched. The radius from the centre of the dish was 4.8 cm (fig. 2) This was done in order to facilitate an easier read when analysing the data and to know exactly where the counting area was located on the filter.

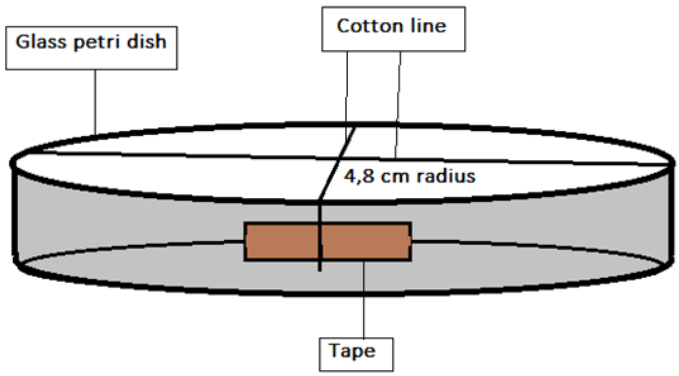

Figure 2. Preparation of petri dish used for counting MP-particles with cotton line taped to the dish wall. Radius of dish = 4,8 cm.

Filtering Tap water by use of a GF/C filter

1L beaker was filled up with tap water and a filtration funnel was prepared with a GF/C filter. The tap water was filtered and stored in a precleaned bottle.

2.4 Wastewater method

2.4.1 Step A vacuum filtration

The wastewater sample was shaken for 60 seconds with the lid on. Then the lid was taken off and the sample inside was stirred with a glass rod for 30 seconds until it was homogenised. 100 ml of the homogenous wastewater was poured into a 500 ml measuring cylinder. 200 ml of 70 % ethanol was added to the sample and stored for 15 minutes to sterilise. The sample was mixed in the measuring cylinder by rotating it in circular motions. After the sample was homogenised it was poured into a vacuum filtration unit using GF/C filter. The amount poured into the vacuum filtration unit was measured when the filter clogged. The remaining content in the measuring cylinder was washed with distilled water into the vacuum filtration unit using a new GF/C filter, to get adhered particles from the glass walls. GF/C filters were then stored in petri dishes covered with aluminium foil and named with place, date and sample type. Before analysing the filters, 7 drops of hydrogen peroxide (H_2O_2 30 %) was treated to cover the filter area. One drop was centre in the middle and 6 drops around the surface of the filter. Then each filter was stored in a heat oven with a temperature below 60°C till it was dry.

2.4.2 Step B Analysing filters

The dried GF/C filter was transferred to the glass petri dish with cotton line. The filter was analysed by using a digital stereo microscope, (Zeiss, Discovery V20). At first the filter was studied by moving over the whole filter (figure 3). This step was done to check the filter for large microplastic (5-3 mm in diameter) and macroplastic particles (above 5 mm in diameter).

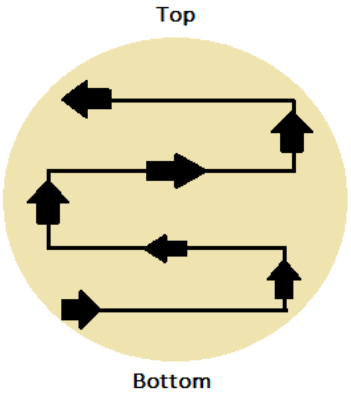

Figure 3. Overview of filter from bottom to top, where the overlook started at the left side at the bottom moving to the right and in the direction to the top.

In the next step the 2 diagonally lines were followed by counting 15 randomly chosen areas of each line analysed by a magnification of 91.4x. The area where the diagonally lines crossed, was skipped in the second diagonally line, preventing it from being analysed twice. Then 5 areas in each quadrant were randomly chosen and counted at the same magnification (figure 4). A total of 50 areas were analysed, the particles that could not be categorised was counted and included in the table of categories as unknown.

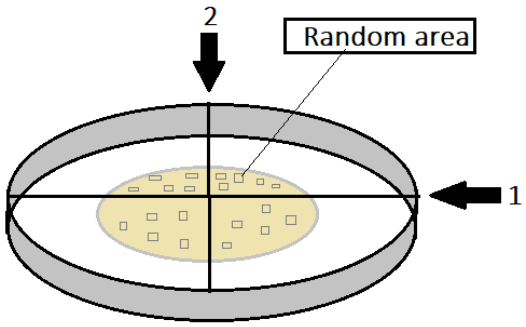

Figure 4. Petri dish with GF/C filter in the middle and two cotton lines marked as 1 and 2 and four quadrants in between. For each line 15 areas were analysed and 5 random areas were observed in each quadrant.

2.5 Calculations

To calculate the total number of MPs at each filter for the wastewater samples, area observed and the total filter area is needed. Area observed (field of view) was measured to be 2.64 mm in length and 2.1 mm in width, the total filter area is calculated based on the filter diameter of 3.5 cm.

Total filter area = $\pi r^2 = \pi * 1.75cm^2 = 9.62cm^2$ or $962mm^2$.

Area of one square: Field of view area $= L * W = 2.64mm * 2.1mm = 5.54mm^2$.

Since the volume of the wastewater samples was 100 ml it has to be multiplied with 10 to get particles per litre, the total filter area and the area observed was put in this formula to calculate number of MPs/L:

$$\frac{MP}{1L} = \frac{tot\ filter\ area}{area\ square * 50} * MP * 10 = \frac{962mm^2}{277mm^2} * MP * 10$$

To get MP/kg dry weight a calculation from gram to kg was needed. This formula was used for converting MP/g to MP/kg dry weight:

$$MP/g\ (d.w.) * 1000 = MP/kg(d.w.)$$

V: volume of the sample (ml, dl, L)

(d.w.): dry weight of the sample

2.6 Sludge method, treatment and flotation

2.6.1 Fenton's reagent

The melted sludge was mixed thoroughly for 1 minute before taking 3 subsamples from the top, middle and bottom with a metal spoon. Each subsample was weighted to be 10 g each in wet weight. Then the subsamples of total 30 g were mixed together for 1 minute and 10 g were transferred to a 250 ml precleaned beaker with aluminium foil as lid. 20 ml of ethanol was added to sterilise the sample for 15 minutes. Then the sample was transferred to a pan of water, placed over a heating plate giving a temperature of 60˚C. Temperature was measured every 30 minutes and when the temperature increased cold water was added to decrease the temperature. When the ethanol had evaporated the sample was transferred to a heating oven to dry at a temperature of 60˚C. When the sludge had dried, the sample was weighted and the total dry weight was noted. Fenton's reagent was then added to the sample and stored in a fume hood at room temperature for 2-3 hours with aluminium foil as lid. When the sample was in the fume hood it was checked 2-3 times and the beakers was rotated to mix the sample.

2.6.2 Sludge step B Tap water flotation

When the reaction had occurred for 2-3 hours the beakers were transferred to a 1L canteen of stainless steel and placed in the middle of the canteen. GF/C filtered tap water was poured into the beaker to the brim, then it was homogenised for 30 seconds by stirring the solution with a precleaned glass rod and settled for 1 hour. Aluminium covered the beaker and the canteen while it settled. After the sample was settled the top layer was gently poured out into the canteen and the beaker was transferred to an another precleaned canteen. The floated layer was then vacuum filtrated using 3 filters, and particles adhered to the canteen were rinsed with a wash bottle into the vacuum funnel. The middle layer of the tap water flotation was gently poured into the canteen, and then poured into the vacuum filtration unit and filtered using 2 filters. The adhered particles to the canteen were rinsed into the filtration unit with a wash bottle. The remaining bottom layer was then mixed by rotating the beaker and then poured into the vacuum funnel. Particles adhered to the beaker walls were also rinsed into the funnel, 1 filter was used for the bottom layer.

2.6.3 Step C Visual inspections

Each filter was inspected for a minimum of 5 to 15 minutes using a digital stereo microscope (Karl Zeiss Discovery V20). A quick overlook of the filter was done with a magnification of 7.6x to 11.5x. Then the filter was studied using a magnification range of 11.5x to 150x by moving from the bottom left to right described above in figure 3. The particles that were not possible to categorise were counted and noted in the table of categories as unknown.

2.7 Criterias

The criterias used for categorising microplastic particles (MPP) were based on two articles namely Nor & Obbard (2014) and Horton. et al. (2017). Basic criteria (1) originated from Nor & Obbard (2014) but since it had uncertainties about including possible non-plastic particles, the study by Horton. et al. (2017) made a second consideration to exclude possible non-plastic particles. Table of categories is based on Hidalgo-Ruz et al. (2012) with the type, shape and colour.

(1) The analysed MP needed to be confirmed by these criteria: (Nor & Obbard, 2014):

1- No cellular or organic structures
2- Filaments and fibres should be equally thick through their entire length
3- Fibres and filaments or particles should not be segmented
4- The particle should have an unnaturally shape

(2) To be considered as MP the particles had to fulfil at least two of the following criterias: (Horton. et al. 2017):

1- Unnaturally coloured compared to the majority of the sample
2- Unnaturally homogenously coloured material or structure
3- Unnaturally coat of colour
4- Shiny/ glassy
5- Flexible/ elastic can be compressed without being brittle
6- Filaments/fibres remains intact with a poke with a tweeze/needle

2.8 Contamination control

A lot of factors could contaminate the samples and result in overestimation of the number of MPs in the samples. Steps were taken to prevent and quantify the possible contaminations:

1- All equipment were pre-cleaned three times with distilled water and ethanol.
2- All surfaces involved with the laboratory work was wiped down with 70 % ethanol three times according to Murphy et al. (2016).
3- Two clean GF/C filters was placed in two petri dishes to be left open for the duration of the laboratory work to collect any atmospheric microplastic that may be present. One of the filters was placed in the right corner of the fume hood where the filtration took place and the second one in the working area.
4- Tap water were vacuum filtered through a GF/C filter and stored in a precleaned bottle.
5- Work clothes and lab coat of cotton was used during the laboratory work.
6- All equipment and beakers were covered with aluminium foil to prevent air contamination.

3 Results

Microplastic was observed in all samples from the four WWTPs. An average of 8544 particles kg^{-1} dry weight (d.w.) were calculated for the sludge samples. MPPs from the sludge consisted of filaments (72 %), fragments (23 %) and beads (5 %). The average amount of MPPs/L inlet wastewater was 13644 particles and 6255 MPPs/L in outlet water of the wastewater treatment plants. The MPs in the wastewater sample consisted of filaments (11 %), fragments (59 %) and beads (30 %). A total of 12 filters was left for the air contamination control, and a total of 130 particles were detected. In the sludge process 12 filters were left for air control and a total of 17 particles were registered.

3.1 Microplastic in wastewater

The average of MPP in inlet and outlet wastewater samples for each treatment plant is shown in figure 5. Location 4 has the highest observed inlet (19937 MP/L) and outlet (9257 MP/L) followed by location 1 as the second highest observed location of inlet (18304MP/L) and outlet (7455MP/L). Location 2 and 3 had the lowest observed inlet and outlet samples in this investigation. In all locations the amount of MP observed was higher in inlet than the outlet samples, and the overall average showed 54% reduction of MP.

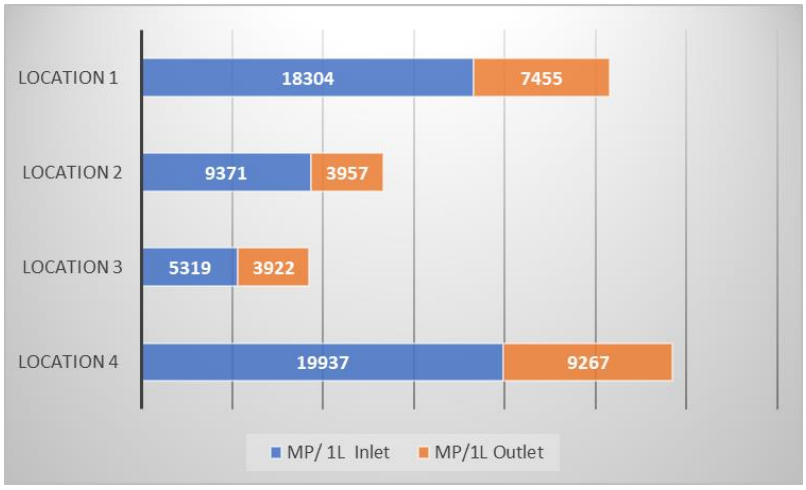

Figure 5. Average amount of MP/L in inlet and outlet samples of four treatment plants.

