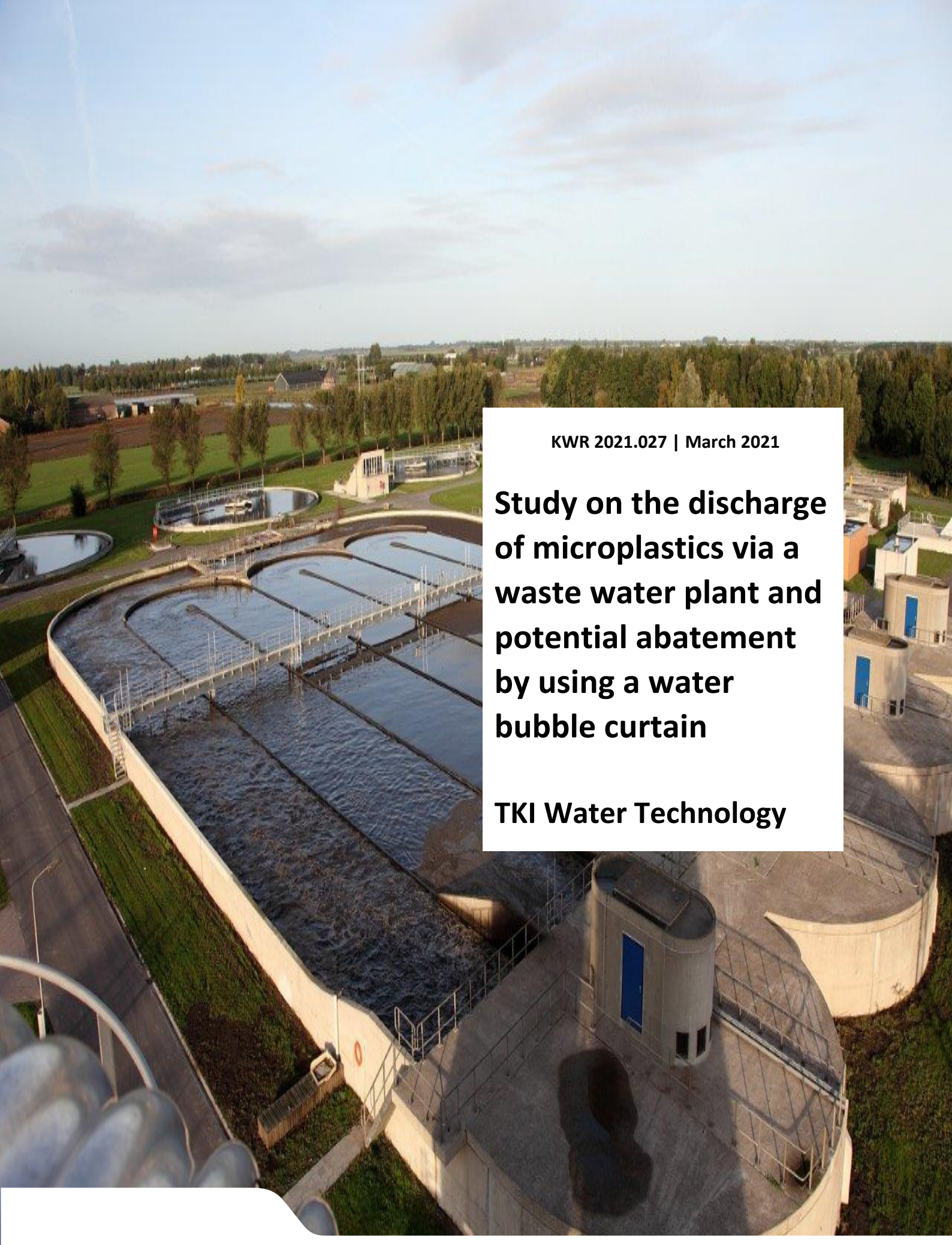

KWR 2021.027 | March 2021

# Study on the discharge of microplastics via a waste water plant and potential abatement by using a water bubble curtain

## TKI Water Technology

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# Report

## Study on the discharge of microplastics via a waste water plant and potential abatement by using a water bubble curtain

**KWR 2021.027 | March 2021**

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**Project manager**
Frank Oesterholt

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**Authors**
Stefan Kools (KWR), Patrick Bäuerlein (KWR), Eelco Pieke (HWL), Frank Oesterholt (KWR)

**Quality Assurance**
Thomas ter Laak (KWR)

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More information
Ir. F.I.H.M. Oesterholt
T 0306069575
E frank.oesterholt@kwrwater.nl

PO Box 1072
3430 BB Nieuwegein
The Netherlands

T +31 (0)30 60 69 511
E info@kwrwater.nl
I www.kwrwater.nl

# Summary

This report describes the research of a consortium to investigate the potential to reduce plastic outflow from a waste water treatment plants (WWTP) with a bubble curtain (The Great Bubble Barrier) The focus lies on plastic in the size ranges of 0.02 mm to 5 mm (microplastic) The ultimate goal of the consortium is to prevent microplastic discharge from effluent towards surface water.

To reach this goal not only an effective removal technique is necessary but also reliable analytical techniques proving the effectiveness. For this, two analytical methods were compared during this research. A pilot set-up was built of a bubble curtain in a WWTP-effluent canal. During the course of six months several samples were taken at multiple points and analysed using two analytical principles: laser direct infrared (LDIR) imaging and optical microscopy. These two methods were evaluated for their comparability for plastic particles analysis in the size range from 0.02 mm to 5 mm.

In the condition where the pilot bubble curtain was set up, it was not possible to conclude that it is capable of reducing the outflow of microplastic particles in the effluent stream of the sewage treatment plant. Changes in particle concentration before and after the curtain were indistinguishable from the variation between samples from the same location. Previous studies however showed that the bubble curtain was capable of blocking buoyant plastic fragments on the surface from 1 mm. The lack of a measurable effect may be due to external influences, including analytical detection limitations. Changing the design of the barrier and the dimensions of the canal may improve the detection of the efficiency towards smaller plastic particles studied here. To evaluate the effect of different properties of the plastic particles we recommend additional research with a focus on (i) how hydrodynamic conditions affect separation (concentration and potential collection of microplastics), considering bubble curtain characteristics, horizontal and vertical water-flow and sampling/collection as well as (ii) the effects of physicochemical characteristics (e.g. size, dimensions, surface characteristics) of the plastic particles on their behaviour in the water column.

In this research we observed a WWTP-outflow of microplastics between 1 – 6 particles/L (optical method) and 40 – 50 particles/L (LDIR method). These results clearly indicate that WWTP-effluent emits as much microplastics as was shown in previous studies. Nevertheless, WWTPs remove a substantial part of the microplastics (90 - 99 %) from the influent water through the sludge line. Comparison of the two analytical strategies shows that these methods have similar results and variation with regard to trends, fluctuations, particle number and fibre number. However, these methods provide different levels of information: LDIR is capable of identifying a range of plastic types and shapes whereas in the optical methods distinction is limited and based on visual assessment only. Additionally, LDIR is able to detect smaller particles explaining why more particle numbers were reported using LDIR. However, the optical method may be more advantageous in samples with a high organic content as this impedes plastic

identification using infrared. Both methods showed that especially fibres are a consistent part of the outflow of plastic fragments, also described in other studies. Most frequently identified polymers were polyamide (PA) PET, isoprene, PU/varnish, PP, PE-Cl, PE.

This study highlights the need of analytical strategies in microplastic analysis. Furthermore, it identifies the necessity for standardisation and a deeper understanding of factors of influence, e.g., sampling depth, weather conditions and day-to-day fluctuations.

Our results confirm other studies that WWTP-effluent is an emission source for microplastics. A preliminary risk assessment shows that no imminent ecological risks are to be expected on the basis of the concentration levels of microplastics measured in this study in the outflow of a WWTP. Note that the risk for particles with a smaller size is not yet fully known and is currently very uncertain. Although no ecological effects are currently expected there is a high chance that microplastics will accumulate in the environment as they show little breakdown. This, together with increasing emissions, lack of recycling or alternatives to plastics, could pose a risk in the future, as it could lead to an increase in the levels of microplastics.

Out study underlines that technology options to reduce the outflow of (micro) plastics to surface water, such as the bubble screen of the Great Bubble Barrier, are urgently needed and worth further development. Besides the need for analytical methods, we recommend policy on preventing plastic pollution and methods to reduce plastic outflow in water.

# Uitgebreide Nederlandstalige samenvatting

Microplastics worden gedefinieerd als polymeerdeeltjes of kunststofvezels die kleiner zijn dan 5 mm maar groter dan 0,001 mm. Microplastics in het milieu worden verdeeld in twee verschillende hoofdgroepen: primaire en secundaire microplastics. Primaire microplastics worden geproduceerd in de vorm van deeltjes met een grootte van minder dan een millimeter en kunnen in het milieu terechtkomen via consumentenproducten of verliezen tijdens opslag of transport. Secundaire microplastics zijn het resultaat van verwering en afbraak van allerlei plastic producten, waaronder vezels van synthetische kleding (bv. PET-vezels van fleece), deeltjes van bandenslijtage, materialen op sportvelden en polymeerverven.
Momenteel is er veel aandacht voor microplastics, zowel vanuit de wetenschap als van de media, en dit heeft microplastics op de agenda gezet bij regelgevende instanties en internationale organen die vragen om meer gegevens. De omvang van het microplastics-probleem is nu nog lastig te bepalen: er is nog maar weinig bekend over de hoeveelheid microplastics in het milieu en nog minder over de impact van die microplastics op het milieu en de (menselijke) gezondheid. Vragen richten zich op: (a) de effecten van plastics zelf, (b) de stoffen die uit de plastics kunnen komen als ook (c) de vragen over mogelijke aangroei van bacteriën en (d) of toxische stoffen aan de deeltjes kunnen ophopen.
Naarmate plastic langer in het water ligt, breekt het onder invloed van zonlicht en wrijving af in steeds kleiner wordende deeltjes die makkelijker in de voedselketen kunnen worden opgenomen. Inmiddels is uit verschillende studies gebleken dat microplastics worden aangetroffen in vogels, vissen, sediment, voedsel en tot op zekere hoogte ook in drinkwater. Deze kleine plastic deeltjes trekken organische microverontreinigingen aan in het oppervlaktewater wat kan leiden tot ecotoxicologische effecten. Er zijn ook aanwijzingen dat microplastics dragers zijn van biofilms waarin zich pathogene micro-organismen bevinden en op die manier zogenaamde water overdraagbare ziekten kunnen overbrengen.

Traditionele rioolwaterzuiveringsinstallaties (RWZI's) verwijderen nitraat, fosfaat en zwevende deeltjes uit afvalwater. Grotere delen plastic en andere materialen worden op de RWZI uit het water gezeefd. Uit literatuurgegevens blijkt verder dat RWZI's ook een groot deel van de microplastics (90 – 99 %) via de sliblijn verwijderen. Over de aanwezigheid van microplastics in het effluent van RWZI's is op dit moment nog weinig bekend. De getallen in de literatuur lopen op dat punt behoorlijk uiteen. Effectieve oplossingen die het mogelijk maken om microplastics tegen te houden en/of te verwijderen uit het RWZI-effluent zijn nog niet duidelijk genoeg beschreven.

De toepassing van The Great Bubble Barrier (TGBB) is een bewezen effectieve technologie om macroplastics uit stromende rivieren en kanalen te verzamelen. De buis van de Bubble Barrier heeft kleine gaatjes waar luchtdruk op wordt gezet waardoor een bellenscherm ontstaat. Door de natuurlijke stroming van een rivier en de diagonale

ligging van het bellenscherm op de bodem wordt plastic afval naar de oever geleid, zonder de scheepvaart of vissen te hinderen. Daar kan het plastic dan verder uit het oppervlaktewater worden gehaald. Met een vergelijkbare opstelling in het effluentkanaal van de RWZI Wervershoof is onderzocht of het bellenscherm ook effect heeft op de verwijdering van microplastics. Het is al bewezen dat de Bubble Barrier effect heeft op microplastics van 1 mm en groter. Dit experiment zou moeten uitwijzen waar de grens qua grootte van de deeltjes precies ligt. Het effluentkanaal van de RWZI Wervershoof is direct verbonden met het IJsselmeer. In het IJsselmeer bevindt zich een waterinnamepunt van het drinkwaterbedrijf PWN. Dit oppervlaktewater wordt, deels direct via geavanceerde waterzuiveringstechnieken en deels indirect, na voorzuivering en infiltratie in de duinen, gebruikt als grondstof voor de drinkwaterproductie. Voor PWN is het daarom van belang onderzoek te doen naar de mogelijkheden om de uitstroom van microplastics naar oppervlaktewater te voorkomen.

In het voorjaar van 2019 is door TGBB een bellenscherm geplaatst in het effluentkanaal van de RWZI Wervershoof. Deze locatie is gekozen omdat het effluentkanaal door zijn afmetingen zeer geschikt is om eenvoudig een bellenscherm te installeren. Daarnaast zijn door TGBB voorzieningen getroffen om de monsterneming rond het bellenscherm mogelijk te maken. Vanaf juni 2019 zijn gedurende een periode van zes maanden op verschillende momenten en posities voor en na het bellenscherm watermonsters genomen. Het onderzoek richt zich naast de werking van het bellenscherm op microplastics ook op het bepalen van de hoeveelheid, type en grootteverdeling van microplastics in het gezuiverde afvalwater (effluent) én op verbetering en standaardisatie van het meetprotocol voor microplastics.

Parallel aan de wens om innovaties te ontwikkelen voor de reductie van plastics in waterstromen zijn ook analytische technieken nodig die de effectiviteit kunnen aantonen. In dit onderzoek zijn om die reden twee recent ontwikkelde analytische methoden toegepast. Het betrof een techniek gebaseerd op laser direct infrared (LDIR) waarmee microplastics in korte tijd met behulp van een gevoelige laser worden geïdentificeerd, die is vergeleken met de meer standaard optische microscopie waarbij microplastics visueel worden gedetecteerd en gekarakteriseerd. Beide methoden zijn geëvalueerd op hun vergelijkbaarheid voor de analyse van plastic deeltjes in het groottebereik van 0,02 mm tot 5 mm. Het gaat hier derhalve over een studie naar een brede range van microplastics.

In deze opstelling van het bellenscherm bleek het niet mogelijk om te concluderen dat het scherm de afvoer van microplastics in de effluentstroom van de rioolwaterzuiveringsinstallatie kon verminderen. Veranderingen in deeltjesconcentratie, voor en na het scherm, waren niet te onderscheiden van de variatie tussen monsters van dezelfde locatie op verschillende tijdstippen. Het ontbreken van een meetbaar effect werd in verband gebracht met verschillende oorzaken en externe invloeden, waaronder beperkingen van de analytische detectiemethoden. Op grond van dit onderzoek is een aanbeveling gedaan voor het uitvoeren van twee typen onderzoek onder gecontroleerde omstandigheden om het gedrag van kleine fragmenten in water beter te begrijpen en zo de intrinsieke zuiveringsprestaties te scheiden van artefacten zoals omgevingsfactoren (weersomstandigheden) die de zuiveringsprestaties onder veldomstandigheden beïnvloeden. Ten eerste zou moeten worden onderzocht hoe

hydrodynamische omstandigheden, zoals de eigenschappen van het bellenscherm, de horizontale en verticale waterstroming en de bemonstering/verzameling, de scheiding (concentratie) en de potentiële verzameling van microplastics beïnvloeden. Ten tweede zouden de effecten van fysisch-chemische kenmerken (bijv. type plastic, grootte, afmetingen, oppervlakte-eigenschappen) van de plastic deeltjes op hun gedrag in de waterkolom moeten worden geëvalueerd. Hiervoor zijn waarschijnlijk meer fundamentele studies op laboratoriumschaal nodig om zo een beter inzicht te krijgen in de beweging van deze deeltjes in een waterkolom en met luchtbellen in relatie tot hun fysisch-chemische kenmerken. Tenslotte moeten deze twee aspecten worden gecombineerd om het potentieel van een bellenscherm voor het onderscheppen van microplastics te optimaliseren voor een specifieke situatie, zoals het effluent van een afvalwaterzuiveringsinstallatie. Pas dan kan definitief worden geconcludeerd of het bellenscherm in deze opstelling geen effect heeft of dat de omstandigheden het scherm tegenwerken.

De resultaten van meer dan 70 metingen geven ook duidelijk aan dat RWZI-effluent vergelijkbare hoeveelheden microplastics uitstoot als vastgesteld in eerdere studies. Door het feit dat zoveel metingen zijn gedaan op deze locaties, zijn (i) de concentraties betrouwbaarder en (ii) wordt duidelijk dat er grote concentratiefluctuaties zijn, zowel tussen de verschillende meetpunten als in de tijd.
Het naast elkaar leggen van de twee analytische meetmethoden laat vergelijkbare resultaten zien met betrekking tot fluctuaties, deeltjesaantal en vezelaantal. Dit onderbouwt de conclusies van het onderzoek omdat ze door beide meetmethoden worden ondersteund. De twee methoden leveren dus dezelfde trends, maar tegelijkertijd ook verschillende informatieniveaus. De twee belangrijkste verschillen tussen beide methoden zijn dat met LDIR deeltjes tot 20 μm (in dit onderzoek) worden gedetecteerd terwijl optische microscopie een ondergrens van 50 μm kent. Daarnaast kan LDIR de deeltjes op basis van hun infraroodspectrum identificeren. Bij de optische methode wordt alleen visueel bepaald (expert judgement) of een deeltjes een plasticdeeltje is of niet. De meest frequent geïdentificeerde polymeren met LDIR waren polyamide (PA) PET, isopreen, PU/vernis, PP, PE-Cl en PE. De hoeveelheid plasticdeeltjes in het RWZI-effluent lag tussen 40 – 50 deeltjes (LDIR) en 1 – 6 deeltjes (microscoop) per liter. Het verschil laat zich verklaren door het feit dat met de LDIR methode meer kleinere deeltjes worden gedetecteerd (tussen 20 en 50 μm). Beide methoden toonden aan dat vezels een consistent onderdeel vormen van de uitstroom van plastic fragmenten, zoals ook in andere studies is vastgesteld. Hoewel als onderdeel van het onderzoek de monstervoorbehandeling en de detectie voor beide methoden is geoptimaliseerd, is ook vastgesteld dat het veel inspanning kost om in sterk verontreinigde monsters via LDIR microplastics te kunnen meten. Dat komt omdat een hoog organisch gehalte de identificatie van kunststoffen met infrarood belemmert. Op dit punt heeft de optische methode een voordeel. Het verwijderen van deze organische stoffen in de monstervoorbehandeling blijkt dus essentieel bij het toepassen van LDIR methode. Verdere ontwikkeling van LDIR als meetmethode is aan te raden om ook calamiteitenmonsters te kunnen meten.
Uit dit onderzoek wordt duidelijk dat bij het plannen van meetstrategieën van tevoren duidelijk moet zijn welke informatie belangrijk is. Dit is bepalend voor selectie van de meest geschikte meetmethode. Dit onderzoek wijst ook op de noodzaak van een gestandaardiseerde methodologie voor de monsterneming, monsterbewerking en analyse van microplastics, aangezien kleine keuzes bij de monsterneming, zoals bemonsteringsdiepte,

weersomstandigheden en dagelijkse schommelingen, aanzienlijke gevolgen kunnen hebben voor de meetresultaten.

Uit de op dit moment beschikbare voorlopige risicobeoordeling blijkt dat op basis van de in deze studie gemeten concentratieniveaus van microplastics in de uitstroom van een RWZI geen dreigende ecologische risico's te verwachten zijn. Het risico voor de deeltjes met een kleinere omvang is overigens nog niet volledig bekend en op dit moment nog onzeker. Hoewel op dit moment dus geen ecologische effecten worden verwacht, bestaan er wel grote onzekerheden en bestaat de kans dat microplastics zich ophopen in het milieu. Dit kan, samen met toenemende emissies, gebrek aan recycling of alternatieven voor kunststoffen, in de toekomst een risico gaan vormen, aangezien dit kan leiden tot een toename van de gehaltes aan microplastics. Daarom zijn mogelijkheden om de uitstroom van (micro)plastics naar het oppervlaktewater te voorkomen, zoals het bellenscherm van The Great Bubble Barrier, dringend gewenst en de moeite waard om verder te ontwikkelen.

# Contents

# 1 Introduction

## 1.1 Research setup

This research has been set up around the Great Bubble Barrier, a bubble curtain designed and operated by a recent founded company. The bubble curtain was installed in an effluent canal transporting the treated waste water from a WWTP. While the issue of plastics in our environment is known for a long time, detection methods on small plastic fragments are in a developing phase and reliable emission data on the entry to the environment is currently lacking. Hence, to perform this research project, several questions were to resolve in parallel.

- The goal was to gather data on a possible mitigation option for WWTPs, namely a bubble curtain to place in an effluent canal. Recent success in active removal by a bubble curtain as demonstrated by The Great Bubble Barrier was chosen as this has proven to be a promising technique [1]. In previous research and trial series (unpublished), the bubble curtain showed continuous removal of (plastic) particles larger than 1 mm. Bubble curtains are interesting as they are non-invasive in the environment, are scalable and usable locally, and focus on the body of water rather than on the treatment process [2, 3]. These techniques could be employed as additional treatment steps of effluent from an existing WWTP, reducing the costs of altering the treatment system itself. The utilization of bubble curtain techniques for the removal of microplastics is therefore an interesting opportunity for wastewater utilities. In our set-up, the wastewater utilities effluent canal was chosen as a suitable test area, considering it a system without strong external influences, with well-known flowrates, and easy-to-determine flow profiles.
- Data for the evaluation of the bubble curtain is also used to get a better insight in the plastic particle number and types of plastics present in the effluent of the WWTP and the canal. This is important as this canal is connected to a lake which serves as a drinking water abstraction point. By using the data in this research, we were able to quantify the discharge of particles from a waste water treatment plant (WWTPs) as WWTPs are identified as entry points of microplastics into freshwater in others studies [4-6].
- As described, the science on analytical methods to sample and detect plastic fragments is currently under development. In this research, we have chosen to compare two methods for microplastic monitoring in the size range 20 µm – 5000 µm: laser direct infrared imaging (LDIR) and a visual microscopic method. These two methods were used by two separate Dutch laboratories (KWR Water Research Institute and PWN's Laboratory, further referred to as HWL). The parallel research set-up was chosen to investigate how these methods may complement each other.

In this report, we describe and discuss the environmental issue, research outcomes and put data in context. Based on this report, a scientific paper is submitted to a journal. In this report all supplemental information is included.

## 1.2 Consortium

The consortium consists of knowledge and technology partners. The consortium is composed of drinking water company PWN, water board Hoogheemraadschap Hollands Noorderkwartier (HHNK), knowledge institute KWR Water Research Institute, and innovative tech partner The Great Bubble Barrier (TGBB). The consortium was formed as an answer to the growing need for more information on microplastics and potential removal options. The Great Bubble Barrier® aims to create a barrier stopping plastics from flowing from the WWTP, but it also allows fish and ships to pass through the barrier unimpeded. The public partners HHNK and PWN in the consortium are interested as the mitigation option may prove to be suitable technology for reducing plastic particles from the WWTP of HHNK, close to a drinking water abstraction point of PWN. In this research project, newly developed detection methods were used and next to KWR laboratories, het Waterlaboratorium, further referred to as HWL was added.

## 1.3 Acknowledgements

We would like to thank all project partners for their combined effort to initiate, execute and finalize this project. The thorough reading and commenting on the draft manuscript and this TKI-report by all project partners was essential. The execution of this project would not have been successful without the field work assistance of the operators of HHNK at the waste water facility Wervershoof. We would like to thank TGBB for installing the system on site and their willingness to always be available for problems and questions. Many thanks go also to the sample takers of HWL and KWR who sometimes had to deal with poor weather conditions and to the laboratories of HWL and KWR for their efforts in detecting microplastics in the samples provided, which is sometimes a rather intensive job.

# 2 Environmental issue of microplastics

Plastics are only being produced since the 1950s but in 2018, the global production was 359 million tons of plastics annually, of which 62 million tons were produced in Europe alone [7]. While production has evidently increased over the years, recycling and waste reduction of plastics has not kept up with environmental emissions as a result. It is only recent that the environmental issues of plastics became apparent by studies on marine systems [8]. In environmental science, large focus is on the microplastics, which are defined as plastic polymer particles or plastic fibres smaller than 5 mm but generally larger than 1 µm. However, the term had no lower boundary size limit but the sub-micron particles can be called "nanoplastics" [9-11]. Around 2014, the research was broadened to freshwaters and terrestrial systems [12, 13]. By now, various studies have shown that plastics are detected in birds, fish, sediment, food, and to some extent drinking water [8, 14-17]. There is also evidence that microplastics are vehicles for biofilm and can carry waterborne diseases [18]. Microplastics in the environment are divided into two different major groups; primary microplastics and secondary microplastics. Primary microplastics are produced as particles in the sub-millimetre size range (e.g., pellets) and may enter the environment via consumer products or losses during storage or transport. Secondary microplastics are the result from weathering and breakdown of all kinds of plastic products, including fibres from synthetic clothing (e.g. PET fibres from fleece), particles from tyre abrasion, materials on sport grounds and polymer paints [8, 11, 19 20].

At the moment there is widespread attention for microplastics from both science and the media, and this has brought microplastics to the agenda of regulators and international bodies that are requesting data [21, 22]. The extent of the microplastics problem is difficult to determine: very little is known about the quantity in environmental media and even less about the impact of microplastics on the environment and (human) health.

## 2.1 Microplastics in waste water

One commonly named source for microplastics is wastewater generated by anthropogenic activities, e.g., industrial or living [5, 23-25]. Wastewater is treated at wastewater treatment plants (WWTP) prior to release in the environment and wastewater effluent may therefore be an important source of environmental microplastics. However, it is poorly understood whether treatment efforts at WWTP are capable of mitigating microplastics discharge into the environment, especially for smaller particles. Most microplastics expected in wastewater are secondary microplastics, i.e. the product of breakdown processes. It is expected that, due to flocculation procceses in the WWTP process, macroplastics, mesoplastics, and larger microplastics are less likely to be present in WWTP-effluent as these are likely more effectively removed by trapping in activated sludge particles and sedimentation[25]. Hence, WWTP-effluent is likely to contain an overabundance of smaller particles contrasted to larger particles which will rarely be found.

# 3 Materials and Methods

## 3.1 Research development

During the course of the research projects, adjustments have been made that are incorporated in this technical report, e.g. in sampling and detection methodology. We have included these learning points in this chapter and indicated which results are presented in the following chapters.

## 3.2 Research location

We conducted research on a traditional waste water treatment plant (WWTP) in Wervershoof, The Netherlands, containing a multiple step treatment process: pre-sieving, aeration tank and sedimentation tank after which water is discharged. The plant is operated by water authority Hoogheemraadschap Hollands Noorderkwartier. It serves around 306.000 people equivalents and discharges on average 40 million litre a day into an effluent canal that eventually leads to Lake IJssel (IJsselmeer). This lake has a Natura 2000 status and serves as a drinking water source for drinking water company PWN. The effluent canal is 10 m wide and 1,500 m long before it discharges into Lake IJssel.


*Figure 1: Aerial photograph of the WWTP Wervershoof. Indicated is the location of the bubble barrier in the effluent canal.*

## 3.3 Installation and operation of the bubble curtain

The bubble curtain was installed in the effluent (outflow) canal of the WWTP, see Figure 1 and Figure 3. The bubble curtain is created by pressurizing common air in a specifically designed tube made of perforated PVC, which is located on the bottom of the waterway. The bubble curtain creates an upwards thrust due to ascending air bubbles, which brings particulate matter and undissolved solids to the water surface. By placing it diagonally in the waterway, the bubble curtain uses natural current to guide gathered matter to the catchment system at the riverside. In practice the system is fully functional when a catchment system is placed at the end of the bubble curtain to collect debris. However, a catchment system which catches plastics smaller than 1 mm has not been developed yet. Therefore, for this specific pilot research into microplastics, a catchment system was not installed.

## 3.4 Weather conditions and water flow rate from the WWTP

The weather conditions for each of the sampling dates were retrieved from the KNMI (The Royal Dutch Metrological Institute, see Table 1). The closest measuring station is in Berkhout, about 14 km southwest of WWTP Wervershoof. In addition, a weather station was mounted on top of the bubble curtain setup and operated by consortium partner The Great Bubble Barrier (for data, see Table 1).

Operators of HHNK provided the data on the flow ($m^3$/hour) in the WWTP, largely influenced by rain fall (Figure 2). For example, the 28$^{th}$ of November was a day with a particularly high rainfall that resulted in a sewage overflow. The data from this day will later not be included when calculating averages. The change of speed and direction of the wind may have an impact on particle numbers. However, as there is no clear pattern in the data, n possible impact of the wind was acknowledged but not substantiated.

*Table 1 Weather conditions in Berkhout and Wervershoof. N.d. means that no data was received due to technical issues.*

| Date | Temp. / ºC | Wind m/s | Wind direction | rainfall mm | Wind m/s | rainfall mm |
|---|---|---|---|---|---|---|
| | Berkhout | Berkhout | Berkhout | Berkhout | Wervershoof | Wervershoof |
| 27-6 | 16.8 | 4.1 | NE | 0 | 2.4 | 0 |
| 4-7 | 15.8 | 3.1 | W | 0 | 4 | 0 |
| 18-7 | 18.5 | 4 | SW | 2 | 2.2 | 0 |
| 6-8 | 18.9 | 5 | SW | 0 | 2 | 0 |
| 4-9 | 15.1 | 5.1 | SW | 4 | n.d. | n.d. |
| 2-10 | 10 | 4.2 | NW | 4.1 | 1.4 | 0.4 |
| 24-10 | 14.3 | 4.4 | SSW | 0.7 | 3 | 0 |
| 31-10 | 4.8 | 4.4 | E | 0 | 1 | 0 |
| 14-11 | 5.8 | 5.2 | EZE | 0 | 3.3 | 0 |
| 28-11 | 9.1 | 6.7 | W | 18.5 | n.d. | n.d. |

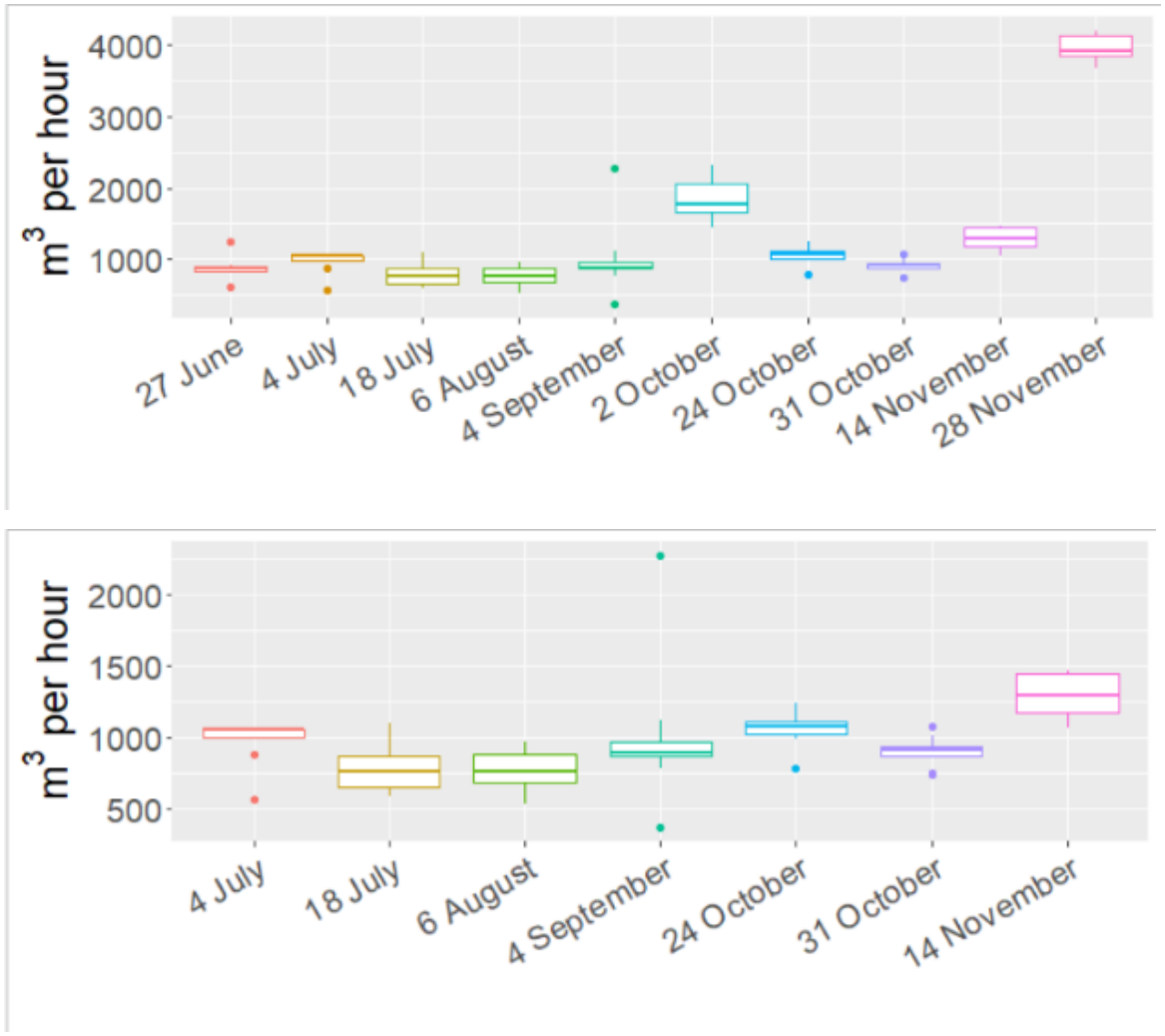



*Figure 2: Flow at WWTP. Top: the flow during bubble curtain experiments. Bottom: flow for the dates for which microplastic data was available.*

## 3.5 Sampling

### 3.5.1 Sampling locations

Samples were taken at five locations in the effluent canal between May and November 2019. Figure 3 shows schematically four of these sampling points. To test the efficacy of the bubble curtain, samples were taken at two positions (front and behind) close to the bubble curtain. We expected that in case the bubble curtain successfully retains microplastic particles, the concentration of microplastic particles should differ at different stages of the bubble curtain process and we have used two hypotheses to test this in more detail on particle numbers and size dependency (see results for more detail).