Location 1 has the highest observed inlet sample (25962MP/L) in July and shows a decreasing trend from July to October, and has its lowest observation in October (10989MP/L). For the outlet the highest observed MP content was in July (13432MP/L) and the lowest observation in September (2325MP/L) (fig. 6a). The amount of inlet wastewater in 24 hours (m^3/d) varied through the period, in which September had the highest amount (912m^3/d) of wastewater and October the lowest (325m^3/d) (fig. 6b).

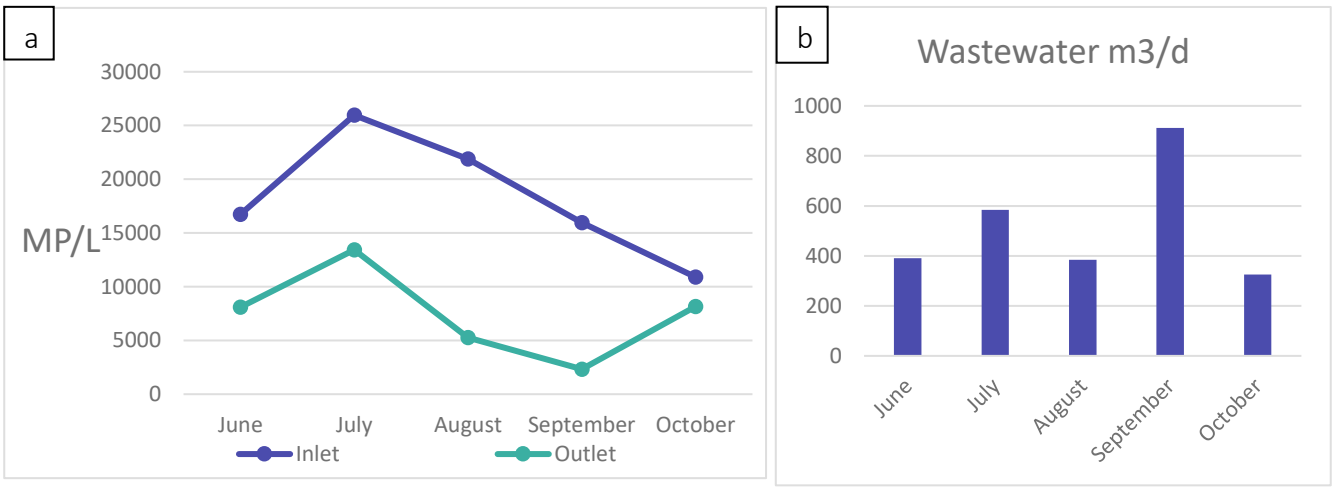

Figure 6. a) Number of MP/L of wastewater in the period from June to October 2018 in location 1. Blue line: inlet sample, green line: outlet. b) Variation in amount of inlet wastewater (m^3/d) during the period from June to October 2018 in location 1.

Location 2 is one of the lowest observed WWTP of this investigation it shows also a decreasing inlet trend from June (9857MP/L) to October (7323MP/L) (fig. 7a). Highest observed inlet month was July (13050MP/L) from the period. While a decreasing trend is also shown in outlet with highest observation from July (7462MP/L) and lowest August (2603MP/L). For m^3/d a small variation between the months was observed with the highest inflow in September (614m^3/d) (fig. 7b).

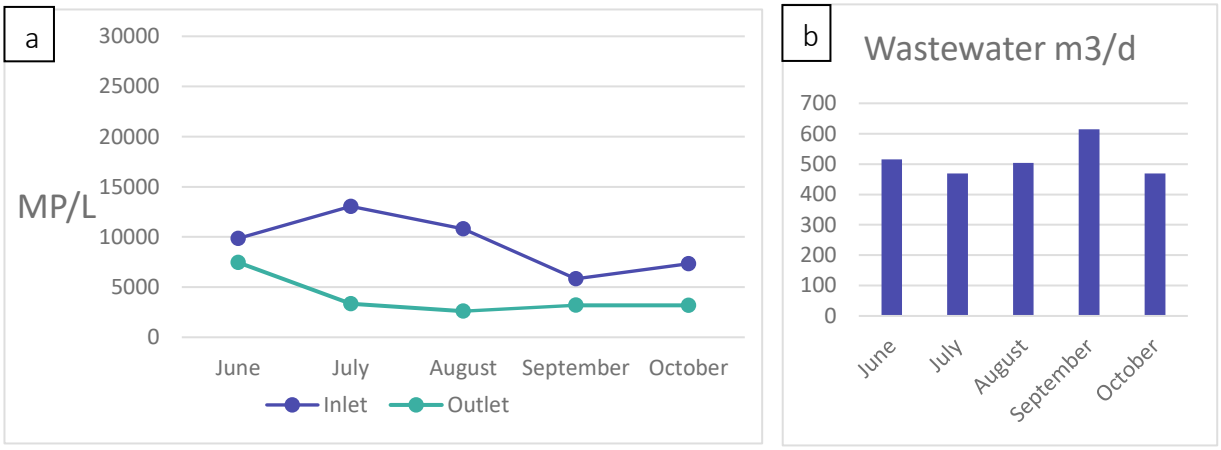

Figure 7. a) Number of MP/L of wastewater in the period from June to October 2018 in location 2. Blue line: inlet sample, green line: outlet. b) Variation in amount of inlet wastewater (m^3/d) during the period from June to October 2018 in location 2.

Location 3 was the lowest observed location without July in the period of this investigation. But inlet shows an increasing trend line from June (2360MP/L) to September (8121MP/L) and then decreasing to October (5171MP/L). While outlet is also increasing from June (1423MP/L) to September (5345MP/L) and decreasing to October (4616MP/L) (fig. 8a). Wastewater m^3/d in the period increased from June (381m^3/d) to September (1077m^3/d) and decreased to October (727m^3/d) (fig. 8b).

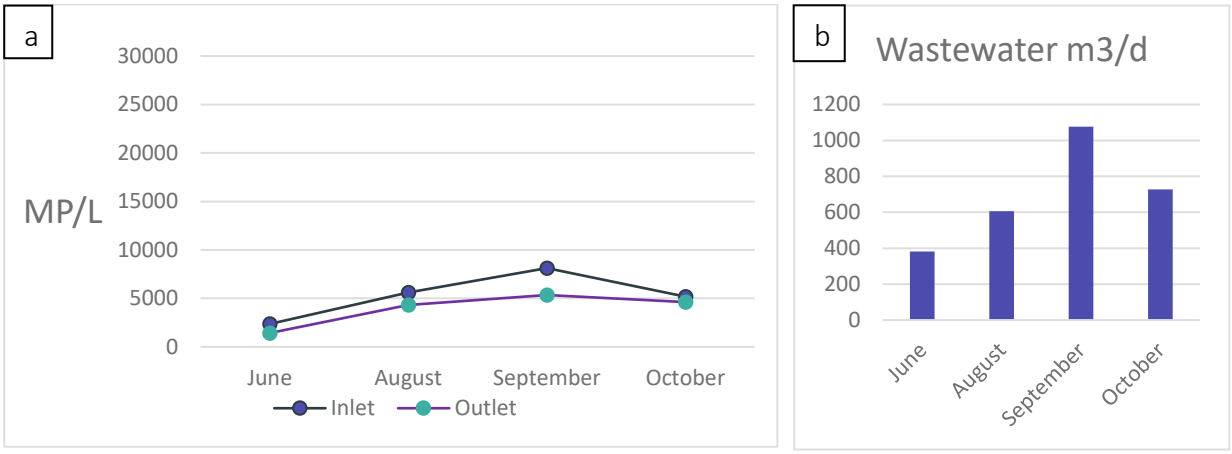

Figure 8. a) Number of MP/L of wastewater in the period from June to October 2018 in location 3. Black line: inlet sample, purple line: outlet. b) Variation in amount of inlet wastewater (m^3/d) during the period from June to October 2018 in location 3.

Location 4 is the highest observed investigation area with highest inlet quantity. And shows a decreasing trend of the period in inlet and outlet, shown in figure 9a. Highest observed month in inlet was August (24747 MP/L) to lowest October (14855 MP/L).
In outlet the highest observed month was June (16555 MP/L) to lowest October (6490 MP/L). Inflow of m^3/d wastewater increased from June (949m^3/d) to July (1132m^3/d) and decreased during the period to October (978m^3/d) (fig. 9b).

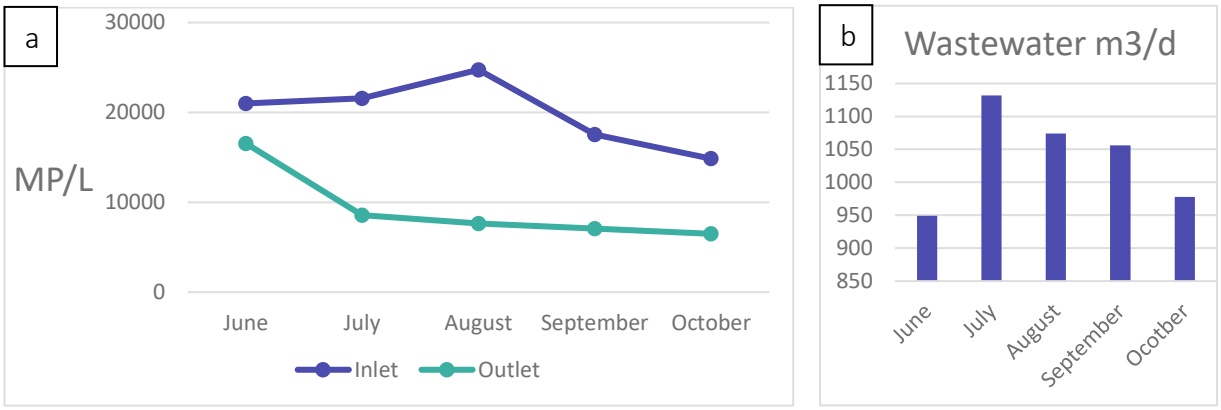

Figure 9. a) Number of MP/L of wastewater in the period from June to October 2018 in location 4. Blue line: inlet sample, green line: outlet. b) Variation in amount of inlet wastewater (m^3/d) during the period from June to October 2018 in location 4.

3.2 Microplastic in sludge

The dry weight (d.w.) of the sludge differed between all subsamples. Highest % (d.w.) 10 g wet weight (w.w.) was 51 % from location 2 in August, and the lowest percentage of dry weight was 15 % from location 1 in June (fig. 10). The average dry weight for all locations was 24 % /10 g (w.w.). The average percentage of dry material in the subsamples from location 2 had the highest average (28 %). The average of wet and dry weights is shown in table 1 for each location, the differences in content of dry substance was 7%.

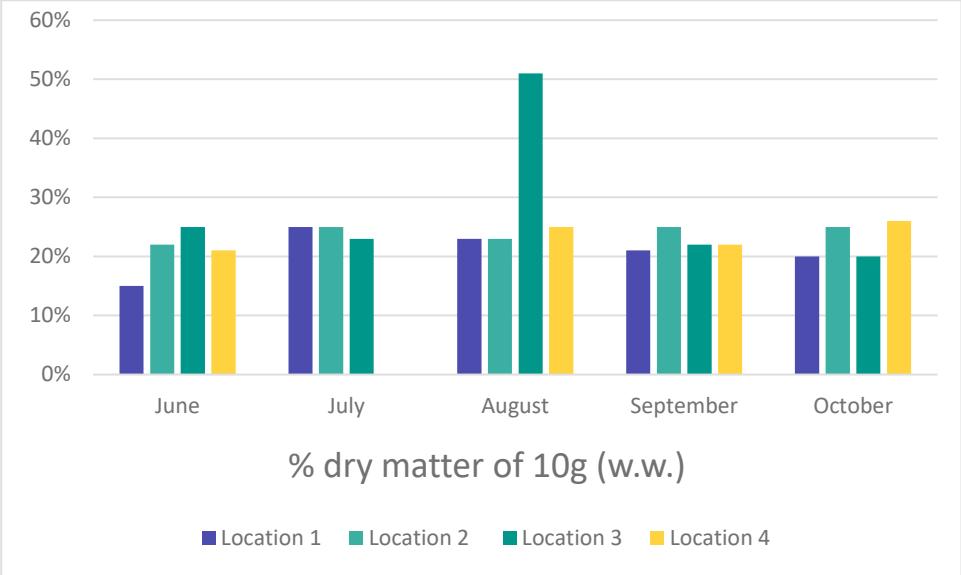

Figure 10. % dry matter of 10 g (w.w.) in the period of June to October, without location 3 in July.

Table 1. Average % dry weight (d.w.) of wet weight (w.w.) for each location.

Average	Wet weight	Dry weight	% of (d.w.) in (w.w)
Location 1	10,01 g	2,09 g	21 %
Location 2	10,01 g	2,80 g	28 %
Location 3	10,00 g	2,36 g	24 %
Location 4	10,01 g	2,41 g	24 %

The average of MP kg/d.w. in sludge varied during the season for each location (fig. 11). Location 4 is the location with highest quantity of MP (10370MP/kg d.w.), followed by location 1 with the second highest average quantity (8255MP/kg d.w.).

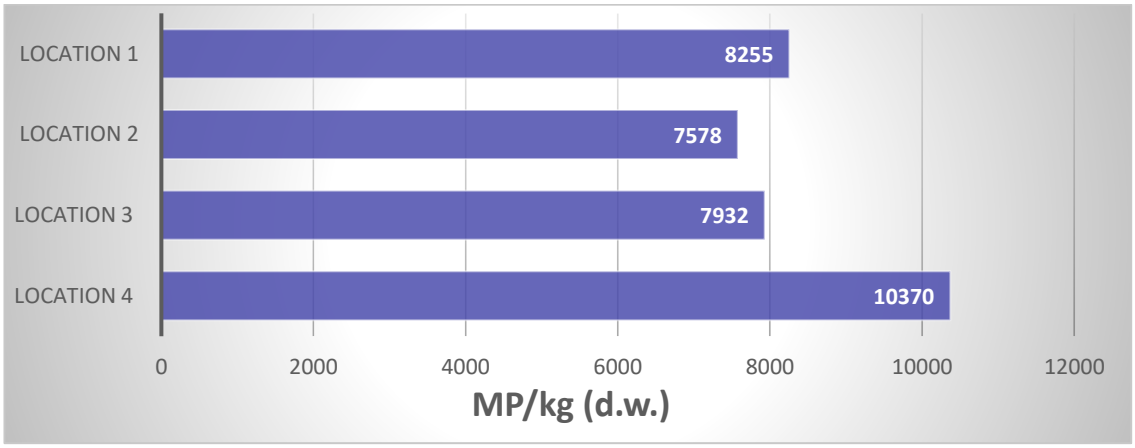

Figure 11. Average amount of MP/kg (d.w.) in sludge for each wastewater plants 2018.

Variation in types of MP was observed between the sludge samples. The majority of MP particles that was observed was fibres and the size was mostly below 1 mm. There was no MP observed larger than 1 mm in the sludge samples. Two of the highest amounts of MP calculated was 16883MP/kg d.w. and 16444MP/kg d.w. from location 1 and 4 in June (fig. 12).

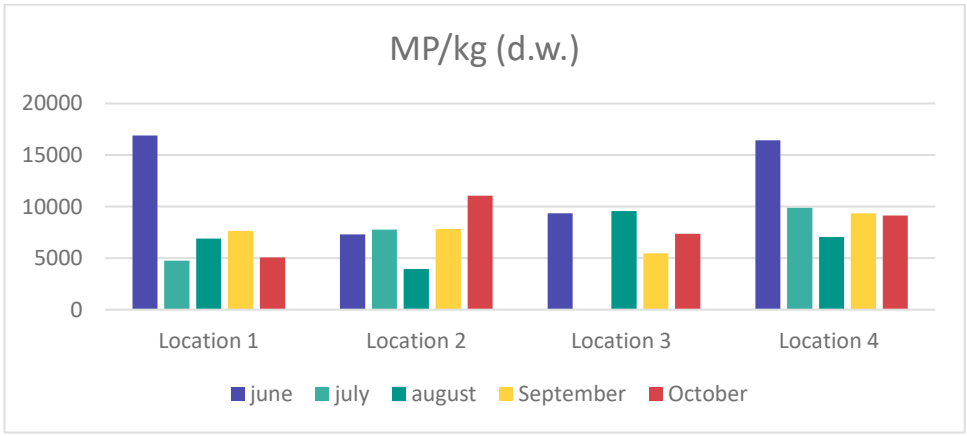

Figure 12. Calculated amount of MP/kg (d.w.) in sludge of the period of June to October for each wastewater plants 2018.

3.3 Microplastic diversity and types

The majority of MPs observed in this investigation had a size below 1 mm. Microplastic groups detected was fibres, beads and fragments. In wastewater samples fibres had the lowest amount of 11 % of the total quantity and the beads had a total of 30% of the total quantity counted. The major quantity (80%) of these beads was observed in the effluent wastewater samples. The largest amount of MPs in the wastewater was the fragments that made up 59% of the total quantity (fig. 13). The amount of particles that couldn't be categorised was 778 particles that was marked as unknown.

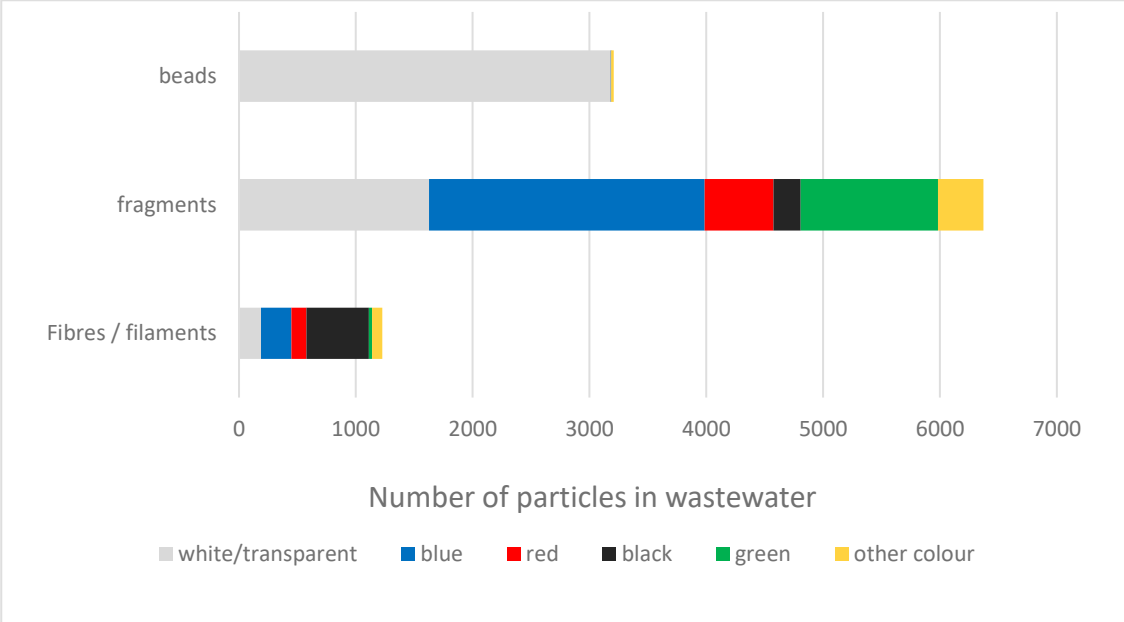

Figure 13. Total observed microplastic particle quantities and composition of the three types beads, fragments fibres/filaments.

In the sludge samples, fibres were the most observed (72 % of total observations) with black fibres as the dominant type. Fragment was the second most observed type after fibres, (23 %) where the majority was transparent fragments. The beads (5 %) of the total observed MPs was dominated by irregular white and transparent particles (fig. 14).

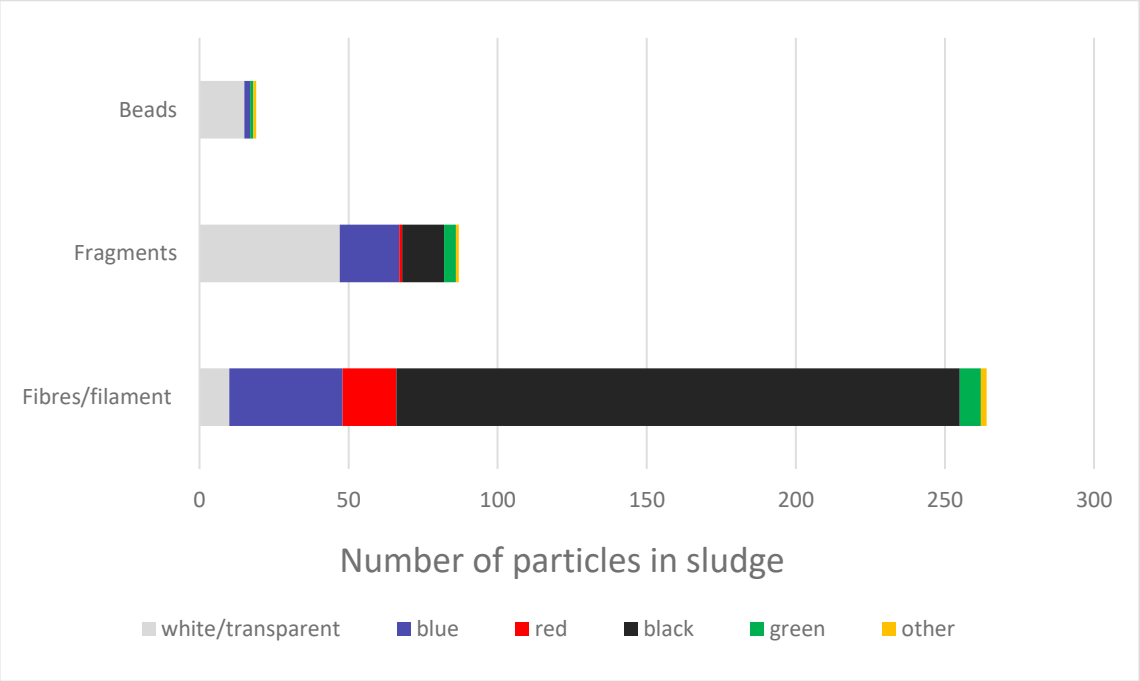

Figure 14. Total observed number of microplastic particles and categorisation into different types in the sludge samples.

The MP observed (of each group) had different structure and colour. The most common type of filaments were black and flat throughout their whole length. White transparent filaments had a helix and a cylindrical shape. The most colourful filaments had a round cylindrical shape and were transparent (fig. 15).