The sample point 'effluent' at the effluent discharge point is not shown on Figure 3. The four locations shown on Figure 3 are in proximity of the bubble curtain and named: 'upstream, front, behind, downstream'. Location upstream was chosen to be 30 meters upstream from the bubble curtain and location downstream 30 meters downstream from bubble curtain. At each sampling location for each sampling event, two consecutive samples were taken. One sample was processed for LDIR analysis and one for microscopic analysis (described below in more detail). However, as a maximum of eight samples could be logistically processed per sampling event, the number of available sample locations per sampling event was limited to four. Consequently, the location "effluent" was sampled five times in the first phase of the research and was later replaced with sampling point "downstream" at expense of the effluent sampling point.

Sampling depth was fixed at 15 cm below water surface. To sample from the bridge above the bubble barrier a device was designed (Figure 4) to ease the sampling and to ensure an equal sampling depth or both sampling devices. For the other two locations the sampling tube was fixated at the bank of the effluent canal.

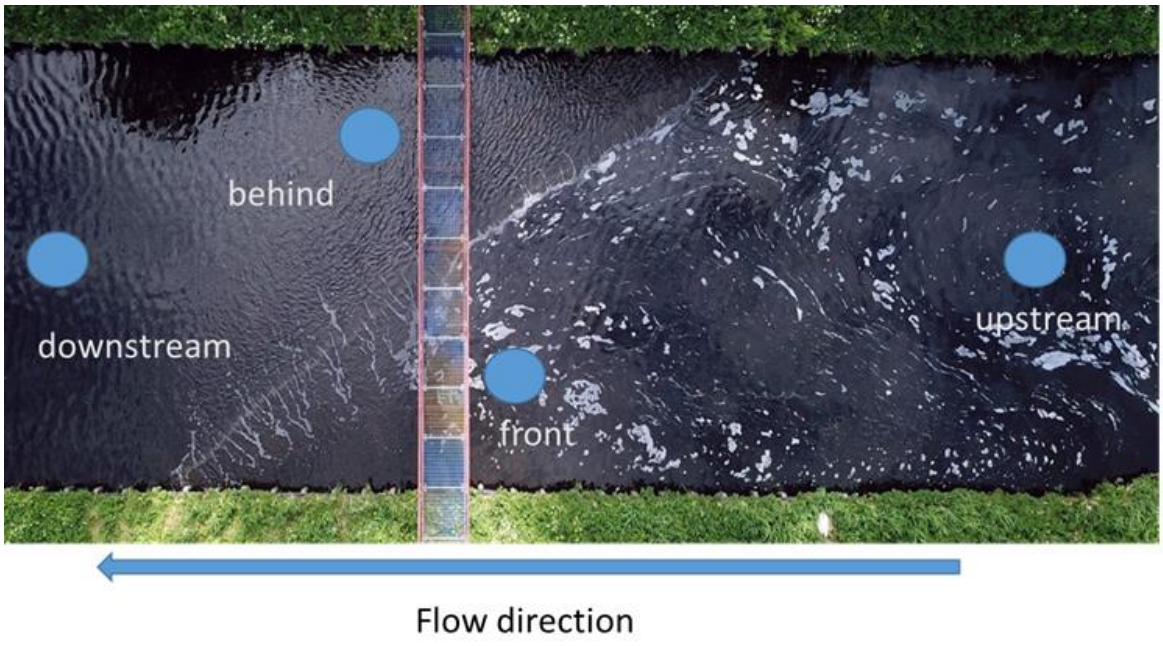



*Figure 3: Aerial picture of the working bubble barrier and the four sampling locations close to the barrier.*

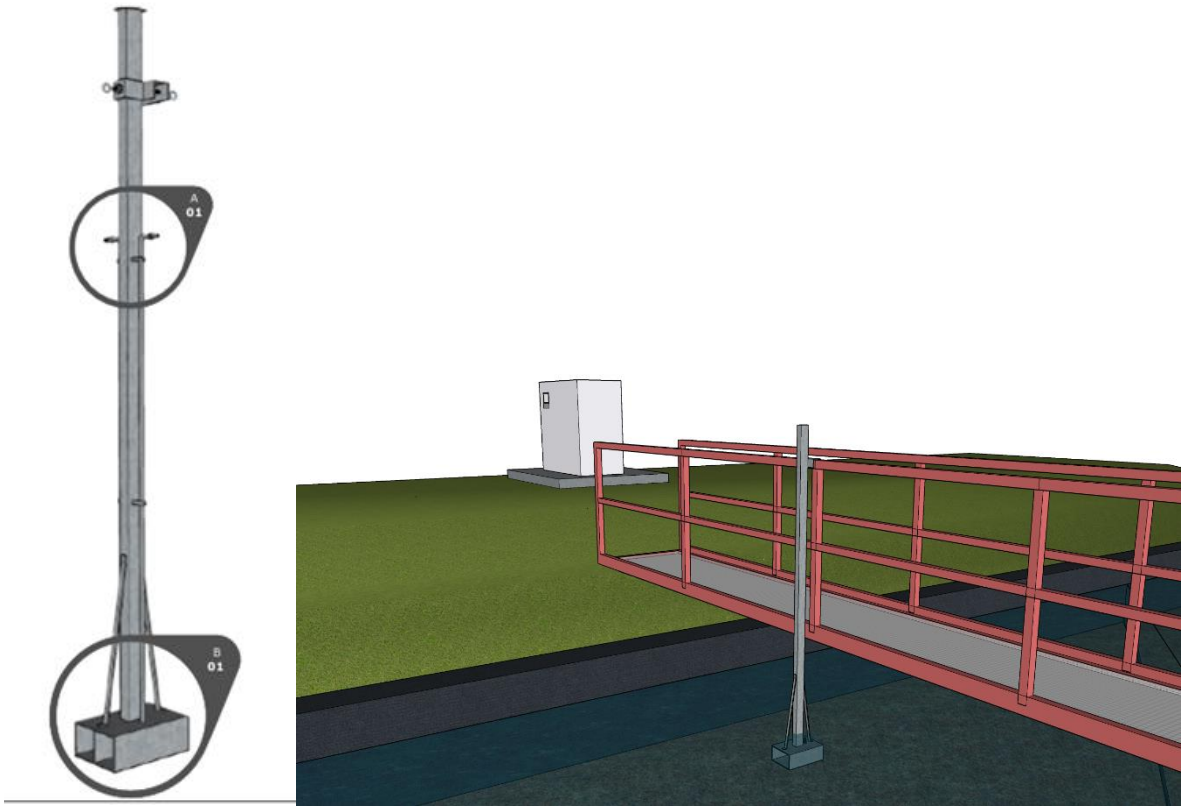



*Figure 4: Sample device attached to the bride above the bubble curtain.*

## 3.6 Analytical methods: LDIR and Microscopic method

Detection principles differ for both methods. The microscopic method detects and characterises plastic particles visually. The LDIR method detects and characterises particles based on their infrared light (IR) spectrum. The two methods applied in this research differ significantly in the identification and determination of size and shape of the plastic particles. (See Table 2). Each method asks different sampling and sample treatment, quality assurance issues and data analysis, described in the next paragraphs.

*Table 2: Comparison LDIR and Microscopic method.*

| | LDIR method (KWR lab) | Microscopic method (HWL lab) |
|---|---|---|
| Sampling volume effluent | Approx. 500 L | Approx. 500 L |
| Sampling device filters | 10; 100; 500 µm | 50; 125; 250; 1000; 5000 µm |
| Size range covered | 10 – 500 µm possible with the setup<br>20 – 500 µm measured and reported for the total PN number. Size fraction 10 – 20 µm was also measured for comparison reasons but these particles were not included in the total PN | Larger than 50 µm |
| Size classification | Continuous, size is determined for each particle individually by the software | Binned, particle is categorised based on the filter it was found on. |
| Particle numbers | Determined in a subsample by the software and corrected for blank | Counted manually under a microscope |
| Particle colour | Cannot be determined | Determined visually |
| Particle shape | Determined based on physical parameters such as circularity, diameter etc. | Determined visually |
| Type of plastic | Determined by the software based on infrared spectra | Cannot be determined. Expert judgement used to assess if a particle is plastic or not |

## 3.7 Sample treatment and detection using LDIR

### 3.7.1 Development of sampling for LDIR

The first samples for the LDIR methods in May and June were taken using 1000 L of water. These were treated as mentioned in the material and method section except for the SDS-step. The result (Figure 5) was clogging of the filters during sample treatment that prevented further work-up and analysis. In the end a grey, greasy and sticky white substance was covering the particles (Figure 5). It was hypothesized that the mass likely was comprised of fatty acids that were not removed during the work-up. To prevent the mass from appearing, subsequent samples were taken under the following altered conditions: a) the SDS treatment step was introduced b) the total sampling volume was reduced to about 500 L. After these adjustments the white substance disappeared. All LDIR data shown later is only from samples with the new, adjusted method.

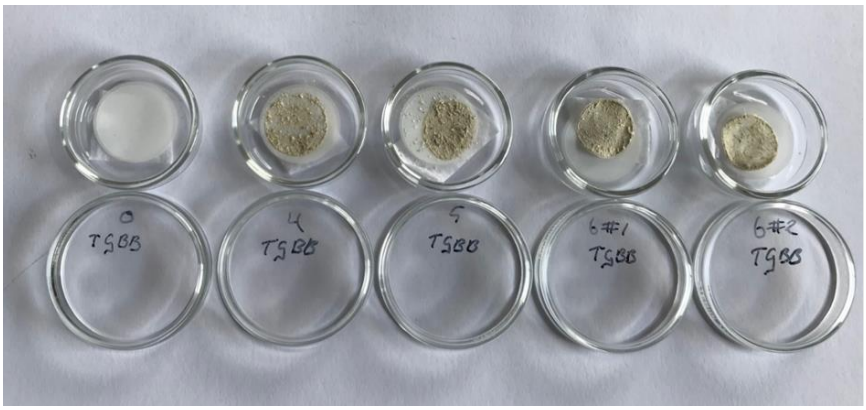



*Figure 5: Filter cake after the chemical work-up of the sample without SDS.*

### 3.7.2 Final sampling description for LDIR

At each sampling event about 500 L of surface water or effluent was filtered through a cascade of two metal sieves (Gilson, USA) of 500 µm and 100 µm, with a 10 µm plankton net (Hydro-Bios, Germany) at the end (see Figure 6). The 500 µm sieve was used to remove particles of that size and to prevent clogging of the smaller sieves. Residue from that filter was not analysed. The other residues were later transferred into separate glass bottles using Milli-Q ultrapure water (Millipore Sigma, USA) and stored at 4˚C.

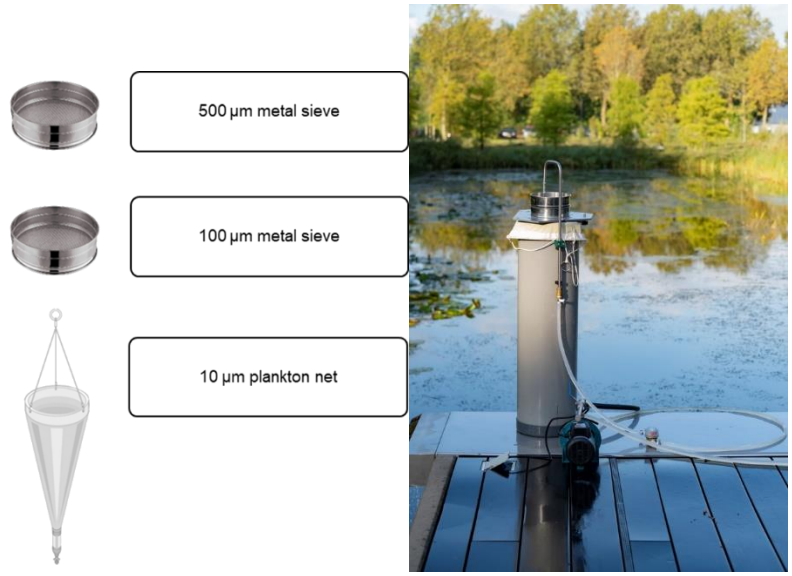



*Figure 6 Sampling device for the LDIR method.*

### 3.7.3 Microplastic measurements using LDIR

Particle analyses were based on previously described methods.[11, 18, 26-28] and depicted in Figure 7. Particle analysis focused on 10 µm and 100 µm residues of the sieves. The suspensions from these two fractions were combined and filtrated over a 10 µm metal mesh. The filter was then transferred into a beaker with a 10% sodium dodecylsulfonate solution. After a day, the suspension was filtrated over a 10 µm metal mesh. The filter was then transferred into a beaker with 75 ml 12.5% potassium hydroxide solution and left standing for 5 days at 35°C. Subsequently, the suspension was filtrated again through the same 10 µm metal filter. The residues were then transferred into a beaker with 50 ml 30% hydrogen peroxide solution and left standing for one day at 35°C. The sample was filtrated again through the same metal filter, and the residues were transferred into a separation funnel using a 100 ml zinc chloride solution (1.6 g/cm$^3$). The funnels were shaken and left standing to enable settling of denser materials. The settled material was discarded by continuously turning the valve of the funnel to prevent clogging, re-suspension and loss of plastics. About 10 mL liquid was allowed to remain in the funnel. These 10 mL were filtrated again over a metal filter. Using 4 mL ethanol, the retained materials were removed from the filter and transferred into a glass vial. A vortex was created in this suspension to distribute the particles evenly. A subsample (2 x 50 µm) was taken and transferred on the microscope slide for analysis. From this subsample the actual particle number is extrapolated. The subsample is necessary as otherwise to many particles will be transferred onto the slide. The sample was analysed using an Agilent chemical imaging laser direct infrared (LDIR). Particles ranging from 20 - 500 µm were measured and quantified. If not stated otherwise particle numbers always mean particles in the size range from 20 – 500 µm per m$^3$ sample. Four samples were analysed in parallel in combination with a negative control sample to detect (cross)-contamination during work-up and a sample spiked with a known amount of polyethylene microbeads (see quality control) to calculate the recovery rate. No negative control check was executed for the steps of sampling at the Waste Water Treatment Plant in Wervershoof. After each set the whole equipment was cleaned using MilliQ water and Ethanol. The equipment was then covered with aluminium foil to prevent contamination from the air.

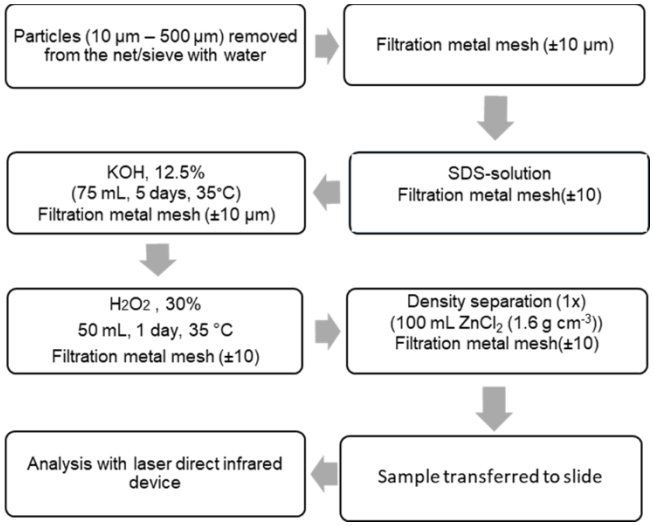



*Figure 7 Workflow of the sample treatment*

### 3.7.4 Quality assurance aspects

Potentially contamination occurring during sample handling was minimized. All laboratory surfaces were cleaned with ethanol, equipment was rinsed and covered immediately with aluminium foil, and a cotton lab coat was worn at all times. Next to this, solutions of chemicals were filtered prior to use. Used materials were not made from plastic wherever possible (*e.g.*, a metal filter setup with a Teflon tube, the separation funnels made of glass). Negative controls were treated in parallel to each batch of actual samples to determine the degree of contamination. For the positive control a known number of plastic particles (green fluorescent PE, average diameter 100 µm) were added to a water sample. The percentage of particle number before and after work-up is the recovery rate. These control particles were counted visually under a microscope.

### 3.7.5 Chemicals used for LDIR

The following chemicals were purchased: Sodium dodecylsulfonate from Merck (Darmstadt, Germany), KOH from Merck (Darmstadt, Germany), H202 (30%) from Boom, ZnCl2 from Boom (Meppel, The Netherlands), Ethanol from Boom (Meppel, The Netherlands), fluorescent green polyethylene microspheres from Cospheric (Santa Barbara, USA), MilliQ water with >18 MΩ from Veolia (The Netherlands). All liquids used, including the cleaning liquids, were filtered prior to usage over 5 µm sieves and stored in closed bottles. These liquids were exclusively used for microplastic research. All equipment was at all times covered when not used to prevent air contamination, except when the samples were taken on location.

### 3.7.6 Data analysis for LDIR

Particle number: The particle number per sample is corrected for the number of particles in the negative control. These numbers are subtracted from the sample. Furthermore, the recovery rate from the positive control is applied to the particle number. E.g., a recovery rate of 90% means that the particle number of the sample is divided by 0.9.

**Particle size/weight:** Particle size was determined by the Agilent software based on the infrared image of the particle. The width and height of the particle were measured. The smallest dimension defines the category the particle belongs to. The third dimension was estimated based on a method used by Kooi et al. to enable to calculate a weight range from the volume of the particle [5, 29]. The lower limit of the plastic mass and the upper limit of plastic mass of all measurements was taken and used for the calculation of the minimum and maximum daily discharge of the WWTP. Due to the large variance between the lowest and the highest possible mass, a pooled value for all the samples was calculated.

**Chemical characterisation:** The particles were identified by the Agilent software (Clarity). As quality cut-off a value based on expert-judgment of 0.6 for the certainty was chosen. Particles with a value lower than those were entirely dismissed.

**Particle shape and colour determination:** Particle colour cannot be determined by this method. The particle shape of the identified particles was determined using the Random Forest Model package (Breiman and Cutler's Random Forests for Classification and Regression – Version 4.6-14) in R. The particles were spilt into six categories (sphere, particle, rod, fibre, fibre/cluster and artefact). Example pictures of each class can be found in the (Table 6). About 500 different particles were categorised and this data set was used to identify the shapes of the other particles. Variables defining the category of a particles were diameter, aspect ratio, area, perimeter, eccentricity, circularity, solidity and the maximum IR adsorption. Using the out of bag method (OBB) [30], the best amount of decision trees is received ($n_{tree}$= 100) and number of branches ($m_{try}$=7).

**Statistical analysis:** Column statistics were performed using GraphPad Prism 5.01. ANOVA (Analysis of variation), Shapiro-Wilt test and t-test were performed in R (v. 1.2.1335) from stats package 3.6.1. For p-values a threshold of 0.05 was used.

## 3.8 Sample treatment and detection using microscopy

### 3.8.1 Development of sampling for microscopy

The sampling volume was reduced after the initial experiments. Initial experiments used sample volumes of 1 $m^3$ for sieves > 125 µm and 0.5 $m^3$ for sieves < 125 µm. Subsequent samples were taken using a reduced sample volume of 500L for sieves > 125 µm and 100L for sieves < 125 µm to prevent sieve overloading.

Like in the LDIR method, the CN filters eventually contained a grey, sticky substance (Figure 8). Due to visual counting, particles could be counted albeit with extra effort. Although the substance was undesirable, it did not prevent further work-up or analysis. It was independently hypothesized that the substance may be saponified fatty acids as a result of treatment with peroxide, causing the formation of soap-like compounds.

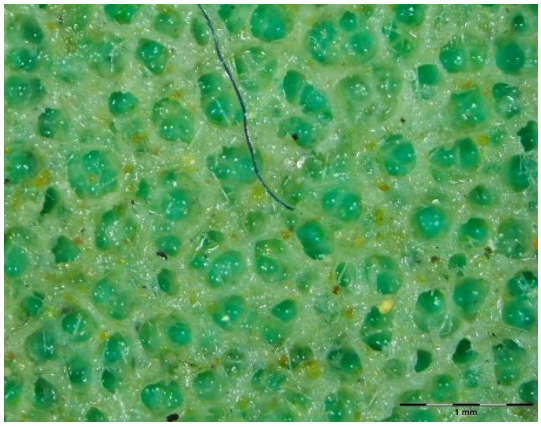



*Figure 8: Example of the white substance on the CN filters.*

### 3.8.2 Final sampling description for microscopy

Microplastics were sampled from 350 L to 600 L of WWTP water by filtering over a stainless-steel sieve cascade containing mesh sizes of 50 µm, 125 µm, 250 µm, 1000 µm, and 5000 µm (Retsch, Germany) at a constant flux between 7-10 L/min for 1 hour. The smallest sieve (50 µm) was removed after 100 L (approx. 15 minutes) to prevent clogging. After sampling the required volume or before clogging of the 125 µm sieve, the sampling was stopped. Each time, exact volumes were recorded per sieve and used in subsequent concentration calculations. All sieves were packed in aluminium foil and transported. Microplastics were recovered from the sieves by reverse flow rinsing and collecting rinse effluent into glass bottles. Microplastic samples were kept refrigerated at 4°C in bottles as a suspension until sample pre-treatment.

### 3.8.3 Microplastic measurements using microscopy

Analysis was performed with an Olympus stereomicroscope SZX10, magnification 6.3-63x, assisted by a light source (Photonische Optische Geräter, LED LichtquelleF3000). The CN filter was visually scanned for the presence of microplastics. If an uncertainty remained whether a particle was a plastic particle, metal tweezers were used to determine the fluidity and tension of the particle. Particles were classified as plastics if they were solid, not elastic, and able to resist tension force (in house protocol). Each confirmed microplastic particle was counted and categorized based on morphology; no size was recorded. At low numbers of microplastics, the entire filter was counted. If high levels of plastics were present, at least 10% of the filter was counted and the results extrapolated.

### 3.8.4 Quality assurance aspects for microscopy

Clean-up of samples was kept as simple as possible as visual counting is not strongly influenced by background contamination. Each glass sample bottle containing different size fractions were concentrated separately by using a 30 µm stainless steel mesh filter. The residue on the 30 µm mesh was back flushed with a minimal amount of pre-filtered MilliQ water into a separate glass beaker. Hence, for each sample, five beakers corresponding to five sieve fractions were prepared. To each sample 10 ml of 30% $H_2O_2$ was added and heated to 75 $^{o}$C under constant stirring. The solution was left to settle for at least 24 hours. After digestion, samples were again filtered over 30 µm

stainless steel filters and the residue was transferred by pre-filtered MilliQ water and separately vacuum filtered over a 0.45 µm bacterial cellulose nitrate (CN) filter (Sartorius Cellulose Nitrate filters, sterile, pore size 0.45 µm, green). CN filters were kept wet and sealed from air under refrigerated conditions until analysis.

### 3.8.5 Chemicals used for microscopy

The following chemicals were purchased: stabilized hydrogen peroxide (Merck, 30%). Demineralized water was produced in-house. Liquid chemicals were filtered over 30 µm mesh filters before usage.

### 3.8.6 Data analysis for microscopy

Size classification: Particles were assigned a size fraction based on the filter these were found on. The sieve on which the particle was found defines the size category. Particle size was not measured for each particle individually.

Particle number: The amount of microplastics per sieve size was counted. If the collection filter was counted completely, the total amount of plastics was used as-is. If the collection filter was partially counted, results were extrapolated to account for the entire collection. To express it as volume-based unit, the total amount of plastics was corrected for the sampling volume.

Particle colour and shape: Microplastics were binned based on morphological qualities (colour, shape, and size). Each individual microplastic particle was assigned a shape category and colour category. The following shape categories were defined: primary, secondary, rod, miscellaneous and fibre. The following colour categories were defined: white, grey-white, black, blue, green, yellow, red, and miscellaneous.

Dataset: Due to the binning methods applied for size, shape, and colour, each analysis resulted in a combination of 55 parameters consisting of bins size, shape, and colour combinations. The total number of parameters is smaller than possible combinations, as not all combinations are compatible or found (e.g., red primary particles). Per parameter the total number of microplastics corresponding to that classification was reported. The data was visualised using 2D and 3D plots.

# 4 Results and Discussion

## 4.1 Introduction

In this chapter, results of the project are described and discussed in light of other findings in literature. We describe the calculation on the outflow of microplastic in the effluent canal, the effectiveness of the bubble curtain in this research, analytical method developments and describe the environmental impact. In the following chapter, the main conclusions are summarized.

## 4.2 Effectiveness of the bubble curtain

The concentration of microplastic particles did not differ at the different stages of the bubble curtain process (Figure *9*). Figure 6 shows the particle concentration and average particle concentration as measured with the LDIR, and using microscopy. The data does not show differences.

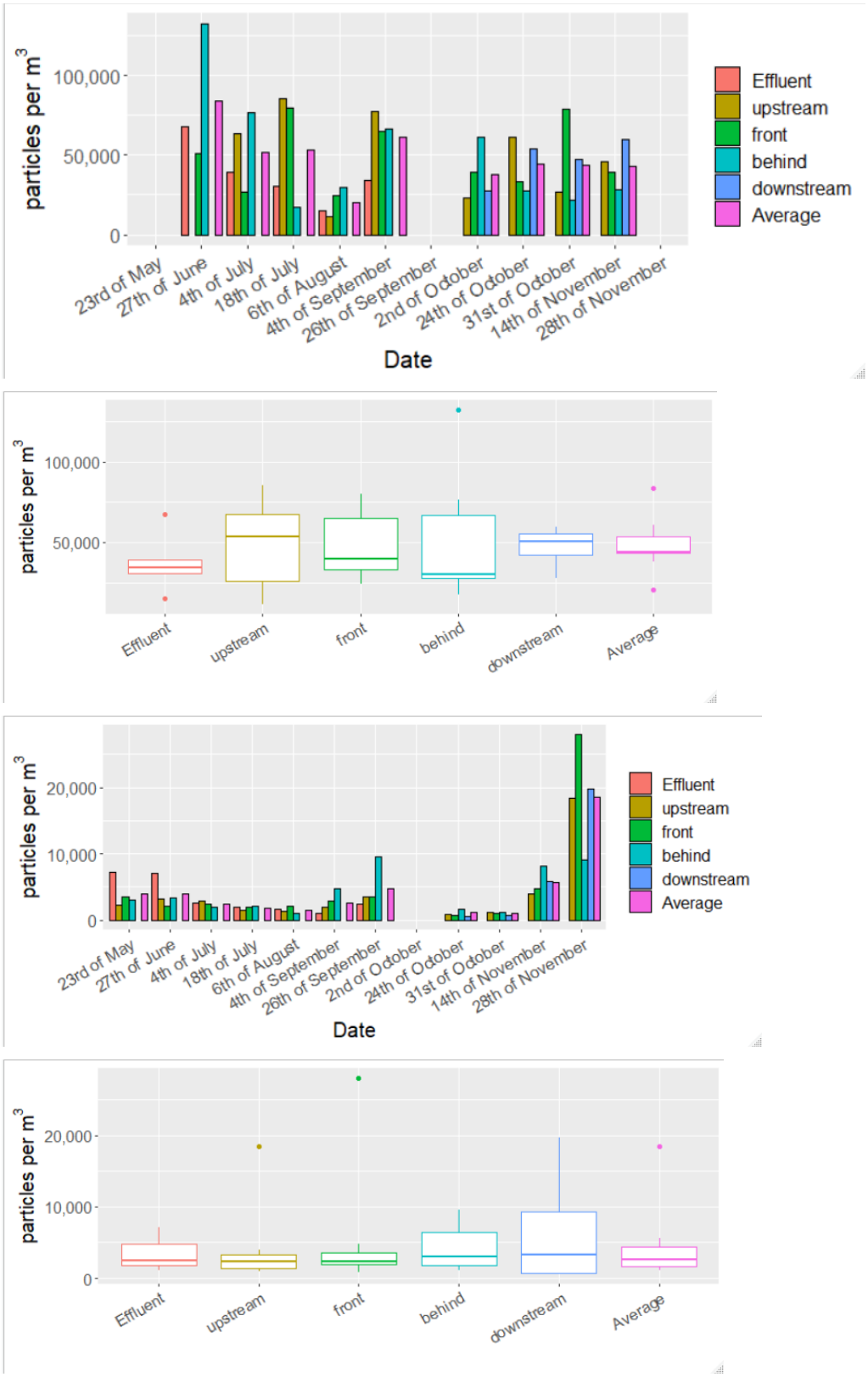



Figure 9: From top to bottom. Particle concentration measured with the LDIR, Average Particle concentration measured with the LDIR, Particle concentration measured using microscopy. Average particle concentrations measured using microscopy

To test the effectiveness issue in more detail, we tested two hypotheses on the ratio of particle numbers and size dependency. The first was that in case the bubble curtain is retaining particles, the ratio between the lean zone and rich zone should be greater than one if more particles accumulate in the rich region, in front of the bubble curtain/barrier. Using data on particle numbers, ratios between the two sampling points were calculated for each individual day. These ratios were then pooled and compared. If the barrier retains particles of all sizes equally, the ratio should be larger than one because particles will accumulate in front of the bubble curtain. The ratios and the results of a t-test for the microscopy and LDIR data are shown in Figure 10 and Table 11 in the SI. The results combined with a one-way t-test show that the ratio is not significantly larger than 1 (p-value = 0.14 (LDIR) and 0.38 (microscopy)). Our observation is that the overall particle number at different sampling points is not affected by the barrier. This still leaves the possibility that certain size fractions or particle types are affected by the barrier despite the fact that the total numbers do not show a significant difference.

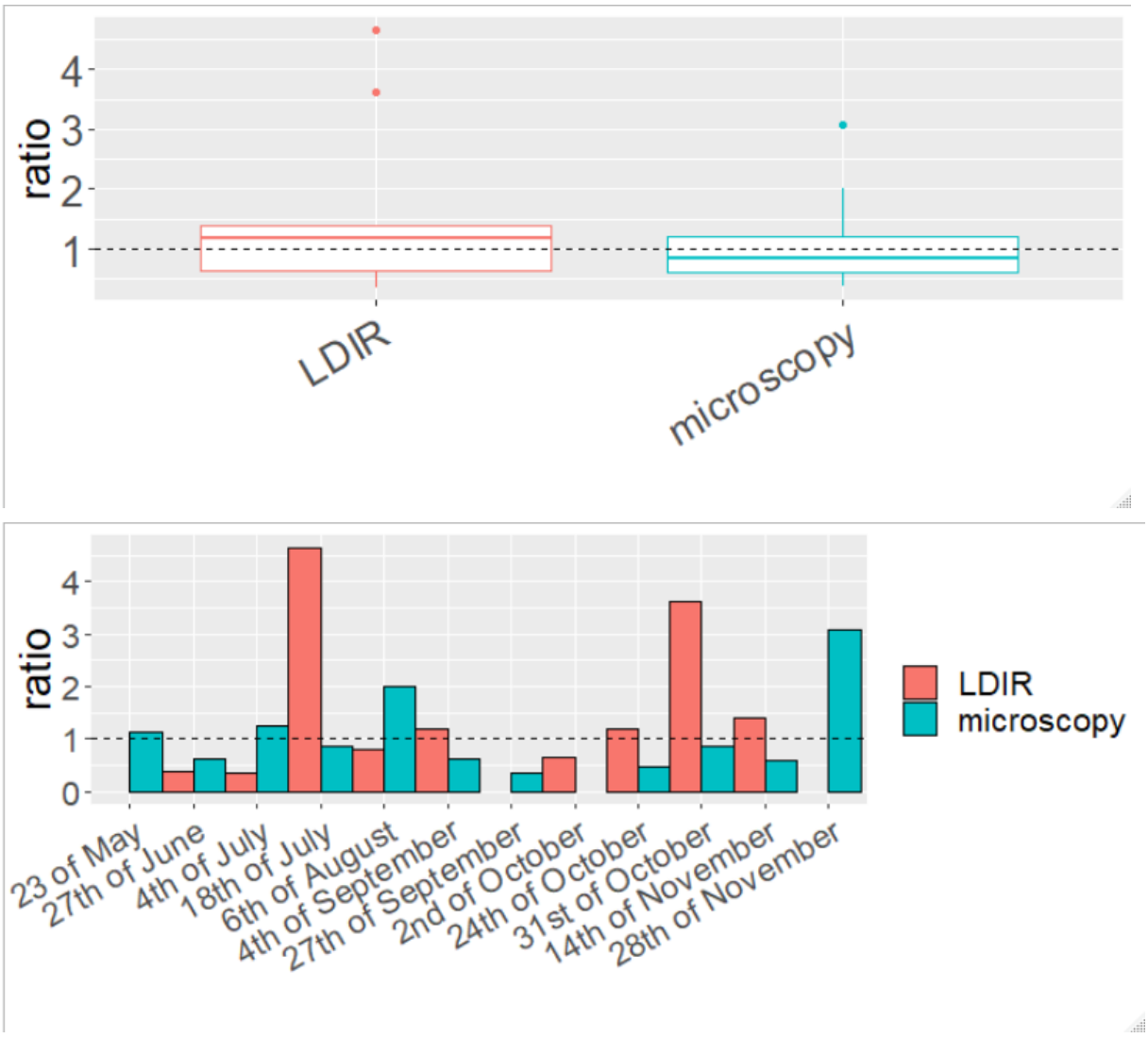



*Figure 10:* Ratio's between the locations front and behind for the LDIR (KWR) and microscopy data (HWL). P-value's for t-test that the ratio is significantly greater than 1 can be found in *Table 11* SI.

The second hypothesis is whether a size dependency on particle concentration effects in the rich zone exists. Larger particles are more easily focused in the rich zone than smaller particles. For this a size dependent effect in a log-log relationship between particle sizes was studied by calculating the slope of each individual linear regression. A change in the slopes would indicate a change in the particle size distribution. The slopes of the sampling point front and behind were compared. Additionally, the pooled slopes of the two sampling points were calculated and compared. Linear regressions for the size range 20 – 200 µm for different days and the two sampling points front and behind are shown for LDIR (Figure 11 and SI Table 12) and microscopy (Figure 12 and SI Table 13). Comparison of the same-day slopes of front and behind shows that there is no significant difference between the size distributions: the particle size distribution in front and behind the barrier appear indistinguishable. The pooled slopes for the two sampling points also support this as there is no significant difference between before and after the barrier (LDIR: –2.04±0.31 (front) and -1.67±0.25 (behind), microscopy: -0.61±0.27 (front) and 0.64±0.19 (behind)).