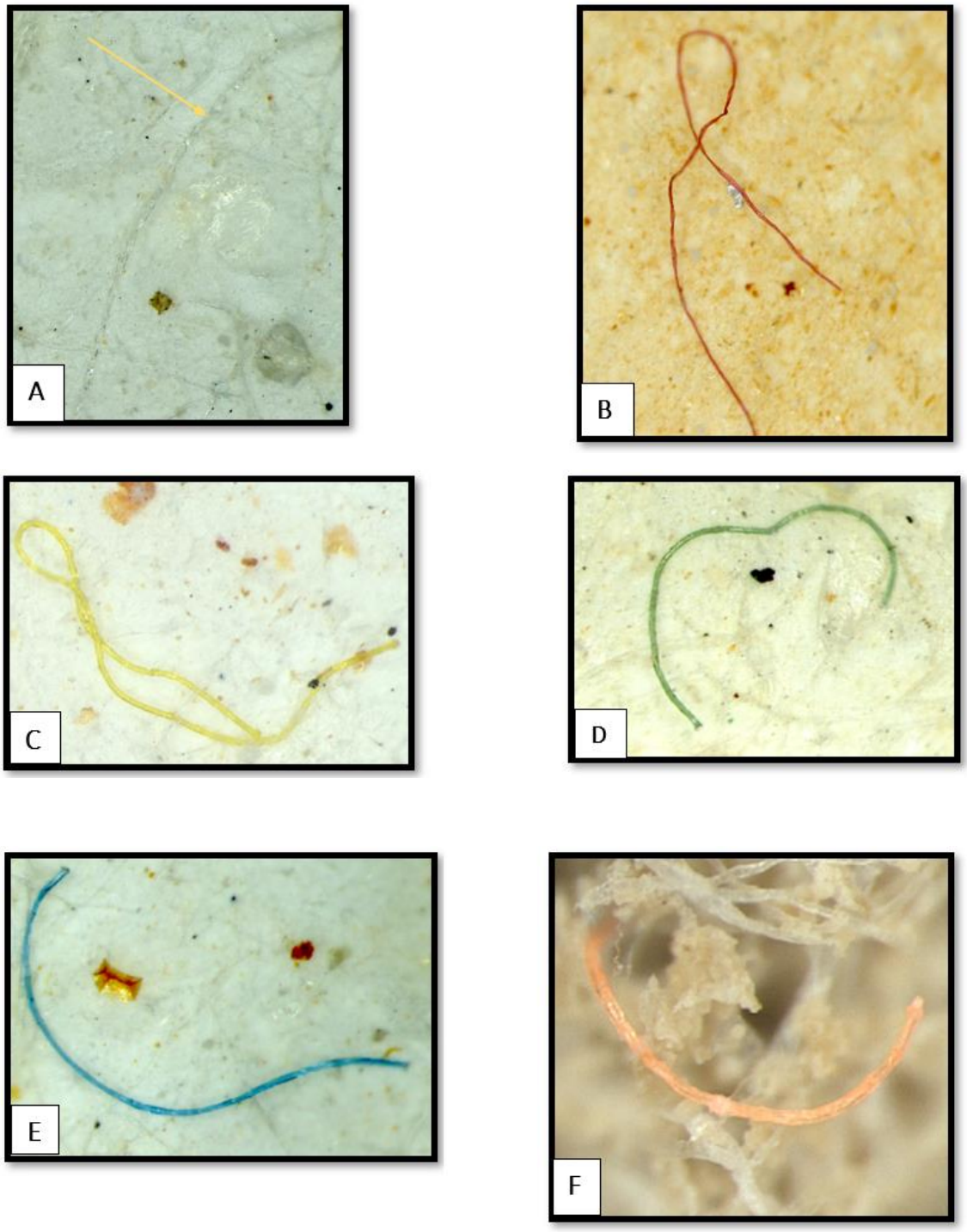

Figure 15. Different types and colour of filaments observed in inlet, effluent and sludge. (A) transparent helix shaped filament, (B) red filament, (C) yellow cylindrical shaped filament, (D) green cylindrical filament, (E) blue cylindrical filament, (F) orange filament. Photo: Asbjørn Ørsland.

Three main types of fragments were observed, granular, smooth shiny, and multicoloured. Red fragments differed also in surface structure, the granular form shown in figure 16A. The major fraction of the coloured fragments were the blue ones, some with granular surface and some with smooth surface (fig. 16B). Green fragments had a similar structure as the red fragments (fig.16C). Other types of fragments that was observed was multicoloured (fig. 16D), and transparent ones with black spots.

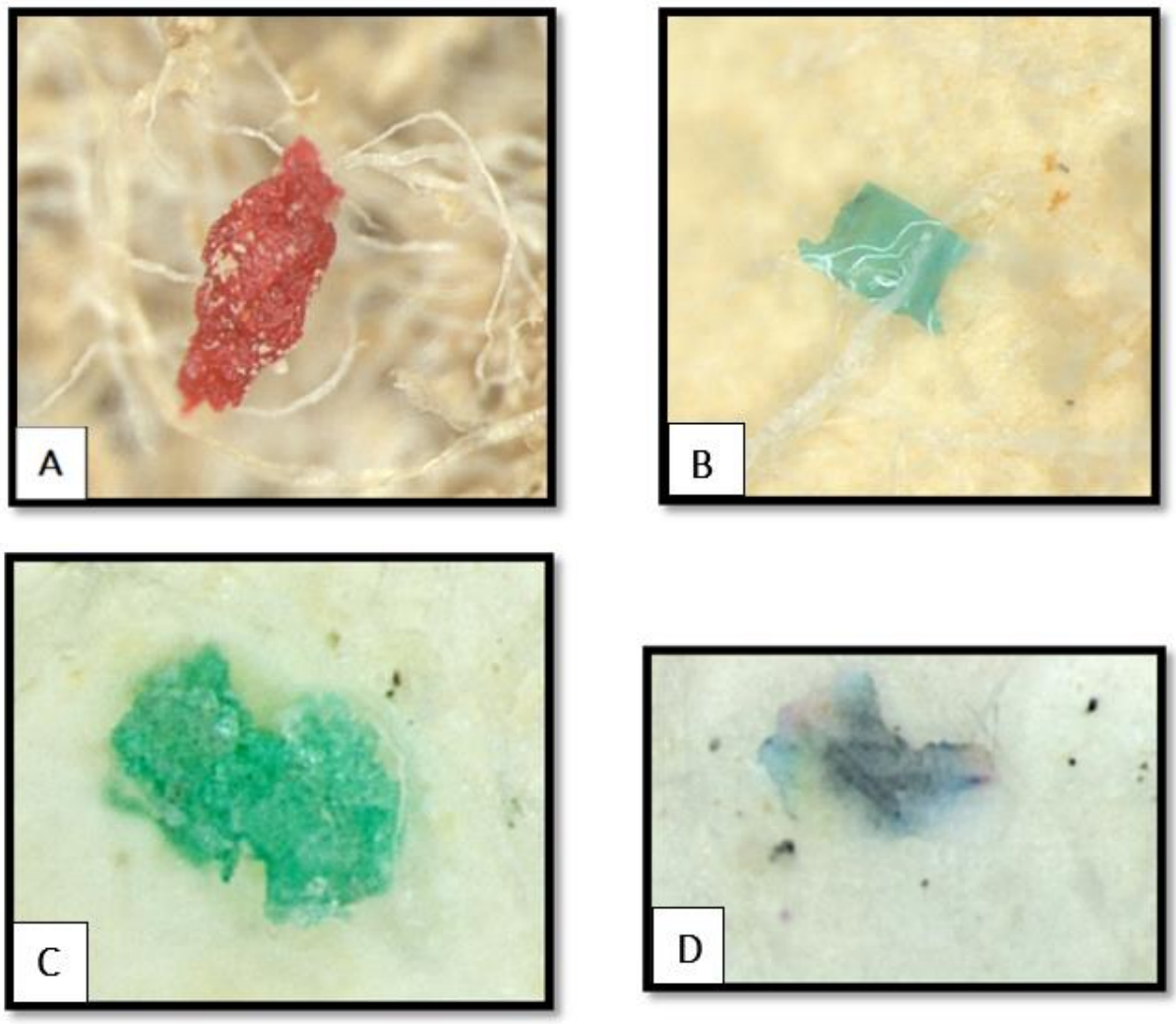

Figure 16. Different coloured fragments observed during the investigation, (A) red granular fragment, (B) blue shiny transparent fragment, (C) green granular fragment, (D) multicoloured fragment. Photo: Asbjørn Ørsland.

The white transparent fragments observed had different structure, some fragments were just like a flake with sharp edges and very transparent (fig. 17A). Some fragments had scrubbed worn and bended edges (fig.17B). Other had a combination of rolled and sharp edges with a waveshape surface (fig. 17C).

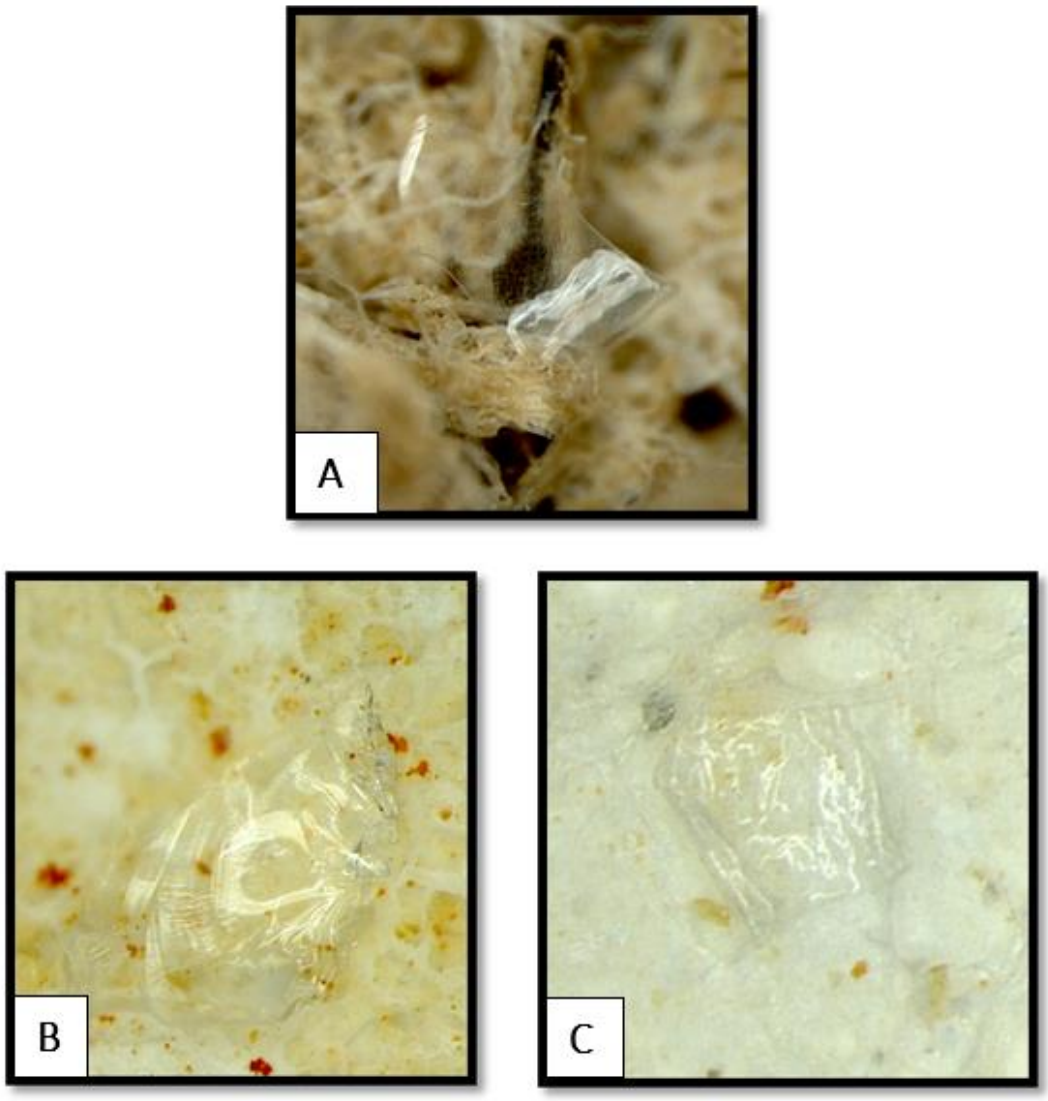

Figure 17. Three different shiny transparent fragments, (A) flat transparent with sharp edges, (B) oval shaped with worn edges bending, (C) waveshape and sharp rolled bending edges. Photo: Asbjørn Ørsland.