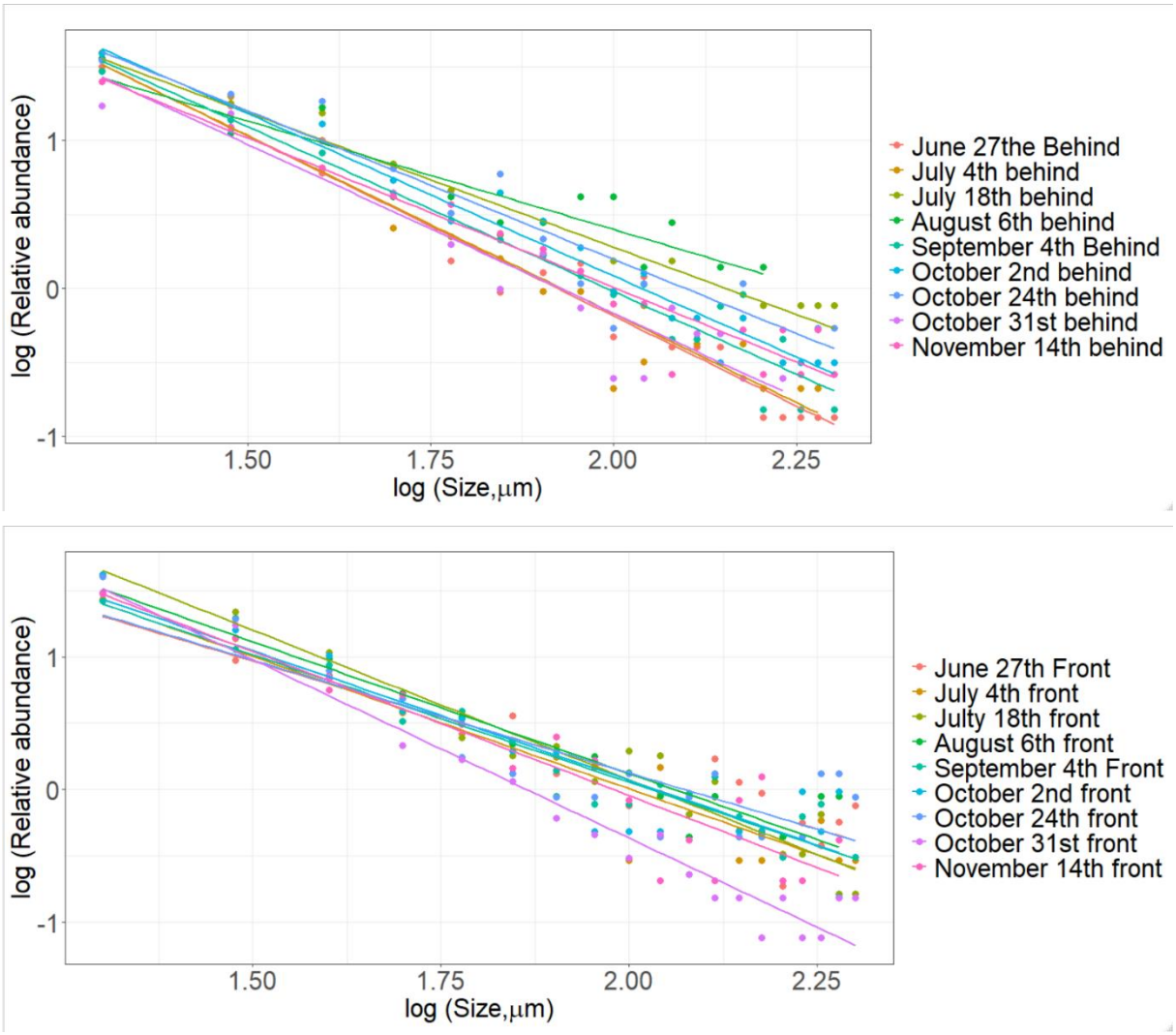



*Figure 11: LDIR logarithmic linear plot for the locations front (top), behind (bottom) (size fractions 20 – 200 µm). The p-values of an Anova test and slopes of each linear regression can be found here (Table 12, SI)*

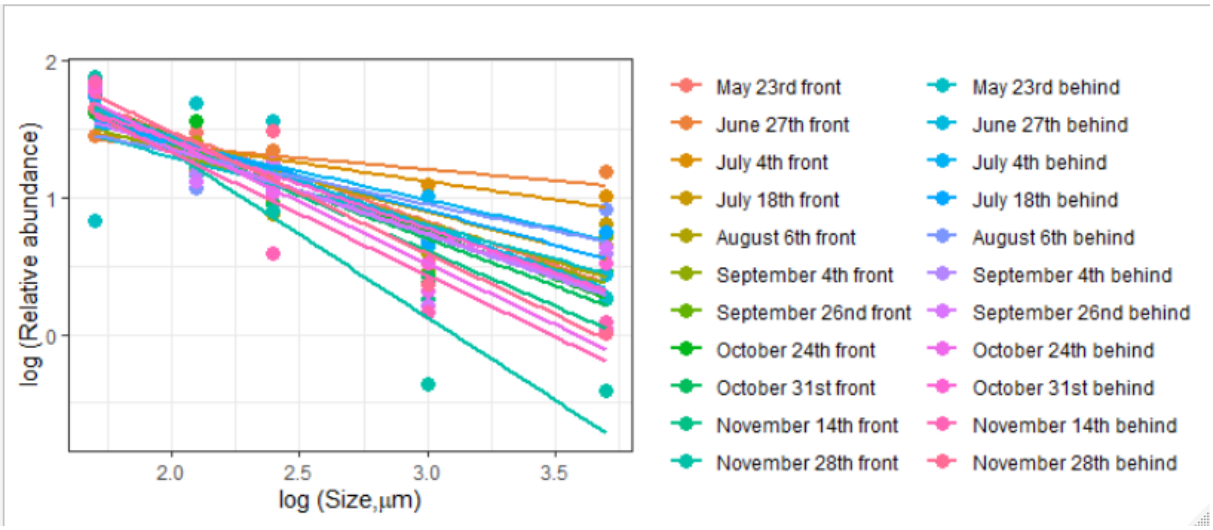



*Figure 12: microscopy logarithmic linear plot for the locations front (top), behind (bottom). The p-values of an Anova test and slopes of each linear regression can be found here (Table 13, SI)*

As the smaller particles are more numerous these will weigh heavy on the slope of the fitted trend line and therefore minor changes in larger particle numbers might go undetected. To ensure no information is missed for larger particles, the particle numbers of the larger particles (>1000 µm) were studied.

Earlier research with the bubble barrier showed that it can effectively remove larger microparticles (> 1 mm) from the water stream on the surface [2, 3]. Figure 13 shows that there is no significant difference between the particle concentration in front of the barrier and behind for the size fraction > 1000 µm up to 5000 µm in this research at 15cm depth below the surface.


*Figure 13: Particle number of particles larger than 1000 µm up to 5000 µm in front and behind the barrier.*

A note of discussion for this setup and future experiments is the sampling depth and technique. In this study, the decision to take the samples at a depth of 15 cm was to avoid contamination from the air. However, the distribution of plastics across a water column is unknown. It can be assumed that plastic is not homogeneously spread over the water column. Moreover, the upstream flux generated by the bubble curtain can influence plastic particles and move these particles to the surface, as was observed for particles larger than 5 mm.

Unfortunately, sampling microplastics over different depths was not feasible within this investigation. It is highly recommended to further investigate the presence and number of plastic particles in the several layers of the water column.

Using all results gathered, including those later discussed in Method Comparison, we see no definitive results that prove that the bubble curtain affects smaller microplastics. This means that based on these data, there is neither an effect on the removal of plastic particles over the whole tested size range, nor an effect for specific size classes.

There are a number of reasons that may explain why the effectiveness of the bubble curtain could not be measured. The first, as previously mentioned, is that distribution of microplastics in the water column could play an important role in which microplastics are sampled during sampling events. In addition, and possibly related, it may be possible that too few particles in the size range of >1mm were available to recognize a measurable difference by the bubble curtain. As WWTPs have been shown to remove the majority of microplastics [31], it is expected that WWTPs will remove a similar fraction of microplastics for which the bubble curtain would be especially effective. As microplastic analysis methods require sufficient particles to be meaningful, it may be that removal by the WWTP reduced the microplastic concentrations to levels at which the bubble curtain could not provide a measurable difference. In this case, the measurable removal efficiency of microplastics would not exceed systematic deviations and scattering in the data due to a too low particle count.

Another explanation is that the bubble curtain cannot remove particles smaller than 5 mm, instead working only for larger plastic particles. However, this explanation is not supported by earlier pilot tests of this particular bubble curtain (unpublished data) where particles between 5 mm and 1 mm were affected.

Finally, external effects such as current, wind direction, convection, or the flow of the water in the effluent canal may have had a significant impact on the results. Testing the bubble curtain under more controlled conditions in a laboratory setting could provide a more complete answer on whether a bubble curtain is effective for microplastics.

## 4.3 Outflow of WWTP

The outflow of plastic was described by using results from the two methods on both particle numbers. The average outflow was observed to be between ca. 2,500 (> 50 µm) and 30,000-55,000 particles per m3 (> 20 µm) (see Figure *9*), depending on the method and size range in the effluent and canal. The presence of plastic particles justified to install the bubble curtain as a means to remove plastic particles from the canal.

### 4.3.1 Plastic release from the WWTP (all sampling points)

As the bubble barrier had no measurable effect on the plastic concentration in the canal, all sampling points have been used to get a better picture of the total plastic particle number and types of plastic in the effluent and canal. We noted that total particle numbers vary between sampling points and days. Our results show that the WWTP releases consistently particles per day (Figure 9), while these numbers do not differ significantly between sampling points (Figure 9). However, the large variation in day-to-day (Figure 14) data underlines the necessity that a sufficient number of samples needs to be taken to get a meaningful particle number on average for a certain location. To describe the outflow, data from a certain sample location may be used, while data may also be pooled

to describe the total outflow better. In our results, we aimed for a robust number to indicate particle load and potential impact (see further below).

The total discharge of microplastics into the environment can be estimated using all experimental data collected from the effluent canal from this WWTP. To include as many types of variation as possible when calculating discharge from the WWTP, all measurements from the same day were used to determine the effective daily discharge of plastic particles. This assumes that sampling is sufficiently adequate to cover the entire water body; however, as the samples are taken over different areas of the water body, it should provide a more robust representation of microplastic concentration than a single sample point. As no significant differences between the various sampling points were detected, data from all locations were included in this dataset. Furthermore, it was established that the variation between the median of various days - with the sole exception of Nov the 28$^{th,}$ when heavy rainfall resulted in a high particle number - is also sufficiently small to allow pooling of all the sampling points. Based on LDIR data, we calculated that on average about 48,000 particles are in one cubic meter of water, see Table 3. This is in accordance with findings from Simon et al [4] 19,000 to 477,000 p/m$^3$ from particles from 10 µm upwards. Currents results of 48,000 p/m$^3$ as estimated discharge coefficient corresponds to 700 trillion particles per year being discharged into the environment from this particular WWTP (40 million m$^3$ of water per year). With the microscopy method about 4,000 particles (p) per cubic metre are found which means an outflow of 58 trillion particles per year. Few comparable studies for microscopy can be found in literature, but a WWTP in China discharged approximately 600 particles per m$^3$ for particles between 43 to 5,000 µm [32].
Overall, studies show that particle numbers fluctuate by several orders of magnitude in various WWTPs around the world [31]. Particle numbers as low as 0.7 p/m$^3$ (USA) and as high as 54,000 p/m$^3$ (Denmark) can be found, so finding large variations in between experimental results at different WWTP-sites is not uncommon. The numbers are impacted by a large number of factors including the size range that is being investigated, the analytical method and the size of the WWTP. Hence, it is difficult to compare results between studies directly. Also, as shown here, *e.g.* heavy rain fall and environmental conditions can significantly influence the number of recovered particles, which further prohibits adequate comparison between studies.
Applying the discharge from Wervershoof to the 0.5 - 11.9 mg/m$^3$ from Simon et al. would result in an emission load of 7 to 173 kg microplastics per year. This means under the assumption that the daily discharge is the same the amount of microplastic discharged on a yearly basis is comparable. Given that WWTPs are relatively efficient for removing microplastics and remove between 90 – 99 % of the microplastics entering as influent [5, 6, 31], this means that between 120 to 1,200 kg of microplastics at WWTP Wervershoof is removed annually. This estimation is based on the microparticles found in the size range 20 – 500 µm, however, as larger particles that are likely to arrive at the WWTP and contain relatively more mass, the actual mass of removed plastics is likely much higher. Note that large plastic fragments are removed by the sieves on intake and other plastic particles may be retained in the WWTP processes.

*Table 3: Calculated discharge from WWTP Wervershoof*

| | LDIR method | microscopy method |
|---|---|---|
| Average PN / $m^3$ | ~48,000 | ~4,000 |
| Average PN / year | $7*10^{11}$ | $5*10^{10}$ |
| Maximum mass discharge kg / year | ~19 | n.d. |
| Average plastic fibres / $m^3$ | 1,000 – 2,000 | 1,000 – 2,000 |

### 4.3.2 Type of plastics in WWTP discharge

We found several polymers and calculated percentages of polymers identified in the investigated WWTP (see Table 4), and data are compared with recent studies that investigated similar samples. In WWTP Wervershoof the most abundant polymer found was polyamide (PA, 18%). The other polymers that were found predominantly were PET, Isoprene, PU/varnish, PP, and PE-Cl, PE. These polymers are commonly found in effluents or surface waters 26. In total 27 different types of polymers were found. Two percent of the particles found in the samples could not been attributed to any plastic. It can be noted that the barrier itself is made from PVC while a low number of fragments were found, most probably not related to the barrier but cannot be excluded. It should be noted that Table 3 does not cover the particle size range, which may impact the relative distribution. The variation between relative particle number distributions in Table 3 can be explained by the fact that different WWTP received different influent depending on the plastic usage in their vicinity. The presence of PA is especially notable, as this is more than expected from the literature, which estimates 1-3% (see Table 4).

One possible explanation could be that there is still organic material in the sample that did not break down completely during the chemical digestion. As a result, these particles were counted as microplastics composed of natural PA. The effect of particle size on polymer type is not discussed, but it is known that each size fraction can have a different distribution [26]. This level of plastic variety concurs with those recently found in two Dutch rivers [26].

Table 4: Relative abundance of the ten most abundant plastics in WWTP Wervershoof, – this research) compared to three other WWTP from literature [4, 6, 32] as listed in Chapter 6 of this report.

| Polymer type | Percentage (Wervershoof) | Percentage (Changzhou) | Percentage (Xiamen) | Percentage (Denmark) |
|---|---|---|---|---|
| **(Natural) Polyamide (PA)** | 18 | 2-3 | 1 | 3 |
| **Polyethylene Terephthalate (PET)** | 16 | 20-35 | 7.5 | 25 |
| **Isoprene/rubber (rubber)** | 13 | 1 | - | - |
| **Polypropylene (PP)** | 9 | 10-20 | 35 | 12 |
| **Polyurethane/varnish (PUR)** | 9 | 1-2 | - | 0.5 |
| **Polyethylene (PE)** | 7 | 5 | 18 | 27 |

| | | | | |
|---|---|---|---|---|
| Polyethylene Chlorine (PE C) | 7 | - | - | - |
| Polyacetal (PA) | 5 | - | - | - |
| Polyvinylchloride (PVC) | 5 | 2-3 | - | 0 |
| Polystyrene (PS) | 2 | 2-10 | 10 | 1 |
| Unknown/other | 2 | - | - | 2 |

Next to the particle numbers also the fibre number can be determined. For the specific plastic fibre discharge, both methods detected levels between 1,000 and 2,000 fibres /$m^3$ effluent. This is much lower than recently reported fibre numbers in WWTP effluents (22,000 ±18,000 p/$m^3$) [33]. Nevertheless, our data suggests that on a daily basis, about 80 million fibres are released [34]. This number is in range of reported fibre numbers in literature (21,000 to 153.4 billion); however, the range of reported values is sizeable. The lowest fibre discharge is reported from a WWTP in Sweden, the highest from a WWTP in Russia. For larger size fractions > 50 µm studied by microscopy in this report, the majority of particles are plastic fibres. This is also in accordance with the literature which shows that between 60 and 90% of all the particles found in effluent are fibres [31]. Nevertheless, for a closer comparing of different WTTPs more data is needed on treatment processes such as sludge retention times and operation details.

## 4.4 Method development and comparisons

Current progress on microplastic analysis shows that comparability between methods is often poor, as minor changes in methodology can greatly influence results. This can be seen e.g. when comparing data from various WWTPS [31]. The amount and type of microplastics found depends greatly on both the sampling process (e.g., lower size, sample pre-treatment) as well as the analytical process (e.g., counting, identification). In this study, two complementary methods were used to maximise the span and scope of to-be-analysed particles. In this section, the methods are compared for performance and similarity between analysis results, where differences are discussed.

### 4.4.1 Total particle number

At first the particle numbers are compared. We noted that particle numbers (PN) found with the LDIR are significantly higher in all samples than the particle number from the optical microscope method. This was expected as the LDIR method can detect smaller particles down to 20 µm whereas the microscopic method is limited to 50 µm particles. It is well known smaller particles are more abundant [29]. For the LDIR method on average between 40,000 – 60,000 particles per $m^3$ were found for the various locations and with the microscope between 1,000 – 6,000 particles per $m^3$ were found (Figure *9*).

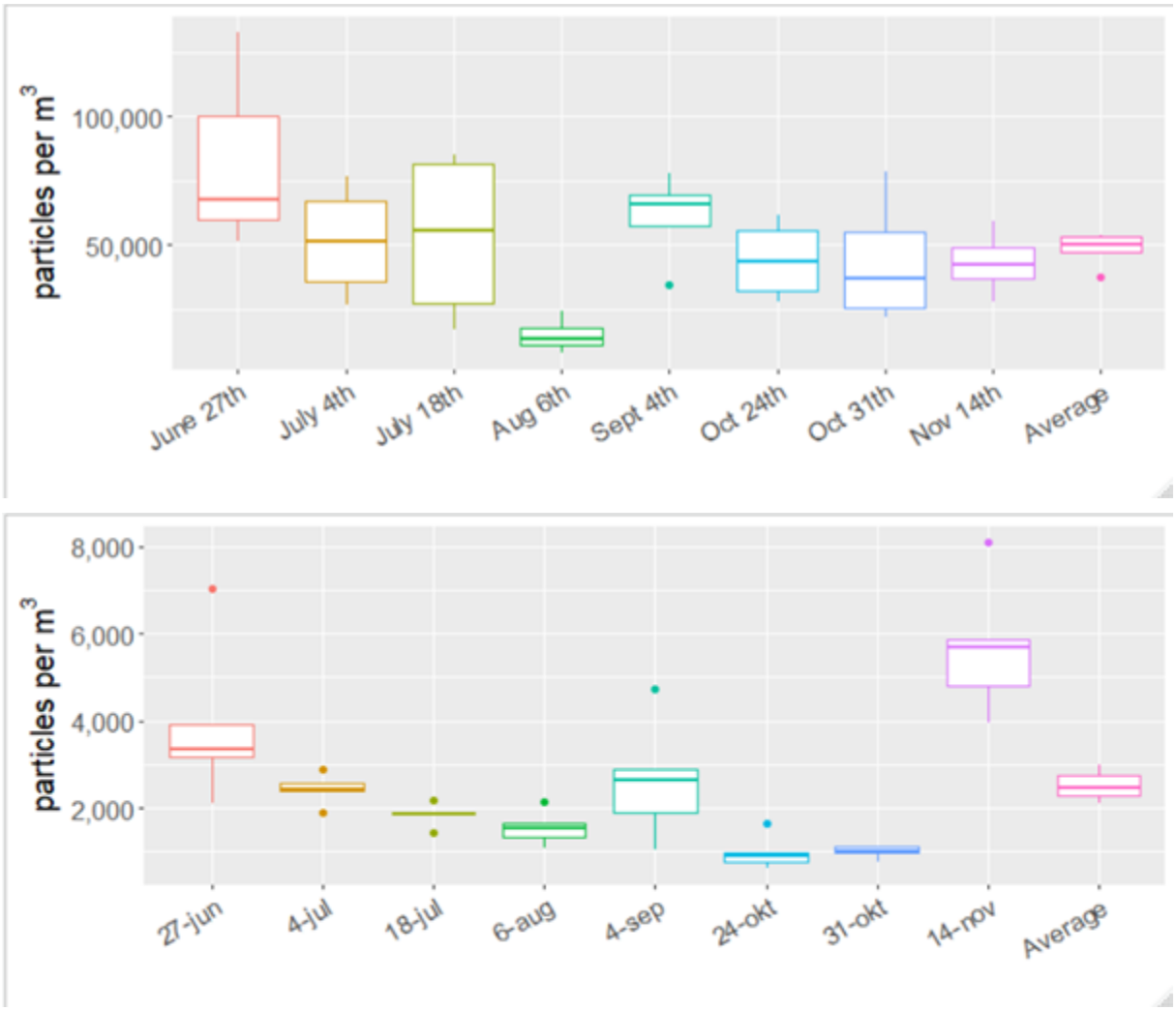
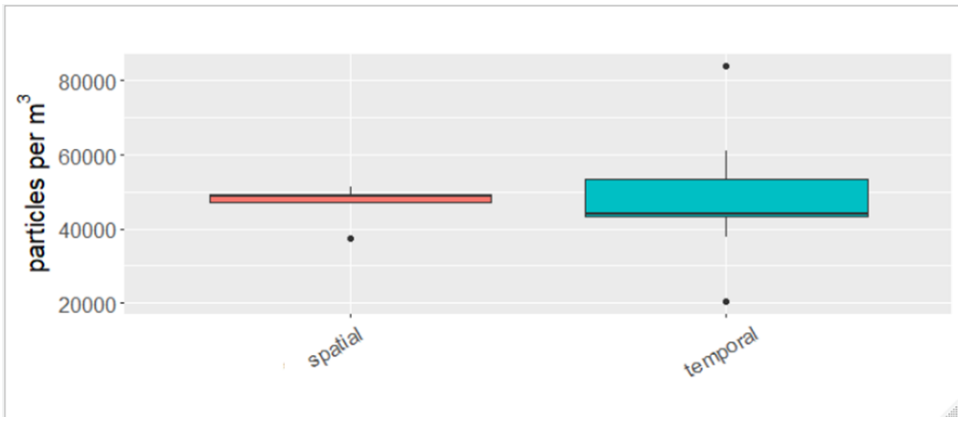



*Figure 14: LDIR (top) and microscopic (bottom) averages per day only for the days of joint sampling.*

Despite the differences in methods, a similar trend between sample events is found by both methods (Figure 14). Figure 15 also shows that scattering of the data is larger between different dates than it is between the different locations. This is true for both methods. Calculating the ratio of the normalised particles numbers between both methods shows that there is no significant diversion from a theoretical mean of 1 (t-test, p = 0.20) (Table 10, SI).



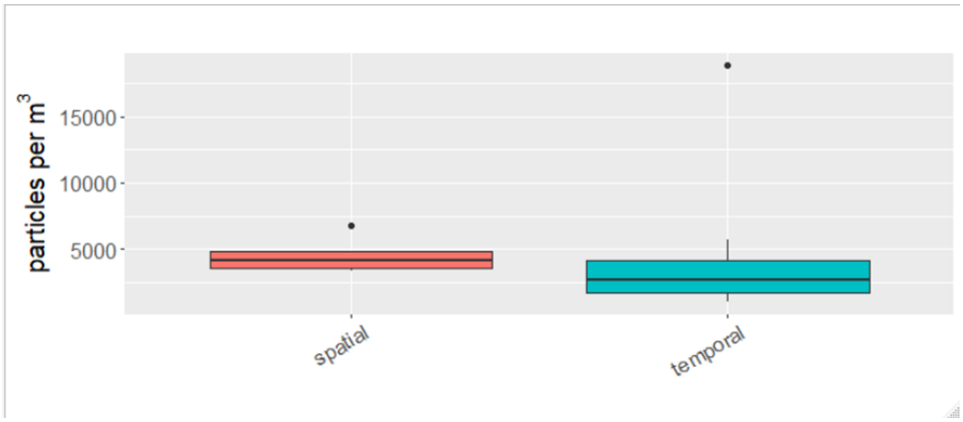



*Figure 15: Variance of particle numbers for LDIR (top) and the microscopy method (bottom). Temporal: Average of each day's average. Spatial: Average of each locations average.*

### 4.4.2 Particle numbers per size fraction

As shown, particle numbers of the two detection methods cannot be compared directly due to the different size limitations. Therefore the particle counts (all measurements) from the two methods were categorised to match the size categories of the optical microscope method (Figure 16). The total particle count of the 50-125 size range is a factor 10 higher for the LDIR method. This difference may be explained by the fact that a 50 µm sieve was the lower limit during sampling for microscopy, whereas in case of LDIR method 10 µm was the lower limit.

It is known that particles with a enlonged shape can pass through sieves if the orientation permits, *e.g.*, a long 1000 µm particle may be able to pass through a 200 µm sieve if the width of the particle is smaller than 200 µm [35]. Therefore, because a 50 µm sieve does not exacly cut off at 50 µm there is a realistic probability that particles of 50 µm may pass through that sieve, causing a lower bound underestimation. For the LDIR method the lower sieve size is smaller (10 µm), so 50 µm particles are likely to be retained. The lower bound underestimation was also observed for LDIR when comparing 10 µm to 20 µm particles. A substantial difference between PN 10 µm and 20 was not observed where it was expected: in five cases the PN for 20 µm particles was larger and in the other cases the numbers were almost equal or slightly more 10 µm particles. Estimated, the 10 µm size fraction should be about four to six times larger than the 20 µm size fraction. Hence, it appears that the closer a particle size is to the smallest filter mesh used, the higher the chance that PNs get underestimated. The means that it is crucial that not only the measured particle sizes are mentioned but also the smallest mesh size used for sampling as well as work-up.

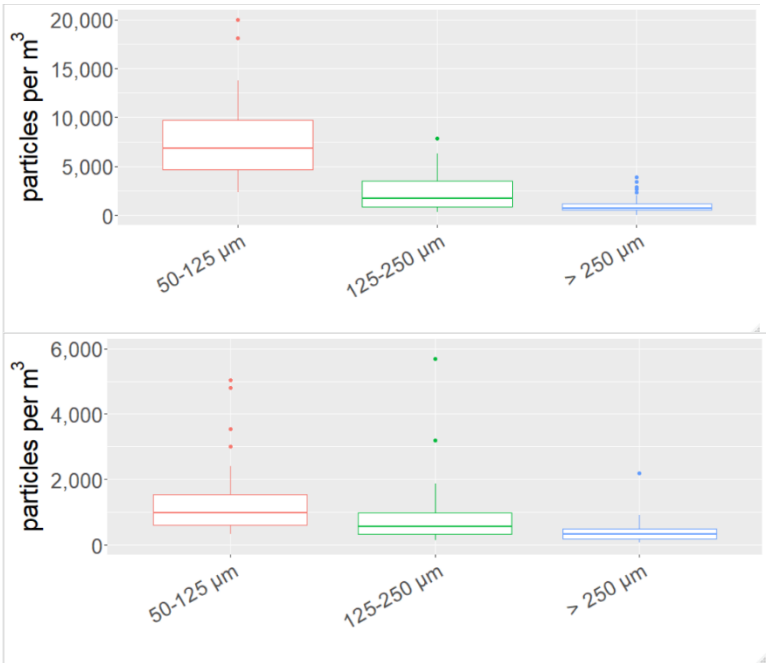



*Figure 16: Average particle number from all dates and locations for the different size classes. Comparison LDIR (top) and microscopy method (bottom).*

In addition, particles can be missed during visual counting, which will likely lead to bias towards large particles and fibre or rod shaped particles that might be easier recognized as plastics [29] . For example, a plastic particle that visually appears as a sand particle will be excluded in the visual microscopic method but included in the LDIR spectroscopic method. Additionally the differences in categorisation of particles with the two methods can also explain the difference. In the LDIR method the smallest dimension defines the size class of the particle, whereas in the optical microscope method the sieve on which the particle was found determines the size class. Hence, especially for fibre-like particles the classification between the two methods differs greatly.
Looking at the other size classes the difference between the two methods is less pronounced. The particle numbers in the size range of 125-250 are slightly higher for the LDIR method, but the difference is not as profound as for the 50 – 125 size range ($p = 0.05$). The, on average, slightly higher counts of particles might be explained by the different definition of size of the two methods. For the larger size range ( > 250) no significant difference was observed ($p=0.23$) and relative differences became smaller. The LDIR size fraction contains slightly more particles on average although the upper limit is 500 µm, whereas the upper limit for the microscopy method is 5000 µm.

All samples from LDIR and microscopy show that with increasing particle size, the particle number decreases. This is in accordance with earlier findings [29]. To assess the goodness of this correlation, log-log plots for all LDIR (Figure 22) and microscopy measurements were made and the quality of the linear regression was evaluated (Table 5).

*Table 5: Calculated slopes and quality parameters for the linear regression of the log-log plots.*

| Size Range | Pooled slope | Pooled $R^2$ | Pooled Sy.x |
|---|---|---|---|
| LDIR | Steps of 10 | | |
| 20-490 | -1.66±0.31 | 0.84 | 0.24 |
| 20-200 | -2.14±0.41 | 0.90 | 0.20 |
| 20-100 | -2.28±0.43 | 0.92 | 0.15 |
| LDIR | 20-50 then steps of 50 | | |
| 20-490 | -2.13±0.41 | 0.95 | 0.18 |
| MICROSCOPY | | | |
| 50-5000 | -0.66±0.23 | 0.84 | 0.25 |
| 50-1000 | -0.91±0.34 | 0.96 | 0.13 |

LDIR: With LDIR, performance characteristics improve when the upper size range is reduced as relatively few larger particles are found. To avoid underestimation by sampling lower bound cut-off, only particles between 20 and 500 µm were included. Detection of larger particles is due to their smaller number much more affected by chance, so removing these from the regression reduces the variation. Choosing a smaller overall size distribution for the linear regression also inevitably comes along with a bias for the smaller particles. However, as 95% of all the particles in this dataset are between 20 –200 µm, fitting between 20 and 200 µm using 10 µm steps while ignoring larger particles is optimal. The value of the slope in this regression is –2.14±0.41, which coincide with a reported average value of –1.6±0.5. Particle numbers sized 200 µm and larger can be calculated based on extrapolating using the formula from the linear regression. These results are decent, as shown when applying this method for data from 4th of July upstream sample point. With the LDIR method actual PN larger than 200 µm is 1,392 particles while calculation yields a PN between 2,404 and 3,180.

MICROSCOPY: Regression of this data cannot be based on particle size, because only binned data is collected per size fraction as recording particle size per particle is too inaccurate and time-consuming. The linear regression reveals that microscopy also has an underestimation of the larger particles. This becomes clearly evident once particles larger than 5,000 are also included: the $R^2$ value drops from 0.96 to 0.84 and the Sy.x rises from 0.13 to 0.25. It should be noted that the generally high $R^2$ value (0.96) for microscopy is based on a linear regression with only four size categories. Therefore, excluding more than one size range (5000 µm) was not viable. The calculated slope for regression based on microscopy data is smaller than the average reported in the literature (–1.6±0.5).

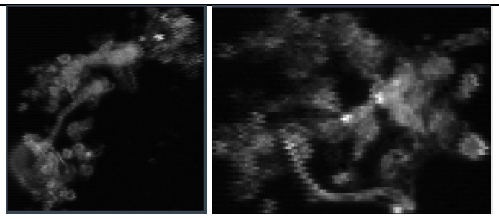
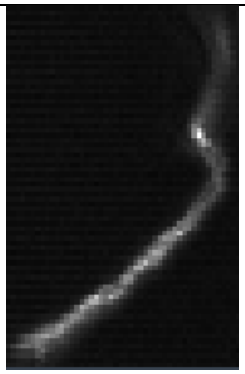
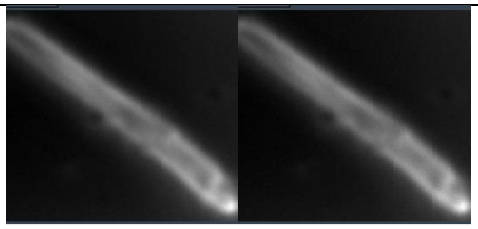
In both data sets there appears to be a negative bias on the lowest size fraction. For LDIR this is based on the 10 µm filtering net, and for microscopy based on a 50 µm sieve. As explained, the closer a particle size is to the smallest

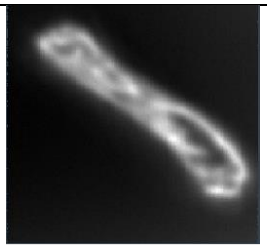
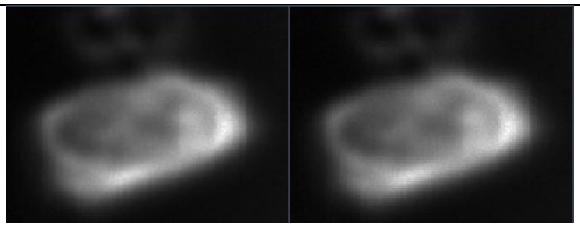
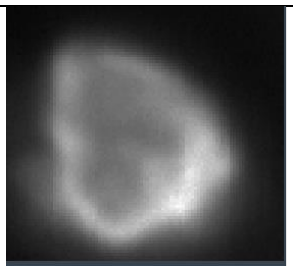
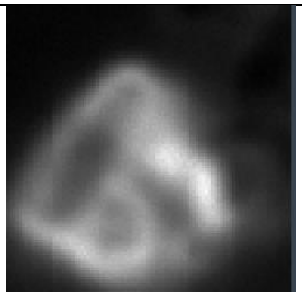
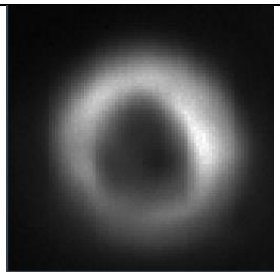
filter mesh used, the higher the chance that PNs pass through the filter and are underestimated in counting. Furthermore, smaller particles are more prone to biofouling and hetero-aggregation which will result in deposition in the environment [36, 37]. Should these small particles have been in the effluent, this could explain their absence in the sampling device.

### 4.4.3 Particle shape and fibre numbers

Particle shape was determined differently for the two methods. Using the LDIR method a particle is labelled based on physical parameters (Table 6), while the microscopy method particles are detected and characterised visually (Figure 17).