There were two different structural types of beads observed in this investigation (fig. 18A) one with irregular shape oval and clumpy form shown in figure 18B. And the most often observed bead was circular white transparent form (fig. 18C).

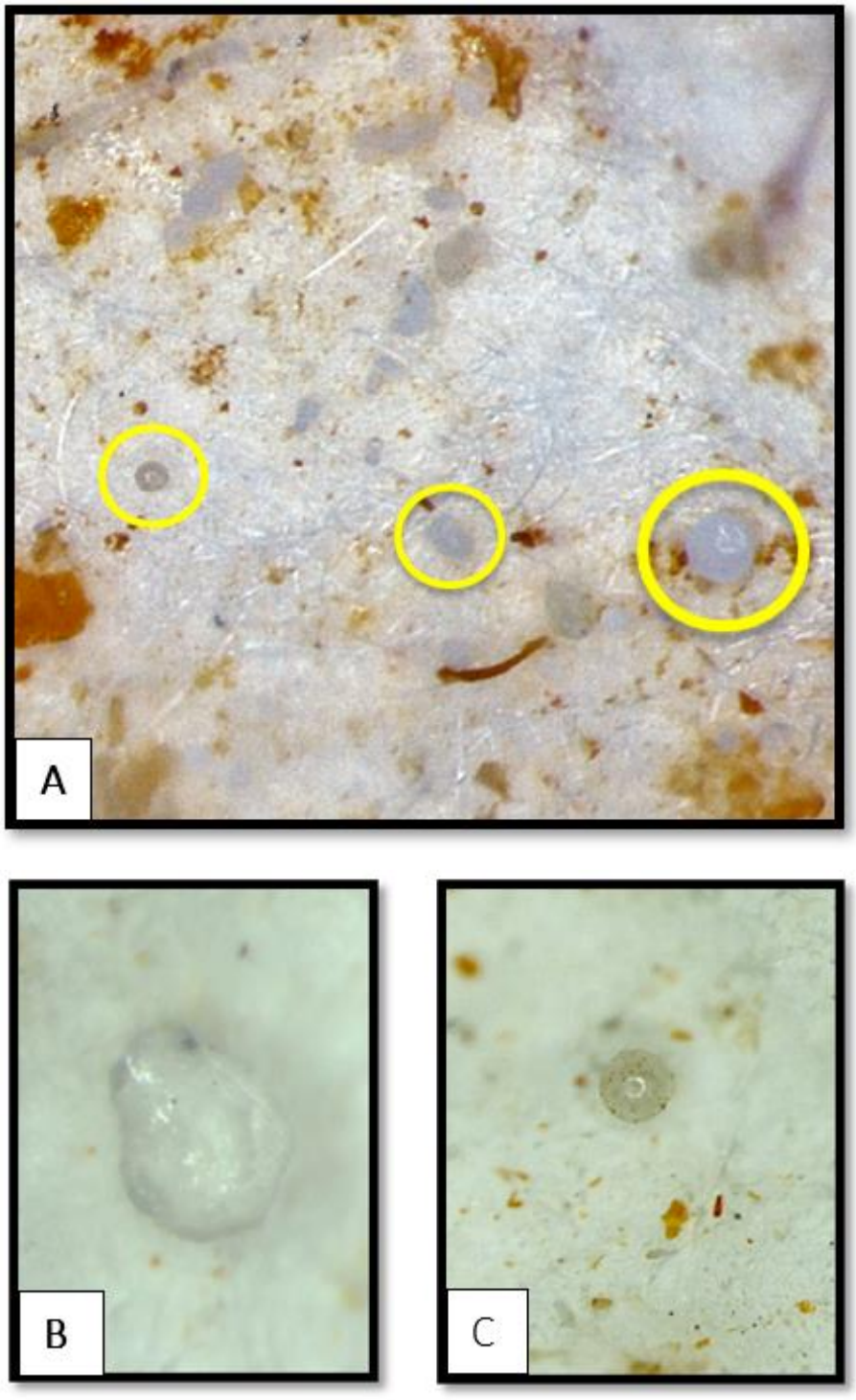

Figure 18. The main type of beads observed in this investigation, (A) to the left and right 2 circular shiny beads, in the middle 2 beads bonded together. (B) irregular transparent shaped bead. (C) round circular transparent shaped bead. Photo: Asbjørn Ørsland.

4 Discussion

4.1 Method Development

The main priority of this thesis was to investigate the amount of MP in 4 WWTPs at three locations: Inlet, effluent and sludge. With the existing variety in research and methods it may attribute to the difficulty of comparison with other results (Hermsen et al. 2018). One project entitled ``*BASEMAN*`` introduced a standardised protocol where the main focus was on MP in marine sediment (Frias et al. 2018). However, since the main focus has been on water and sediment samples, no standard methods for sludge has been recommended. Since sludge has different composition from sediment, different treatments need to be combined. A study by the Norwegian institute of Water research (NIVA) (Lusher et al. 2017) is the closest to a standard sludge protocol that exists.

Before starting with the wastewater, a pre-test with a water sample was executed. Granules from a cosmetic product was added to the water sample to observe the separation by the GF/C filter. Because of the high amount of granules added, it clogged after a short period. Most of the granules was observed on the GF/C filter, but there was also observed granules in the filtered solution. This means that some of the granules went through the filter, mostly because the vacuum pressure was too high. Because of this finding a second filtration was included in the procedure. When the second filtration was done some GF/C filters was analysed and no visible particles was observed, therefore this step was excluded to save time.

For safety reasons it was decided to sterilise the samples prior to performing the lab work, even though hydrogen peroxide and Fenton's Reagent is stated as basic disinfectant (Yoo, 2018). 70 % ethanol was used to sterilise the sludge and according to Rutala (1996) it should be sufficient enough to inactivate HIV-1 and hepatitis B virus. This decision was implemented for laboratory-safety reasons before the basic disinfectant was added.

The intention was to use a sediment method for sludge based on Horton. et al. (2017) but it proved to be difficult to sieve the organic material since major amounts of the sample contained cellulose. The sample was dry which made it very compact and had to

be manually crushed through the sieves by a metal spoon after a period of sieving. The possibility for the MP to either get stuck between the pores or fragmented was an issue. This could result in overestimation by fragmentation of MPPs combined with open air contamination. Moreover, another issue was of underestimation because particles could get stuck in the pores of the sieves. New methods were considered since the sludge samples was very complex, further degradation was needed to be able to observe the MP on the GF/C filters.

A study by Lusher et al. (2017) demonstrated various protocols for sludge which presented two possible methods for this thesis. The first protocol was using 30 % Hydrogen Peroxide at 60-70°C for 6 hours to degrade the sludge. This method was very time consuming therefore, a second protocol was tested in room temperature and the reaction took only 2-3 hours. Fenton's reagent was used and appeared to be more efficient with time and to degrade organic materials.

A test with Fenton's reagent was done to let the reaction occur for a longer period. It seemed to degrade more organic materials but a pressing problem was the reaction still occurred during the flotation process. One possibility could be that the reaction produced gas within the organic material that made it to float after a period. It was then decided to use filtered tap water as first flotation to dissolute and slow down the reaction. A pre-test was performed with biogas processed-sludge and worked very well with Fenton's reagent. However, with different sludge samples from the 4 locations, it did not work as well as the first time. Due to the high amount of cellulose in the sludge sample it still floated in the filtered tap water, so $ZnCl_2$ solution was excluded. Even with this problem, Fenton's reagent showed its efficiency on the other organic material, and it was included in the method. A study by Hurley et al. (2018) exposed no changes in structure or degradation of MP by this method. Moreover, it functioned as a disinfectant as well (Selvakumar et al. 2009), with the reaction of ferrous sulphate ($FeSo_4$), forming hydroxyl radicals (°OH) and this ran as a high-level disinfectant (Yoo 2018). Furthermore, the study of Tagg et al. (2017) showed Fenton's reagent to be an efficient reagent. Without harming microplastic by changing chemistry or size, and with a concentration of 6,67mg ml^{-1} it provided good balance between the reaction and time-efficiency.

The problem occurring in the flotation step could have been prevented by washing the sample after the Fenton's reagent had occurred as Frias et al. (2018) recommends for the H_2O_2. But the subsample of sludge was stored in 250 ml graduated beakers to prevent the sample to be exposed to air contamination when transferred to different compartment. If the subsamples were washed after the Fenton's reagent it would be more vulnerable for contamination. An issue that was not mentioned by Lusher et al. (2017) or by Tagg et al. (2017) of the Fenton's reagent was the pH value. It seemed that the pH had an impact of how efficient the reaction occurred, and if this was thought about earlier in the method development, the flotation step might have been more efficient than it turned out to be. However, even though the pH was not measured the reagent worked very well. In the control filters for sludge, less particles on the GF/C filters than in the wastewater was observed. One of the possibilities could be the cleaning stage, as the floor was more thoroughly cleaned the second time prior to the laboratory work of sludge. One could suspect that this may have had an influence on why there is less particles from the air contamination control. A study during workdays in classrooms found the highest number of atmospheric particles to be during and after human activity (Fischer et al. 2015). Under the circumstance during the laboratory work the activity around the samples seems to be one of the contributors for air contamination.

Related to the most optimal type of method to analyse MP in sludge the Fenton's reagent worked very well with organic matter that had minor compounds of cellulose. For further method optimization, there is a possibility to use Schweitzer reagent to separate cellulose from the sludge. A recently study by Gupta et al. (2018) shows a method based on Schweitzer reagent to recover cellulose from sludge and wastewater. The Schweitzer reagent consist of cupric hydroxide ($CuOH_2$) and ammonium hydroxide (NH_4OH). It was prepared in the study by adding 5.5 g of $CuOH_2$ to 1 litre of NH_4OH with a concentration of 28 to 29 percent. The study concluded that Schweitzer reagent was found to be a rough and reliable method to recover cellulose, in this case to remove it.

4.2 Wastewater method and microplastic

More MPPs were observed in the inlet wastewater samples than outlet, highest amounts of MPs observed was in location 4 with an average of 19937 MP/L for the whole period. Location 4 has also the highest amount (9257MP/L) in the outlet for the period. It seems to be lower amounts of MPs leaving the wastewater plants than entering. The MP amount observed at each location does not seem to match with the amount of wastewater (m^3/d) passing through, except for location 3. The MPs observed in this location seems to match with the income of wastewater (m^3/d). The sample from July was not collected for this location and it could have given different results if it was sampled. Location 3 had the lowest inlet average (5319MP/L) and also the lowest (2360MP/L) observed inlet-sample of MP through this investigation.

The amount of particles observed in the control filters demonstrated some variations with more particles observed in the laboratory work of wastewater than the sludge. One of the possible sources of contamination could be the floor since it was washed one time before the lab work. A study of atmospheric particles indicated that indoor concentrations of particles could be between 3 and 15 particles/m3. It was suggested that MPs was mainly resuspended from the floor due to human activity and movement (Gasperi et al. 2015).

The reason for the high amount of MP in the outlet samples could be the number of beads observed. These beads were detected in all locations and constituted up to 80 % of the numbers of beads, and was not observed in inlet wastewater. One of the possible reasons for these beads to be more frequently observed in the outlet sample, could be that the cellulose in the inlet samples was located above the beads and therefore they were not observed. It could also be from the treatment process itself where polymer is added to bind the sludge together. No further investigation was done. In this topic particles detected by the overview was unfortunately added to the total observations instead of dividing it in 2 categories. Through the first step of analysing, the quick overlook was not separated from the counting of the 50 square area. That resulted in an overestimation between 100 and 650 MPPs/L for each GF/C filter.