*Table 6: Examples of the various types of plastics found the samples from WWTP Wervershoof, as described by LDIR method.*

| Shape | Picture |
|---|---|
| Artefact |  |
| Fibre/cluster |  |
| Fibre |  |
| Fibre |  |

| | |
|---|---|
| Rod |  |
| Rod |  |
| Particle |  |
| Particle |  |
| Sphere |  |

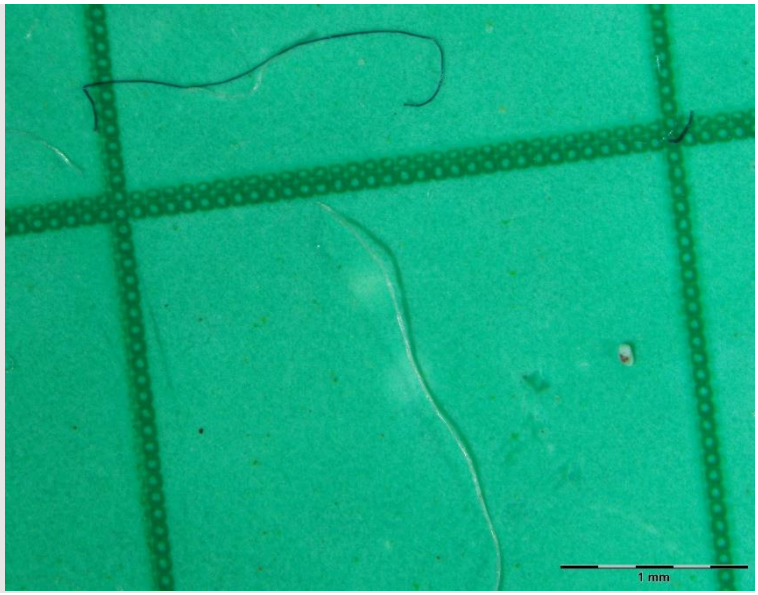



Figure 17: Typical fibre found with the microscopic method.

For both methods the relative amount of particle types differed (Figure 18). With microscopy the dominant microplastic species are identified as plastic fibres in 75% of the cases. By LDIR, the relative abundance of fibres was only between 2 - 4% and reported dominantly particles, spheres, and rods. This difference is supported by recent studies as reported earlier for a sewage treatment plant (WWTP) in Changzhou [6], where plastic fibres are dominant in sizes >100 µm and fragments or rods are dominant in sizes <100 µm.

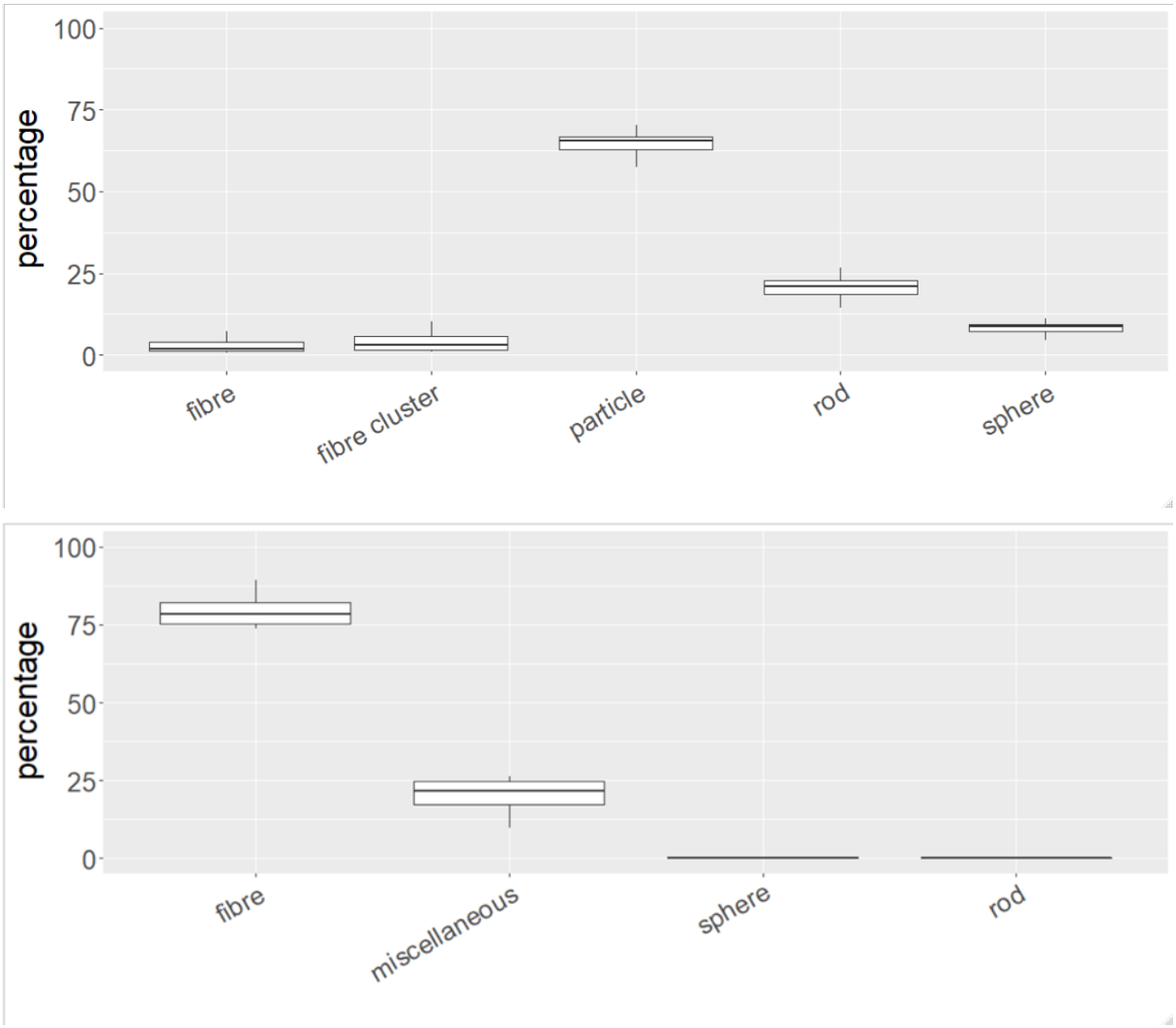



*Figure 18: Relative abundance of different particle shapes (Top: LDIR, Bottom: microscopy)*

As fibres are usually larger particles (Figure 23, SI), these are expected to be less abundant in the smaller size fraction. Smaller particles are less prone to be plastic fibres as the maximum possible ratio between thickness and length is limited for small particles. As the number of particles in sub-50 µm fractions is expected to be exponentially higher, this also has a profound effect on the calculated average particle size.
Total fibre numbers determined by the two methods were compared (Figure 19 and Figure 20 SI). Statistical analysis of the data (Table 10, SI) and the figures show that the numbers are comparable. Both LDIR and microscopy find similar quantities of plastic fibres in the samples. Between approximately 1,000 and 2,500 fibres per $m^3$ were found for all locations. The ratio of the two methods (t-test) per locations also shows that only for the effluent the diversion from 1 is only just significant ($p = 0.09$).

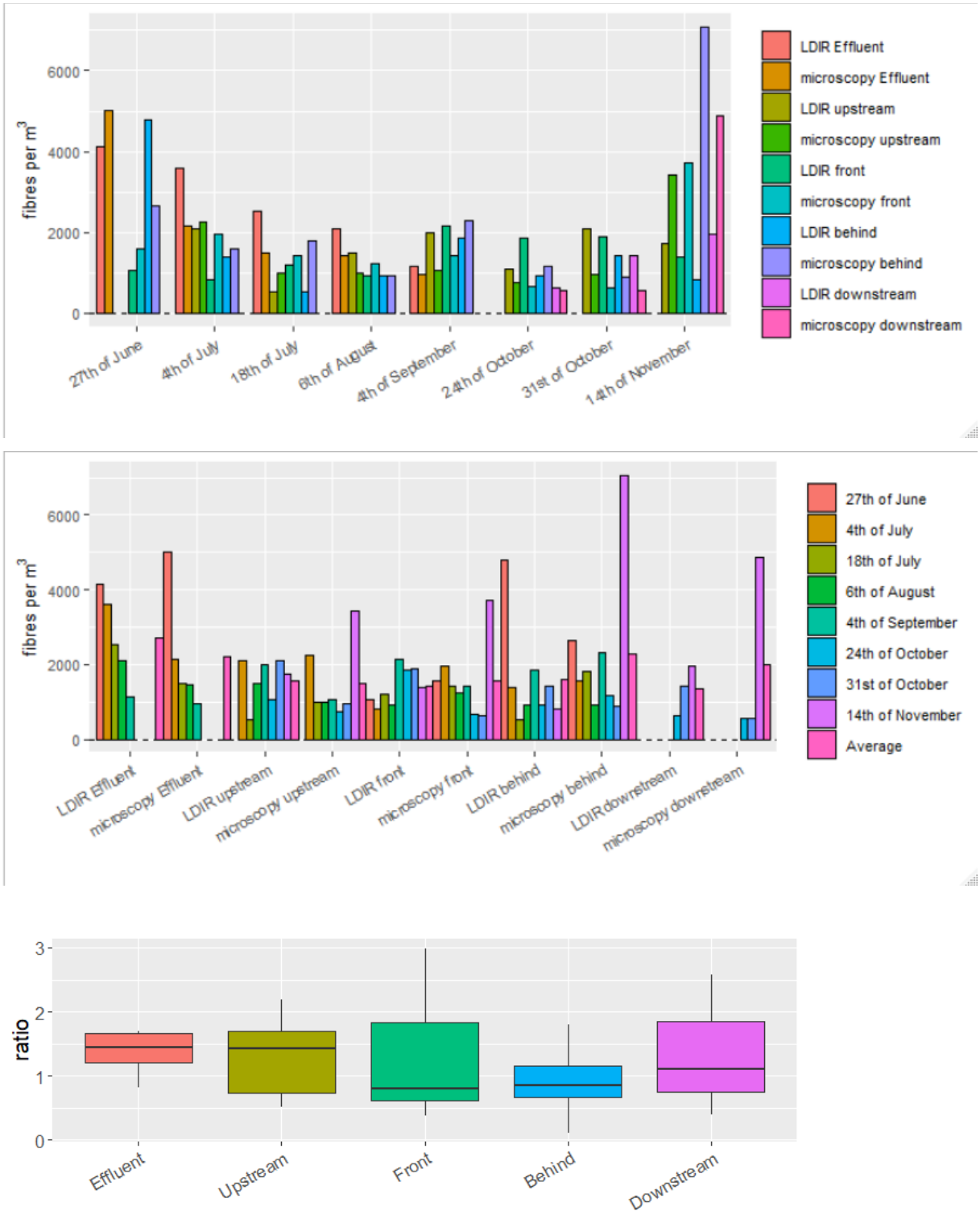



*Figure 19: Fibre concentration per location and date. ANOVA test and t-test can be found in the SI (Table 8). KWR is the LDIR method and HWL is the microscopy method.*

### 4.4.4 Performance comparison

The overall comparison between the applied methods (microscopy and LDIR) appear to indicate the methods show similar results towards the conclusion but are not similar. For example, the LDIR method finds more particles on average. However, trends in regards to WWTP- and bubble curtain hypotheses are similar and give the same results. Day-to-day variation between methods was similar. In addition, PN in comparable size classes are similar with similar trends, and the number of plastic fibres detected is proportionate. Excess of particles was detected by both methods for dates with heavy rainfall. The most obvious difference between the methods is found when size distributions are compared. Comparison of the slopes shows that the microscopy underestimates the smaller size fraction, whereas the LDIR method is prone to underestimation of the larger size fractions. Consequently, size regression slopes of these two methods are significantly different.

It must be noted that comparing results from literature sources with results from this study or with other literature results is not a straightforward comparison. As mentioned, selected sample strategies, applied sampling devices, the measuring technique and types of plastics that can be detected have a significant impact on the measured particle number or their size classification. These differences can readily reach one order of magnitude or perhaps more as shown in this study. Hence, relating values from a study to another study is precarious and, so far, has not been demonstrated to be successful. The medley of sampling and analytical methods that comes along with wildly distributed particle numbers is well documented[4-6]

It must be noted, however, that expressing the discharge of microplastics in mass units is especially prone to error: not only will the different method influence this number but also the way how the mass was calculated or estimated. For example, to calculate the mass the density and the volume of the particles must be determined which includes assumptions that will inevitably be different between various studies and algorithms

Despite the difficulty comparing literature results, the method performance in this study is on-par with that in literature results, fortifying these findings. In conclusion, the presented methods appear to be sufficiently comparable.

## 4.5 Environmental effects of particles in waters

The ultimate endpoint for a feasibility study of microplastic abatement options is to determine whether these options reduce the concentration of microplastics to acceptable levels. However, no defined safety threshold for microplastics currently exists, although studies have derived preliminary ecological effect thresholds. In this research project, we attempt to define the ecological risk based on current, yet limited knowledge. Next, note that we calculate the risk for a worst-case approach, assuming no further dilution of treated waste waters (WWTP-effluent) which may occur in more realistic environmental conditions.

### 4.5.1 Risk calculation

In short, a classical risk calculation in (eco)toxicology is performed comparing microplastic concentrations in the environment (upper part of risk calculation) to levels on which effects are shown to occur or a derived safety value is derived (lower part).

$$Risk\ Index\ (RI) = \frac{Measured\ Environmental\ Concentration\ (MEC)}{Predicted\ No\ Effect\ Concentration\ (PNEC)}$$

The upper part is most often the measured environmental concentrations (MEC) of microplastics, which we can see as the measured particle numbers in this research. Here, the measured environmental concentration of the outflow of the WTTP was described a 40-50 particle per litre (LDIR method) and 1-6 particle p/L (microscopy). The lower part of the equation is the level on which effects are expected to occur. This is called the predicted no-effect concentrations (PNEC) or hazardous concentration for protecting 95% of the species (HC5). In case the Risk Index is above 1, the value of measured is higher than the PNEC indicating an ecological risk.

### 4.5.2 Literature data and comparison

Very little data is known on this, as science has only started to describe the toxicity of particles. Nevertheless, using the limited data, some authors have described the (preliminary) risk calculation of microplastics to aquatic organisms. Up to now, we noted three papers ([38-41]), and note that the paper of Burns and Boxall was corrected later, described in the Corrigendum[42] . We used these studies to define the ecological perspective of the particles in the effluent canal of WWTP Wervershoof. All articles provided quantitative risk estimates for microplastics, based on comparison of measured (MEC) or predicted exposure concentrations (PEC) and predicted no effect concentration (PNEC) data ([38-40]). Burns and Boxall (2018) constructed a species sensitivity distribution (SSD) based on effect thresholds obtained with a limited set of laboratory studies on aquatic organisms [38, 40, 41]. By this study, they calculated a HC5 value of $3.5\times10^3$ particles/L ([38, 40]). A more recent paper derived a predicted no-effect concentration (PNEC) of 12 particles/L with a 95% Confidence Interval of 2.7-52.3[41]. Using their PNEC value would imply that the risk calculation based on the LDIR method was above 1 indicating risk. However, these authors stated that toxicity may depend on size and that their data must be used for the size range of 20 to 300 micrometre, different from the LDIR detection capacity ad more fitting to the range of microscopic derived data. So while the data for LDIR indicted a risk, the risk index based on the microscopy counts (1-6) are below 1 and no ecological risk is expected. The authors state that their risk is based more accurate for the fraction of 20-300

micrometre, as comparable for the microscopy method. Therefore, we could also state that the effects for the smaller fractions is not yet known and risks based on LDIR methodology cannot be described accurately.

In all, we note that in line with other authors, the MECs of microplastics were lower than their PNEC/HC5 values; therefore, no significant ecological concern was anticipated at this time. Note that the ecological risk is calculated for the effluent canal and not for receiving waters such as Lake Ijssel itself. Hence, we did not include how dilution of particles may take place. But, we may expect that the outflowing plastic particles may accumulate in certain regions which locally may pose a risk, as suggested in other studies[39].

Although ecological effects are not expected at this point, large uncertainties remain. For example, a complete risk assessment is not available, especially for the smaller fragments. Next to ecological risks, human health risks are also not fully understood but acute effects in for example drinking water is not expected 21, 22.

While more and more data becomes available on the effects, the risk calculation becomes more reliable. Yet, as concentrations in the environment may increase in case future emissions remain constant, or even increase, ecological systems may be at certain risk. Current concerns associated with microplastics are chemicals leaching from particles, understanding the toxicity of the physical presence of microplastic particles themselves, and the potential for pathogen growth on microplastics acting as vectors. It is paramount to discover the effects of microplastics on the environment. Therefore, within the "*Kennisimpuls Waterkwaliteit*[1]" the ecotoxicological effects described in the scientific literature are currently being summarised. Yet, understanding their presence and discovering possible abatement options should not be delayed in this process. Therefore, it is also required to know the mass balance of microplastics across the ecological system and investigate possible abatement options to reduce microplastics outflow.

[1] https://www.stowa.nl/deltafacts/waterkwaliteit/kennisimpuls-waterkwaliteit/microplastics

# 5 Conclusions and recommendations

This study investigated microplastic particle outflow and abatement options at Dutch wastewater treatment plant (WWTP) using a novel bubble curtain pilot setup (Bubble Barrier). Studies have shown that WWTPs are capable of removing a majority of incoming plastics from influent, but especially microplastics are susceptible to pass through a WWTP.

- In this study we observed a WWTP-outflow of microplastics between 40 – 50 particles/L and 1 – 6 particles/L depending on the size range.

This is in the same scale as other findings reported in literature. Currently available preliminary risk assessment indicates that at microplastic levels measured in this study, no imminent ecological risks are to be expected from the particle outflow from WWTP. However, with increasing emissions, lack of recycling, or alternatives to plastics these concentrations are likely to increase and thereby pose a potential future risk. In addition, the data collected in this study is susceptible to further discussion and placed in context of a pilot-scale test setup, where external influences (e.g., weather, flux) and internal influences (e.g., sampling conditions) may have a pronounced role.