The main problem when observing was the quantity of cellulose in inlet wastewater samples. Hydrogen peroxide was tested and worked well to degrade some organic materials but not everything. The reason could be the amount of H_2O_2 and time on each filter, the recommended time by Frias et al. (2018) is that the H_2O_2 should work for 18 h in a fume hood. In my analyses 7 drops were used and stored in 60°C till GF/C filters were dry. The combination of 60°C with H_2O_2 could have evaporated the solution faster resulting in less reaction time. This problem occurred mainly in the inlet sample since the sludge was treated after the biological process and less cellulose was visible on the outlet filters. Particles defined as MP based on the criteria was only visually observed and could be observed differently between individuals. Each specific type of MP observed should have been analysed by Fourier-transformed infrared spectroscopy (FT-IR) to be sure that it was MP. A combination of photos and FT-IR would make it easier for further investigation to recognise which type of plastic is observed.

4.3 Sludge method and microplastic

Location 4 has the highest average number of MPs/kg d.w. observed (10370 MP/kg d.w.) followed by location 1 (8255 MP/kg d.w.). There is a greater difference between the lowest and highest observation of wastewater than sludge, one of the reasons could be that the visual method in sludge was done differently than the wastewater. The high composition of cellulose could make the MP to be located underneath the sample on the GF/C filters. Since the subsample was a small portion of the total sample, more samples should be investigated to give a more precise amount of MP/kg (d.w.). Difference in the dry weight varied trough the period from the lowest measured for location 1 in June (1,54 g/d.w.) to the highest from location 2 in August (5,06 g/d.w.). The sludge sample from location 2 in August was the only sample with heavy quantity of dry material. Without this sample, the differences of dry weight between each sample was 1,54 to 2,58g (d.w.). Since cellulose is a lightweight organic material, and the sample from August Location 2 displays more heavier compounds. The calculation gets a lower amount of MP/kg (d.w.) than it would appear if the compounds was lightweight.

The average of sludge in this investigation was 8544 MP particles kg^{-1} (d.w.) with the observed range from 4762 to 16883 MP particles kg^{-1} (d.w.) from location 1 in July and June. The study by Lusher et al. (2017) with a range from 1701 to 19 837 particles kg^{-1} (d.w.), indicates a larger variation in numbers of particles than in my investigation. One of the reasons could be that there are larger WWTPs involved in the study with different sampling methods and more variety in industry and sources.

Comparing the different visual methods between wastewater and the sludge, the visual method used on sludge seemed to be more efficient and reliable. By using a certain amount of time instead of looking through 50 areas makes this method less time consuming. The risk of counting particles twice is less in the visual method of sludge. Since no detailed description of the visual step on the filter has been recommended for MP identification, it is important to have a standard for further research so different studies could be comparable.

4.4 Types and impacts of microplastics

Three main types of MPs were observed during this investigation, beads fragments and filaments. The major type of MP observed was fragments, but there are still some uncertainties about the classification that needs further research. The study by Murphy et al. (2016) presented some MPPs to have the possibility to be brittle, therefore a poke with a needle is an uncertain classification criterion. One other issue encountered, was some particles classified as blue fragment with a size below 50µm. These fragments could have been blue-pigment on cellulose but since it followed the criterias it was classified as MP. A study of Norwegian lakes by Lusher et al. (2018) observed more MPs close to urban locations such as industries and municipal WWTP and the most observed type of MP was blue fragments. Blue fragments were most observed within the fragment types in this investigation, one of the reasons could be that it is easily detected on a white background.

Fibres was the MP type that was less observed in the wastewater samples mainly because most of the transparent fibres was difficult to detect between the cellulose on the filter. In the sludge sample the black coloured fibres was most observed due to the differences

in colour, that made these types more easily to detect. The main types of beads observed in this investigation was irregular and circular shaped and the majority was observed in outlet. It seems that the irregular shaped beads could have been concentrated in the sludge since some of the same beads was observed in the sludge samples.

To not damage the MPs it was based on past studies, chosen to use temperatures below 60°C. A study by Munno et al. (2018) performed certain tests with different reagents and MPs. It was observed that at temperatures above 60°C, certain beads from cosmetics product melted. They recommended that methods involving temperatures higher than 60°C should be revised. Since the Fenton's reagent have an exothermic reaction it produces heat but the study by Tagg et al. (2017) showed no degradation of MPPs.

5 Conclusion

MPPs were observed in all the samples in this study. The total average MP observed in inlet, outlet and sludge was 13232 MP/L, 6150 MP/L and 8544 particles kg-1 (d.w.). The types of microplastic observed were beads, fragments and filaments where fragments were the major type followed by beads. During the visual steps, it was difficult to observe the filaments in the samples due to the amount of cellulose. Fenton's reagent worked very well with degrading organic material except of the cellulose within the sludge. More MP was observed in inlet wastewater than the outlet wastewater samples and more MP got stored in the sludge than was passing through with the outlet. The difference of MPs quantity varied more from lowest to highest in the wastewater samples than the sludge. And the amount of m^3 wastewater does not seem to be related with the amount of MPs observed. The visual method for sludge was more suitable for detecting MP since it was less time consuming and showed less uncertainties of overestimation.

As the MP have become an environmental issue affecting biological life living in the aquatic environments, the results of this thesis provides useful and timely indications of how much MP that is released through the WWTP. The MP results demonstrated in this thesis findings has to be considered particularly for the sustainable use of sludge in the agriculture and consider the suitability of the use of sludge for this purpose. A useful contribution put forward and demonstrated in this thesis, is a clarification of the methodology used in studies of MPs. This thesis has highlighted procedures and methods that worked and did not work, based on the composition of samples and the contamination sources. As an example, 70% ethanol did not seem to have any impact of MP trough this investigation.

Overall, this thesis provides a fruitful step in the study of MPs in WWTPs and further studies such as investigating the removal of cellulose with the Schweizer reagent and how it effects the sludge on MPPs should be further investigated. The standard manual of description of MP types and structure of the most commonly observed MP highlighted in this master thesis would be helpful for further research. By combining the manual description and a standard protocol for the visual step, future studies would be more comparable.

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6 Annexes

Contents

1. Wastewater data

Table 1. The income of water/m3 during 24 hours

wastewater m3/d	June	July	August	September	October
Location 1	391	584	384	912	325
Location 2	515	469	504	614	469
Location 3	381		606	1077	727
Location 4	949	1132	1074	1056	978

Table 2. Total numbers of MP observed and counted before further calculations

Inlet	june	July	August	September	october
Location 4	605	621	713	505	428
Location 2	280	376	311	168	211
Location 3	68		162	234	149
Location 1	482	748	631	460	314
Outlet	june	July	August	September	october
Location 4	477	247	220	204	187
Location 2	212	96	75	92	92
Location 3	41		124	154	133
Location 1	233	387	152	67	235

Table 3. The numbers of MP in 100ml wastewater after calculations of the total number of MPs.

MP/100ml Inlet	June	July	August	September	October
Location 1	1673	2596	2190	1597	1090
Location 2	986	1305	1079	583	732
Location 3	236		562	812	517
Location 4	2100	2155	2475	1753	1486
MP/100ml Outlet	June	July	August	September	October
Location 1	809	1343	528	233	816
Location 2	746	333	260	319	319
Location 3	142		430	535	462
Location 4	1656	857	764	708	649

Table 4. Numbers of MP in 1L after calculation of MP in 100ml.

MP/ 1L Inlet	June	July	August	September	October
Location 1	16793	25962	21901	15966	10899
Location 2	9857	13050	10794	5831	7324
Location 3	2360		5623	8122	5172
Location 4	20999	21554	24747	17528	14855
MP/1L Outlet	June	July	August	September	October
Location 1	8087	13432	5276	2325	8157
Location 2	7462	3332	2603	3193	3193
Location 3	1423		4304	5345	4616
Location 4	16556	8573	7636	7081	6491

Table 5. Total amount of MP types and colours observed in wastewater before calculation.

Total counted MP	White transparent	Blue	Red	Black	Green	Another Colour
Fibres/filaments	187	262	129	533	27	88
Fragments	1625	2359	590	234	1174	389
Beads	3182	1		1		22

2. Raw-data of MP observed in 100ml of wastewater per sample.

2.1 Location 1 inlet and outlet.

Inlet wastewater

Location 1	Inlet06	Inlet07	Inlet08	Inlet09	Inlet10
Filter 1	220	280	158	160	137
Filter 2	262	233	249	179	177
Filter 3		235	224	121	
Tot	482	748	631	460	314

Inlet06-1	white/trans	blue	red	black	green	other
fiber	4	8		6	1	7
fragments	40	74	16	4	30	11
beads	19					
other						
unknown						41

Inlet06-2	white/trans	blue	red	black	green	other
fibers	2	18	1	3	8	2
fragments	21	114	24	29	3	16
beads	20					
other						
unknown						51

Inlet07-1	white/trans	blue	red	black	green	other
fibers	7	18	15	35	2	2
fragments	58	61	24	19	30	
beads	7					
other						
unknown						

Inlet07-2	white/trans	blue	red	black	green	other
fibers	3	6	3	10		1
fragments	51	58	23	26	34	2
beads	16					
other						
unknown						

Inlet07-3	white/trans	blue	red	black	green	other
fibers	5	7	2	12		
fragments	67	40	21	27	46	
beads	8					
other						
unknown						1

Inlet08-1	white/trans	blue	red	black	green	other
fibers	2	6	2	5		
fragments	39	39	18	1	27	1
beads	18					
other						
unknown						20

Inlet08-2	white/trans	blue	red	black	green	other
fibers	2	3	3	2	1	
fragments	30	93	30	3	53	4
beads	24					1
other						
unknown						31

Inlet08-3	white/trans	blue	red	black	green	other
fibers	2	3	3	5	1	3
fragments	14	71	29	4	50	11
beads	28					
other						
unknown						36

Inlet09-1	white/trans	blue	red	black	green	other
fibers		2	2	8		1
fragments	18	76	15	4	26	8
beads						
other						
unknown						18

Inlet09-2	white/trans	blue	red	black	green	other
fibers	1	5	4	9	2	5
fragments	13	87	10		34	6
beads	3					
other						
unknown						13

Inlet09-3	white/trans	blue	red	black	green	other
fibers	3	7	2	9		2
fragments	17	42	8	1	23	4
beads	3					
other						
unknown						8

Inlet10-1	white/trans	blue	red	black	green	other
fibers		6		5	2	6
fragments	14	35	8	1	29	9
beads	22					
other						
unknown						10

Inlet10-2	white/trans	blue	red	black	green	other
fibers	1	5		3		3
fragments	26	53	21		36	8
beads	21					
other						
unknown						11

Outlet wastewater

Location 1	Outlet06	Outlet07	Outlet08	Outlet09	Outlet10
Filter 1	233	387	152	67	235
Tot	233	387	152	67	235

Outlet06-1	white/trans	blue	red	black	green	other
fiber	3		1	5		1
fragments	98	5	11		10	7
beads	91					
other						
unknown						13

Outlet07-1	white/trans	blue	red	black	green	other
fibers	1		1	8		
fragments	13	2	3	6	10	3
beads	340					
other						
unknown						

outlet08-1	white/trans	blue	red	black	green	other
fibers		4		12		1
fragments	21	1		1	5	
beads	103					
other						
unknown						41

Outlet09-1	white/trans	blue	red	black	green	other
fibers	1	1		4	1	
fragments	43	6		2	2	
beads	7					
other						
unknown						29

Outlet10-1	white/trans	blue	red	black	green	other
fibers	4		1	12	1	3
fragments	17				13	4
beads	177					3
other						
unknown						15

2.2 Location 2 MP observed in 100ml per sample of wastewater.

Inlet wastewater

Location 2	Inlet06	Inlet07	Inlet08	Inlet09	Inlet10
Filter 1	284	205	171	105	114
filter 2		171	140	63	97
Tot	284	376	311	168	211

Inlet06	white/trans	blue	red	black	green	other
fiber	5,2	10,3	0,9	7,9	0,5	1,4
fragments	61,9	63,6	8,9	7,5	38,4	19,9
beads	56,9					
other						
unknown						

Inlet07-1	white/trans	blue	red	black	green	other
fiber	2	2		18		1
fragments	20	80	5	4	33	24
beads	16					
other						
unknown						4

Inlet07-2	white/trans	blue	red	black	green	other
fiber	4	10	1	5		3
fragments	7	66	4		23	23
beads	25					
other						
unknown						23

Inlet08-1	white/trans	blue	red	black	green	other
fiber	4	3	2	7		
fragments	9		10		16	13
beads	12				1	
other						
unknown						22

Inlet08-2	white/trans	blue	red	black	green	other
fiber	7	5		6		
fragments	5	76	10	1	15	10
beads	5					
other						
unknown						6

Inlet09-1	white/trans	blue	red	black	green	other
fiber	5	6		5		1
fragments	18	37	2	2	13	6
beads	10					
other						
unknown						5

Inlet09-2	white/trans	blue	red	black	green	other
fiber	3			3		
fragments	8	33	3		9	1
beads	3					
other						
unknown						

Inlet10-1	white/trans	blue	red	black	green	other
fiber		4	1	5	1	1
fragments	9	41	6		28	12
beads	6					
other						
unknown						3

Inlet10-2	white/trans	blue	red	black	green	other
fiber	2	2	1	4		1
fragments	14	48	1	1	10	9
beads	4					
other						
unknown						4

Outlet wastewater

Location 2	Outlet06	Outlet07	Outlet08	Outlet09	Outlet10
tot	215	96	74	92	92

Outlet06	white/trans	blue	red	black	green	other
fiber	5,8	0,3	0,2	3,5	0,1	0,2
fragments	15,1	0,1	2	0,6	0,2	7,4
beads	178,4					
other						
unknown						

Outlet08-1	white/trans	blue	red	black	green	other
fiber	8			6		
fragments	11			2	2	1
beads	45					
other						
unknown						11

Outlet09-1	white/trans	blue	red	black	green	other
fiber	3	2		11		
fragments	7			1		
beads	66					1
other	1					
unknown						1

Outlet10-1	white/trans	blue	red	black	green	other
fiber	7			8		1
fragments	18	2				1
beads	55					
other						
unknown						

2.3 Location 3 MP observed in 100ml per sample of wastewater.

Inlet wastewater

Location 3	Inlet06	Inlet08	Inlet09	Inlet10
Filter 1	68	95	133	149
Filter 2		67	101	
Tot	68	162	234	149

Inlet06-1	white/trans	blue	red	black	green	other
fiber	3		2	6		
fragments	23	12	3		6	
beads	9	4				
other						
unknown						9

Inlet08-1	white/trans	blue	red	black	green	other
fiber	2	1	1			
fragments	28	28	6	2	9	
beads	18					
other						
unknown						6

Inlet08-2	white/trans	blue	red	black	green	other
fiber	1	2	1	3		
fragments	21	24	2		8	2
beads	3					
other						
unknown						13

Inlet09-1	white/trans	blue	red	black	green	other
fiber		4	1	6		
fragments	12	51	12	1	20	4
beads	22					
other						
unknown						12

Inlet09-2	white/trans	blue	red	black	green	other
fiber	1			2		
fragments	10	47	7		15	8
beads	11					
other						
unknown						10

Inlet10-1	white/trans	blue	red	black	green	other
fiber	11	3	6	10	1	1
fragments	9	30	24	4	29	14
beads	7					
other						
unknown						13

Outlet wastewater

Location 3	Outlet06	Outlet08	Outlet09	Outlet10
Filter 1	41	124	154	133
Filter 2				
Tot	41	124	154	133

Outlet06-1	white/trans	blue	red	black	green	other
fiber				5		
fragments	3	1				
beads	30					2
other						
unknown						4

Outlet08-1	white/trans	blue	red	black	green	other
fiber	3	1		4		
fragments	35	4				
beads	76			1		
other						
unknown						2

Outlet09-1	white/trans	blue	red	black	green	other
fiber	3	1		10		
fragments	7	4		4	2	
beads	123					
other						
unknown						4

Outlet10-1	white/trans	blue	red	black	green	other
fiber	8	2	1	8		2
fragments	4	4		2		
beads	100					2
other						
unknown						3

2.4 Location 4 MP observed in 100ml per sample of wastewater.

Inlet wastewater

Location 4	Inlet06	Inlet07	Inlet08	Inlet09	Inlet10
Filter 1	262	214	226	222	196
Filter 2	256	225	272	283	232
Filter 3	87	182	215		
Tot	605	621	713	505	428

Inlet06-1	white/trans	blue	red	black	green	other
fiber	2	7	6	19	2	
fragments	93	32	11	17	37	6
beads	30					
other						
unknown						4

Inlet06-2	white/trans	blue	red	black	green	other
fiber		13	6	13		1
fragments	82	35	13	24	36	4
beads	29					
other						
unknown						

Inlet06-3	white/trans	blue	red	black	green	other
fiber	5	1	2	5		
fragments	43	7		6	8	1
beads	9					
other						
unknown						1

Inlet07-1	white/trans	blue	red	black	green	other
fiber	12	14	9	13		2
fragments	7	77	20	5	26	11
beads	18					
other						
unknown						18

Inlet07-2	white/trans	blue	red	black	green	other
fiber	2	5	3	10		3
fragments	22	97	18	1	23	17
beads	24					
other						
unknown						27

Inlet07-3	white/trans	blue	red	black	green	other
fiber	2	4		4		1
fragments	29	56	16	6	37	8
beads	17					
other						
unknown						27

Inlet08-1	white/trans	blue	red	black	green	other
fiber	3	10	6	6	2	1
fragments	57	71	20		28	7
beads	14				1	
other						
unknown						8

Inlet08-2	white/trans	blue	red	black	green	other
fiber		11	9	18		8
fragments	61	103	20	2	23	6
beads	10			1		
other						
unknown						

Inlet08-3	white/trans	blue	red	black	green	other
fiber		7		8		3
fragments	32	101	18	1	24	7
beads	14					
other						
unknown						18

Inlet09-1	white/trans	blue	red	black	green	other
fiber		6	8	21	1	4
fragments	30	56	19	2	40	15
beads	20					
other						
unknown						6

Inlet09-2	white/trans	blue	red	black	green	other
fiber	2	3	1	2		
fragments	82	46	15		67	12
beads	53					
other						
unknown						15

Inlet10-1	white/trans	blue	red	black	green	other
fiber	10	4	7	8		1
fragments	11	85	21		29	10
beads	10					
other						
unknown						12

Inlet10-2	white/trans	blue	red	black	green	other
fiber		15	10	46		9
fragments	4	72	30		33	9
beads	4					
other						
unknown						23

Outlet wastewater

Location 4	Outlet06	Outlet07	Outlet08	Outlet09	Outlet10
Filter 1	477	247	220	204	187
Tot	477	247	220	204	187

Outlet06-1	white/trans	blue	red	black	green	other
fiber	3			12		1
fragments	18			6		17
beads	420					
other						
unknown						

Outlet07-1	white/trans	blue	red	black	green	other
fiber	1	1	1	12		2
fragments	10	2		2	11	3
beads	202					
other						
unknown						16

Outlet08-1	white/trans	blue	red	black	green	other
fiber	1		2	13		1
fragments	16	1		1		1
beads	180	1				
other						
unknown						78

Outlet09-1	white/trans	blue	red	black	green	other
fiber	3			16		
fragments	20	5			10	2
beads	134					14
other						
unknown						14

Outlet10-1	white/trans	blue	red	black	green	other
fiber	5			2		
fragments	28	2	2		1	3
beads	144					3
other						
unknown						5

3. MP observed in 10g of Sludge samples from each location.

Table 6 Total MP observed in 10-gram wet weight of the subsamples for each location.

Tot mp/ 10g (w.w.)	june	junly	august	September	October
location 4	37	25	16	23	23
location 1	26	12	16	16	10
location 3	20		24	12	19
location 2	18	18	20	17	22

Table 7 Dry and wet measured samples in gram for each month.

Dry weight g	June	July	August	September	October
location 1	1,54	2,52	2,32	2,09	1,97
location 4	2,25	2,53	2,27	2,46	2,52
location 2	2,47	2,32	5,06	2,17	1,99
location 3	2,14		2,51	2,2	2,58
Wet weight	June	July	August	September	October
location 1	10.00g	10.07g	10.01g	10.00g	9.99g
location 4	10.03g	9.99g	10.01g	10.01g	10.01g
location 2	10.00g	10.00g	10.00g	10.00g	10.00g
location 3	10.03g		10.00g	10.00g	10.00g

Table 8 The percentage of dry matter of 10 g wet weighted sludge of each months from each location.

% (d.w) in (w.w)	June	July	August	September	October	Average %
location 1	15	25	23	21	20	21
location 4	22	25	23	25	25	24
location 2	25	23	51	22	20	28
location 3	21		25	22	26	24

3.1 Sludge Location 1 MP observed in 10g of Sludge samples from each location.

Location 1	june	July	August	September	October
Tot MP	26	12	16	16	10
Dry weight g	1,54	2,52	2,32	2,09	1,97
Wet weight g	10	10,07	10,01	10	9,99

Sludge-061	white/trans	blue	red	black	green	other
Fibres	2	4		8		
Fragments	4	1		3		1
Beads	2	1				
Other						
Unknown						

Sludge-071	white/trans	blue	red	black	green	other
Fibres				7		
Fragments	3			2		
Beads						
Other						
Unknown						

Sludge-081	white/trans	blue	red	black	green	other
Fibres		1		10		
Fragments	5					
Beads						
Other						
Unknown						

Sludge-091	white/trans	blue	red	black	green	other
Fibres		4		8		1
Fragments	1	1	1			
Beads						
Other						
Unknown						

Sludge-101	white/trans	blue	red	black	green	other
Fibres		1		5		
Fragments	3	1				
Beads						
Other						
Unknown						

3.2 Sludge Location 2 MP observed in 10g of Sludge samples from each location.

Location 2	june	July	August	September	October
Tot MP	18	18	20	17	22
Dry weight	2,47	2,32	5,06	2,17	1,99
Wet weight	10	10	10	10	10

Sludge-06	white/trans	blue	red	black	green	other
Fibres		2	2	10	1	
Fragments	1	1		1		
Beads						
Other						
Unknown						

Sludge-07	white/trans	blue	red	black	green	other
Fibres		1	2	9	1	
Fragments	1	3		1		
Beads						
Other						
Unknown						

Sludge-08	white/trans	blue	red	black	green	other
Fibres		2	1	10	2	
Fragments	3	1		1		
Beads						
Other						
Unknown						

Sludge-09	white/trans	blue	red	black	green	other
Fibres		1	1	13	1	
Fragments					1	
Beads						
Other						
Unknown						

Sludge-10	white/trans	blue	red	black	green	other
Fibres	2	3	1	14		
Fragments	1					
Beads	1					
Other						
Unknown						

3.3 Sludge Location 3 MP observed in 10g of Sludge samples from each location.

Location 3	june	August	September	October
Tot MP	20	24	12	19
Dry weight	2,14	2,51	2,2	2,58
Wet weight	10.03g	10.00g	10.00g	10.00g

Sludge-061	wh	blue	red	black	green	other
Fibres	2			7		
Fragments	7			1	1	
Beads	1					
Other						
Unknown				1		

Sludge-081	wh	blue	red	black	green	other
Fibres			1	11		
Fragments	1	2		1		
Beads	6	1			1	
Other						
Unknown						

Sludge-091	wh	blue	red	black	green	other
Fibres				9		
Fragments	1	1			1	
Beads						
Other						
Unknown						

Sludge-101	white/trans	blue	red	black	green	other
Fibres		2		10	1	
Fragments	3	1				
Beads	1					1
Other						
Unknown						

3.4 Sludge Location 4 MP observed in 10g of Sludge samples from each location.

Location 4	june	July	August	September	October
Tot MP	37	25	16	23	23
Dry Weight	2,25	2,53	2,27	2,46	2,52
Wet Weight	10,03	9,99	10,01	10,01	10,01

Sludge-061	white/trans	blue	red	black	green	other
Fibres		10	4	10	1	
Fragments	8	1		1	1	
Beads	1					
Other						
Unknown						

Sludge-071	white/trans	blue	red	black	green	other
Fibres	1	3	1	12		
Fragments		7		1		
Beads						
Other						
Unknown						

Sludge-081	white/trans	blue	red	black	green	other
Fibres		3	1	9		
Fragments	3					
Beads						
Other						
Unknown				14		

Sludge-091	white/trans	blue	red	black	green	other
Fibres			3	18		
Fragments				2		
Beads						
Other						
Unknown						

Sludge-101	white/trans	blue	red	black	green	other
Fibres	3	3	1	9		1
Fragments	2					
Beads	4					
Other						
Unknown						

4. Calculation of location 2 inlet06

Formula to Calculate MP/100ml and MP types/100ml

Location 2, period June

Inlet 880ml

tot MP counted for the sample = 2502

Outlet 870ml

tot MP counted for the sample = 1880

Tot V= Total Volume for sample

First, I had to calculate the total microplastic per litre;

$$tot\ mp * \frac{1000ml}{tot\ V} = MP/L$$

Inlet sample:

$$\frac{mp}{1L} = 2502 * \frac{1000ml}{880ml} = 2843mp/1L$$

$$\frac{mp}{100ml} = 2843\ mp * 0{,}1L = 284mp/100ml$$

Outlet sample:

$$\frac{mp}{1L} = 1880 * \frac{1000ml}{875} = 2157mp/1L$$

$$\frac{mp}{100ml} = 2157\ mp * 0{,}1L = 215mp/100ml$$

Inlet MP/100ml = 284

Outlet MP/100ml = 215

Formula of percentage of MP type of 100ml

$$100 * \frac{Tot\ mp\ types}{inlet - outlet\ mp/100ml}$$

5. List of Materials/Equipment's and chemicals

Materials

Name	Manufacturer	Explanation
Face mask	VWR International	Protect from virus and bacteria contained in the sludge
Cotton lab coat	FRISTAD AB	Protection clothes
Latex and vinyl gloves	VWR International	Protection for hands
Vacuum funnel top	Millipore® Pyrex®	Top unit of the vacuum filtration where GF/C filters filtrated the wastewater sample.
250-1000 ml graduated beakers	VWR and Schott DURAN	To making $Zncl_2$ Solution and storing sludge sample trough the method.
1L canteen	Norengros	To let sample overflow to the canteen.
Metal cans	(Local paint shop in BØ)	Store all the samples from the wastewater treatment plants.
Aluminium	VWR International	Cover all equipment's as lid and covering petri dish for storage.
Plastic bottle 5L	Emballator and VWR International	For storing distilled water and filtrated tap water.
Zeiss, Discovery V20 (Stereo microscope)	Karl Zeiss	To look for microplastic on the filter
Weight measurement (BL 1500S)	Sartorius	To weight the beakers and the dried sludge

Metal spoon	Unknown	Taking subsamples of the sludge with.
Glass rod	VWR International	Homogenise samples
Gf/C filters 1,2 µm	VWR International	Separate the liquid from the particles.
Wash bottle, KJS E500	Unknown	Washing, and flushing beakers and cylinder measurement. To get adhered particles from the glass walls.
Thermometer	KEBO LAB	To measure the temperature of the evaporation of 70% ethanol.

Chemicals

Chemicals	Molecular Formula	Manufacturer
Hydrogen Peroxide 30%	NaOH (H_2O_2)	MERCK KGaA
Iron sulphate heptahydrate	$FeSO_4$ * $7H_2O$ M= 278.02 g/mol	MERCK KGaA
Ethanol 70%	C_2H_5OH	Kemetyl
Zink Chloride	$ZnCl_2$	MERCK KGaA

6. Sludge Procedure

Pre-treatment

1- Clean the table with ethanol 70%
2- Clean beaker, petri dish and all equipment for use with distilled water – 3x
3- Put 2 petri dish with G/F C filters – Air contamination control.
4- Weight the glass beaker.
5- 10 g sludge wet weight, per sample total 3 subsamples.
6- Fenton`s reagent.

Step A

1- Mix thoroughly the melted sludge sample for 1 minute before taking 3 subsamples Collect 3 subsamples, top, middle and bottom of 10 g each wet weight.
2- Mix the 30 g of subsamples 1 minute and take out 10 grams to a 250ml precleaned beaker.
3- Add 20 ml of ethanol in the subsample of 10 grams and wait 15 minutes.
4- Put the beaker with lid on, into a pan with water over a heating plate on maximum 60 ˚C to evaporate the ethanol. Measure the temperature every 30 minutes, if the temperature increase pour cold water to decrease the temperature.
5- Dry the sample in a heating oven with aluminium foil as lid until its dry (maximum 60 ˚C).
6- Weight the dried sludge to get the total dry weight.
7- Add 20 ml of 30% H_2O_2 and 0.0667 g/ 10 ml of (Fe^{2+}) ferrous sulphate. (concentration 6.67mg/ml)
8- Wait 2-3 hours to let the Fenton's reagent to work in room temperature.

Step B Flotation

Step B1 Tap water

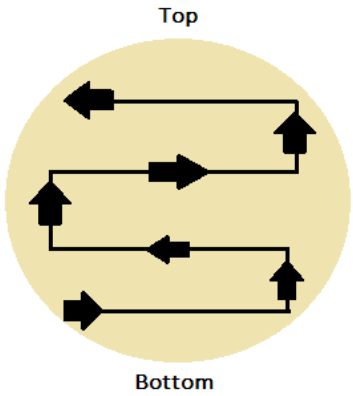
1- In the same beaker add filtered tap water (GF/C filtered tap water) to the brim.
2- Mix for 30 seconds to make it homogenous, then let it settle for 1 hour.
   1- When the layer has sedimented pour out the tap water to the beaker allowing overflow to the larger vessel.
3- Vacuum filter the floated top layer.
4- Pour gently out the remaining freshwater till the bottom layer is disturbed then stop.
5- Vacuum filter the remains of freshwater in the larger vessel.

Step B2 $ZnCl_2$

2- In the same beaker add concentration of $ZnCl_2$ (1.8 g/cm3) to 1 cm below the brim. (180g $ZnCl_2$ / 100 ml distilled H2O)
3- Stir it vigorously for 60 s till its homogenous. (stir in 8 numbers)
4- Make a big lid of aluminium foil to cover the vessel and the beaker.
5- Let the sediment settle for 2 hours placed in a larger vessel.
6- Remove the Aluminium foil and any attached particles rinsed back to the beaker.
7- Additional Pour $ZnCl_2$ (1.8 g/cm3) in the beaker allowing overflow to the larger vessel.
8- Then rinse any adhered particles from outside of the beaker into the overflow container.
9- Vacuum filtrate the overflow liquid.
10- Flush GF/C 1.2 µm filter with distilled water. (to collect floated particles and remove $ZnCl_2$)
11- Mix the sample for 15 seconds and Vacuum filtrate.
12- Flush GF/C 1.2 µm filter with distilled water (to collect floated particles, and remove $ZnCl_2$)
13- Dry GF/C filters at maximum 60˚C before analysis.

Step D Visual inspections

Inspect each filter for a minimum of 5 to 15 minutes starting at the bottom left moving to the right and in direction to the top as figure shows below.



1- Use- Digital stereo microscope for visual inspection.
2- Take a quick overlook of the filter with 7,6x to 11,5x in magnification.
3- Do the same thing and use the magnification range of 11,5x to 150x.
4- Follow criteria: colour, shape, and size.

Example of counting table:

	white	Black	Blue	Green	other
Filaments/fibres					
Fragments					
Beads					
Other					
Unknown					

7. Wastewater procedure

Pre-treatment:

1- Clean the table with ethanol 70%.

2- Clean beaker, petri dish and all equipment for use with distilled water – 3x.

3- Place 2 petri dishes with GF/C filter for air contamination control.

Step A

6- Shake the metal pail for 60 seconds with the lid on.

7- Take off the lid and stir with a glass pin for 30 seconds.

8- Pour 100ml of the homogenous wastewater into 500ml cylindrical beakers with a funnel of glass on the top.

9- Measure the volume of wastewater in the cylindrical beaker (100ml).

10- Add 200ml of ethanol 70% and wait 15 minutes.

11- Mix it before pouring the amount into the vacuum filtration with G/F C filter.

12- Wash the remaining content in the cylindrical beaker with distilled water into the vacuum filter (to get adhered particles).

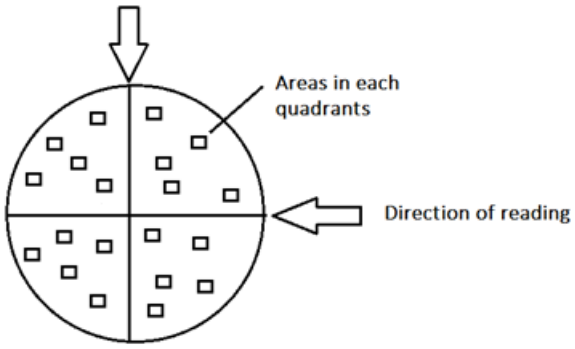
13- Then put the GF/C 1.2 µm filter in a petri dish with aluminium as lid and bottom, identify: place, date and type.

14- Add approximately 7 drops of H_2O_2 on the filters and store them in the heating oven till it`s dry (The oven temperature should be below or maximum 60ºC).

15- Look on the digital stereo microscope.

Step B

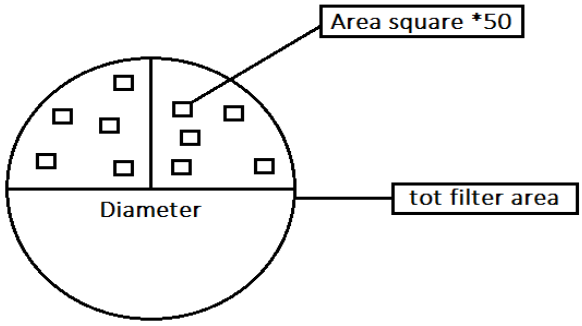
Pre-treatment: The petri dish used for detection of microplastic is treated with a line of 100% cotton to make the diagonal lines easier to follow.



1- Take a quick overlook for big microplastic or macroplastic.
2- The GF/C filters is analysed through a Digital stereo microscope. (Zeiss, Discovery V20)
3- The filter is divided by diagonally lines, where 15 areas of each line is analysed. (2,5mm field of view)
4- And 5 (2.5mm field of view) areas in the quadrants shown in the example above.
5- Read the first line from right to left, and the other one from the top to the bottom.
6- Particles is categorised by type, shape and colour.
7- The particles that can't be defined is counted and wrote down in the unknown label in the table of categories.

Calculation

Total of 50 areas should be analysed to calculate the MPs on the filter.



1- Calculate the mm^2 of the field of view by measuring the length and width.

2- Measure the diameter of filter area to calculate the total area of the filter

Formula: (Area of a circle is 3.14*R^2).

$$\frac{MP}{1L} = \frac{tot\ filter\ area}{area\ square * 50} * MP * 10$$