- We noted that storm events may cause short term peaks of plastic outflow and the potential effects of these events are not yet well understood.

A bubble curtain installed in an effluent canal was evaluated for possible microplastic removal from WWTP effluent water. Earlier research showed that a bubble curtain can be effective to remove larger plastic particles (> 1 mm) from flowing water.

- From our observations it was, however, not possible to conclude that the pilot bubble curtain, in the condition that it was set up, is also capable of reducing the outflow of microplastic particles at 15cm depth, although it is capable of blocking buoyant plastic fragments on the surface from >1mm in pilot experiments.

The lack of a measurable effect was placed in context of several causes and external influences, including analytical detection limitations. Changing the design of the barrier and the dimensions of the canal may improve the detection of the efficiency towards smaller plastic particles studied here. However, it does not evaluate the potential effect of the different properties of the plastic particles. Therefore we recommend two types of research carried out under controlled conditions to better understand the behaviour of small fragments in waters in order to separate the intrinsic treatment performance from artefacts such as environmental (weather) conditions affecting treatment performance under field conditions. First, studies need to focus on how hydrodynamic conditions, considering bubble curtain characteristics, horizontal and vertical water-flow and sampling/collection affect separation (concentration) and potential collection of microplastics. Secondly, the effects of physicochemical characteristics (e.g. size, dimensions, and surface characteristics) of the plastic particles on their behaviour in the water column need to be evaluated. This likely requires more fundamental lab scale studies to better understand the movement of these particles in a water column and with air bubbles in relation to their physicochemical

characteristics. Finally, these two aspects need to be combined in order to optimize the removal potential of a bubble screen for a specific situation, such as the effluent of a wastewater treatment plant.

Two analytical methods were employed in parallel. The comparison of the two methods showed that visual microscopic counting and Laser Direct Infrared (LDIR) mainly differ due to minimum size detection characteristics.

- Smaller microplastics are better detected in the WWTP outflow by LDIR and microscopy is better suited for larger fragments and for samples that cannot be treated with chemicals for clean-up, a step that is essential for the LDIR or similar techniques that suffer from background noise such as FTIR or Raman.
- Sample treatment and analyses were optimized for both techniques and our studies underlined that blank correction and positive controls are essential for LDIR, both in the lab as in the field. This combination of techniques was complementary and showed comparable trends at sampling locations.
- Both methods showed that fibres are a consistent part of the outflow of fragments, as seen in other studies. However, using LDIR showed that a higher relative contribution of particles other than fibres than using the optical microscopy method, possibly explained by the range of smaller particles investigated.
- In total 27 different types of polymers were found, including polyamide (PA), Polyethylene terephthalate (PET), isoprene, Polyurethane (PU)/varnish, Polypropylene (PP), Polyethylene (PE)-Chloride and Polyethylene (PE). We noted that a relative higher proportion of polyamide particles were detected compared to literature. All these polymers are commonly found in effluents or surface waters and also corresponds to those recently found in two Dutch rivers. Yet, there is no clear explanation for the relatively high contribution of polyamide particles compared to literature. One possibly is that despite the chemical work-up, still natural polyamides are present in the sample.

This study shows the merit of using analytical strategies to investigate and provide data on the outflow of microplastics into the environment. We emphasize the need of systematic testing of abatement options and recommend that more data is needed for the interpretation of results. The need for standardised methodology for sampling and analysis of microplastics is highlighted, as minor choices in sampling can have significant effects in the results.

While currently available preliminary risk assessment indicated that at microplastic levels measured in this study, no imminent ecological risks were expected. Note that this is a worst-case approach using concentrations from the particle outflow from a WWTP. Next, we also highlight the preliminary status of this field, as risks for the lower size particles is not yet fully understood and lacks certainty. Although we see that ecological effects are not expected in the outflow itself, large uncertainties exist and it is clear that plastic particles may accumulate in certain regions. Hence, more research is recommended to better identify hazard and risk.

We note that microplastic particles and their possible effects on the environment are keenly followed by science and regulators. While the impact of these particles in the environment is largely unknown it is also expected that microplastic concentration in the environment will continue to increase due to increased usage of plastics and

further degradation of current environmental plastics. In all, abatement options, such as the bubble curtains by the Great Bubble Barrier are asked for and still worth developing further.

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# I Supporting information

*Table 7: Average particle number for the different size classes. Comparison LDIR and microscopy method.*

| data: plot.data.melt7$value and plot.data.melt7$variable | | | | | |
|---|---|---|---|---|---|
| | HWL > 250 | HWL 125-250 | HWL 50-125 | KWR > 250 | KWR 125-250 |
| HWL 125-250 | 0.40588 | - | - | - | - |
| HWL 50-125 | 0.08062 | 0.35680 | - | - | - |
| KWR > 250 | 0.23111 | 0.71297 | 0.57930 | - | - |
| KWR 125-250 | 0.00027 | 0.00446 | 0.05204 | 0.01291 | - |
| KWR 50-125 | < 2e-16 | < 2e-16 | < 2e-16 | < 2e-16 | < 2e-16 |

*Table 8: ANOVA test for fibre numbers*

| | HWL behind | HWL downstream | HWL Effluent | HWL front | HWL upstream | KWR behind | KWR downstream |
|---|---|---|---|---|---|---|---|
| HWL downstream | 0.731 | - | - | - | - | - | - |
| HWL Effluent | 0.903 | 0.823 | - | - | - | - | - |
| HWL front | 0.273 | 0.638 | 0.400 | - | - | - | - |
| HWL upstream | 0.238 | 0.580 | 0.353 | 0.901 | - | - | - |
| KWR behind | 0.276 | 0.642 | 0.403 | 0.995 | 0.896 | - | - |
| KWR downstream | 0.280 | 0.538 | 0.363 | 0.785 | 0.861 | 0.781 | - |
| KWR Effluent | 0.592 | 0.462 | 0.554 | 0.137 | 0.120 | 0.138 | 0.158 |
| KWR front | 0.177 | 0.509 | 0.286 | 0.796 | 0.900 | 0.791 | 0.934 |
| KWR upstream | 0.288 | 0.642 | 0.410 | 0.996 | 0.908 | 0.992 | 0.791 |

| | KWR Effluent | KWR front |
|---|---|---|
| HWL downstream | - | - |
| HWL Effluent | - | - |
| HWL front | - | - |
| HWL upstream | - | - |
| KWR behind | - | - |
| KWR downstream | - | - |
| KWR Effluent | - | - |
| KWR front | 0.088 | - |
| KWR upstream | 0.146 | 0.806 |

*Table 9: Column statistics for the locations front and behind for LDIR.*

| | June 27th Front | July 4th front | Julty 18th front | August 6th front | September 4th Front | October 2nd front | October 24th front | October 31st front | November 14th front |
|---|---|---|---|---|---|---|---|---|---|
| Best-fit values | | | | | | | | | |
| Slope | -1.688 ± 0.1596 | -1.995 ± 0.1766 | -2.254 ± 0.1386 | -1.987 ± 0.1854 | -1.919 ± 0.1404 | -1.951 ± 0.2401 | -1.701 ± 0.2405 | -2.689 ± 0.1398 | -2.170 ± 0.2392 |
| Y-intercept when X=0.0 | 3.501 ± 0.3173 | 3.996 ± 0.3458 | 4.583 ± 0.2755 | 4.095 ± 0.3591 | 3.894 ± 0.2790 | 3.973 ± 0.4643 | 3.528 ± 0.4749 | 5.013 ± 0.2779 | 4.295 ± 0.4665 |
| X-intercept when Y=0.0 | 2.074 | 2.003 | 2.033 | 2.061 | 2.029 | 2.036 | 2.074 | 1.864 | 1.979 |
| 1/slope | -0.5923 | -0.5012 | -0.4436 | -0.5033 | -0.5211 | -0.5124 | -0.5879 | -0.3718 | -0.4608 |
| 95% Confidence Intervals | | | | | | | | | |
| Slope | -2.025 to -1.352 | -2.371 to -1.619 | -2.547 to -1.962 | -2.384 to -1.589 | -2.215 to -1.623 | -2.470 to -1.433 | -2.211 to -1.191 | -2.984 to -2.394 | -2.680 to -1.660 |
| Y-intercept when X=0.0 | 2.832 to 4.171 | 3.259 to 4.733 | 4.002 to 5.164 | 3.324 to 4.865 | 3.305 to 4.483 | 2.970 to 4.976 | 2.521 to 4.534 | 4.427 to 5.599 | 3.301 to 5.289 |
| X-intercept when Y=0.0 | 2.017 to 2.139 | 1.950 to 2.061 | 1.997 to 2.072 | 2.003 to 2.131 | 1.986 to 2.075 | 1.959 to 2.132 | 1.989 to 2.182 | 1.830 to 1.896 | 1.915 to 2.049 |
| Goodness of Fit | | | | | | | | | |
| $r^2$ | 0.8681 | 0.8949 | 0.9396 | 0.8914 | 0.9166 | 0.8356 | 0.7576 | 0.9561 | 0.8458 |
| Sy.x | 0.1940 | 0.2042 | 0.1685 | 0.2042 | 0.1706 | 0.2664 | 0.2865 | 0.1700 | 0.2686 |
| Is slope significantly non-zero? | | | | | | | | | |
| F | 111.9 | 127.7 | 264.4 | 114.9 | 186.9 | 66.06 | 50.00 | 369.9 | 82.30 |
| DFn, DFd | 1.000, 17.00 | 1.000, 15.00 | 1.000, 17.00 | 1.000, 14.00 | 1.000, 17.00 | 1.000, 13.00 | 1.000, 16.00 | 1.000, 17.00 | 1.000, 15.00 |
| P value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Deviation from zero? | Significant | Significant | Significant | Significant | Significant | Significant | Significant | Significant | Significant |
| Data | | | | | | | | | |
| Number of X values | 19 | 17 | 19 | 16 | 19 | 15 | 18 | 19 | 17 |
| Maximum number of Y replicates | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Total number of values | 19 | 17 | 19 | 16 | 19 | 15 | 18 | 19 | 17 |
| Number of missing values | 0 | 2 | 0 | 3 | 0 | 4 | 1 | 0 | 2 |
| Runs test | | | | | | | | | |
| Points above line | 9 | 12 | 8 | 9 | 11 | 8 | 8 | 7 | 9 |
| Points below line | 10 | 5 | 11 | 7 | 8 | 7 | 10 | 12 | 8 |
| Number of runs | 11 | 7 | 13 | 7 | 11 | 7 | 5 | 10 | 9 |
| P value (runs test) | 0.6814 | 0.3654 | 0.9434 | 0.2308 | 0.7217 | 0.2960 | 0.0134 | 0.6241 | 0.5000 |
| Deviation from linearity | Not Significant | Not Significant | Not Significant | Not Significant | Not Significant | Not Significant | Significant | Not Significant | Not Significant |

| | June 27the Behind | July 4th behind | July 18th behind | August 6th behind | September 4th Behind | Ocotber 2nd behind | October 24th behind | Ocotber 31st behind | November 14th behind |
|---|---|---|---|---|---|---|---|---|---|
| Best-fit values | | | | | | | | | |
| Slope | -2.432 ± 0.1353 | -2.407 ± 0.1826 | -1.819 ± 0.1286 | -1.466 ± 0.1864 | -2.229 ± 0.1457 | -2.195 ± 0.1107 | -2.006 ± 0.2090 | -2.281 ± 0.2179 | -2.018 ± 0.1312 |
| Y-intercept when X=0.0 | 4.677 ± 0.2675 | 4.644 ± 0.3523 | 3.918 ± 0.2493 | 3.331 ± 0.3480 | 4.436 ± 0.2870 | 4.474 ± 0.2186 | 4.209 ± 0.3963 | 4.395 ± 0.4157 | 4.042 ± 0.2584 |
| X-intercept when Y=0.0 | 1.923 | 1.929 | 2.153 | 2.272 | 1.990 | 2.038 | 2.098 | 1.927 | 2.003 |
| 1/slope | -0.4112 | -0.4155 | -0.5496 | -0.6819 | -0.4486 | -0.4556 | -0.4985 | -0.4384 | -0.4956 |
| 95% Confidence Intervals | | | | | | | | | |
| Slope | -2.719 to -2.145 | -2.801 to -2.013 | -2.103 to -1.536 | -1.877 to -1.056 | -2.538 to -1.920 | -2.429 to -1.960 | -2.466 to -1.546 | -2.752 to -1.810 | -2.297 to -1.738 |
| Y-intercept when X=0.0 | 4.110 to 5.244 | 3.883 to 5.405 | 3.369 to 4.466 | 2.565 to 4.097 | 3.828 to 5.045 | 4.010 to 4.937 | 3.336 to 5.081 | 3.497 to 5.293 | 3.491 to 4.593 |
| X-intercept when Y=0.0 | 1.889 to 1.957 | 1.883 to 1.977 | 2.097 to 2.222 | 2.159 to 2.456 | 1.952 to 2.030 | 2.008 to 2.071 | 2.023 to 2.198 | 1.872 to 1.985 | 1.963 to 2.046 |
| Goodness of Fit | | | | | | | | | |
| $r^2$ | 0.9528 | 0.9304 | 0.9479 | 0.8490 | 0.9360 | 0.9609 | 0.8933 | 0.8940 | 0.9404 |
| Sy.x | 0.1619 | 0.2001 | 0.1448 | 0.1737 | 0.1709 | 0.1319 | 0.2181 | 0.2222 | 0.1562 |
| Is slope significantly non-zero? | | | | | | | | | |
| F | 322.9 | 173.7 | 200.1 | 61.87 | 234.0 | 392.9 | 92.11 | 109.6 | 236.6 |
| DFn, DFd | 1.000, 16.00 | 1.000, 13.00 | 1.000, 11.00 | 1.000, 11.00 | 1.000, 16.00 | 1.000, 16.00 | 1.000, 11.00 | 1.000, 13.00 | 1.000, 15.00 |
| P value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| Deviation from zero? | Significant | Significant | Significant | Significant | Significant | Significant | Significant | Significant | Significant |
| Data | | | | | | | | | |
| Number of X values | 18 | 15 | 13 | 13 | 18 | 18 | 13 | 15 | 17 |
| Maximum number of Y replicates | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Total number of values | 18 | 15 | 13 | 13 | 18 | 18 | 13 | 15 | 17 |
| Number of missing values | 1 | 4 | 6 | 6 | 1 | 1 | 6 | 4 | 2 |
| Runs test | | | | | | | | | |
| Points above line | 7 | 7 | 8 | 6 | 8 | 8 | 7 | 8 | 11 |
| Points below line | 11 | 8 | 5 | 7 | 10 | 10 | 6 | 7 | 6 |
| Number of runs | 10 | 8 | 5 | 9 | 11 | 10 | 6 | 8 | 8 |
| P value (runs test) | 0.6821 | 0.5136 | 0.1515 | 0.8788 | 0.7822 | 0.6209 | 0.2960 | 0.5136 | 0.4357 |
| Deviation from linearity | Not Significant | Not Significant | Not Significant | Not Significant | Not Significant | Not Significant | Not Significant | Not Significant | Not Significant |

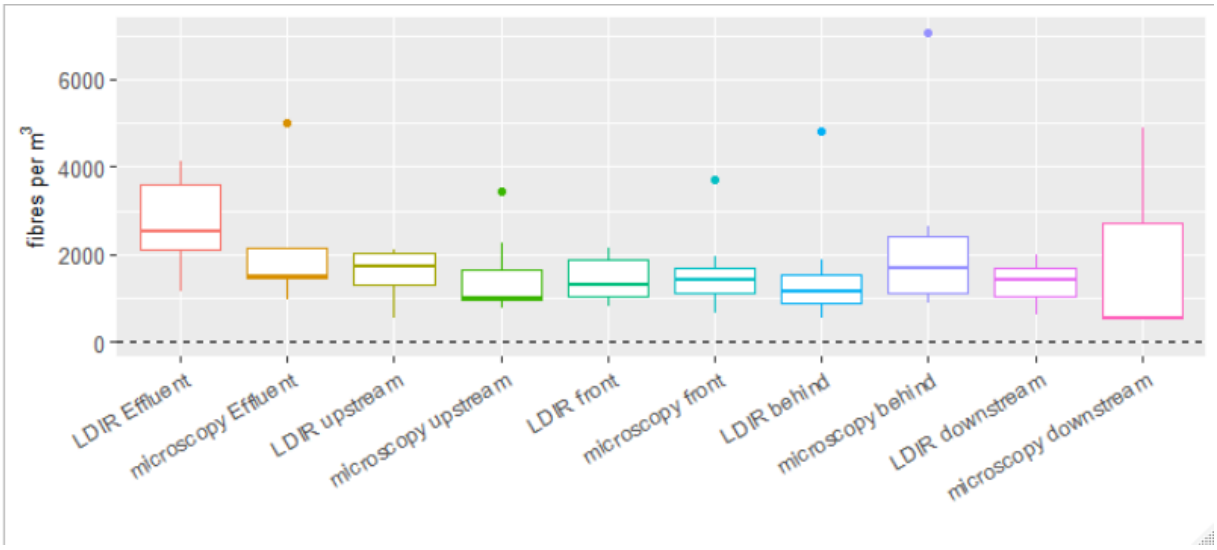


*Figure 20: Fibre number per location and method (KWR=LDIR; HWL=microscopy), see paragraph 3.5 of this report for details on sampling locations*

*Figure 21: LDIR. Particle number from top to bottom: Particle number corrected for negative control, particle number not corrected for the negative control and particles on slide.*

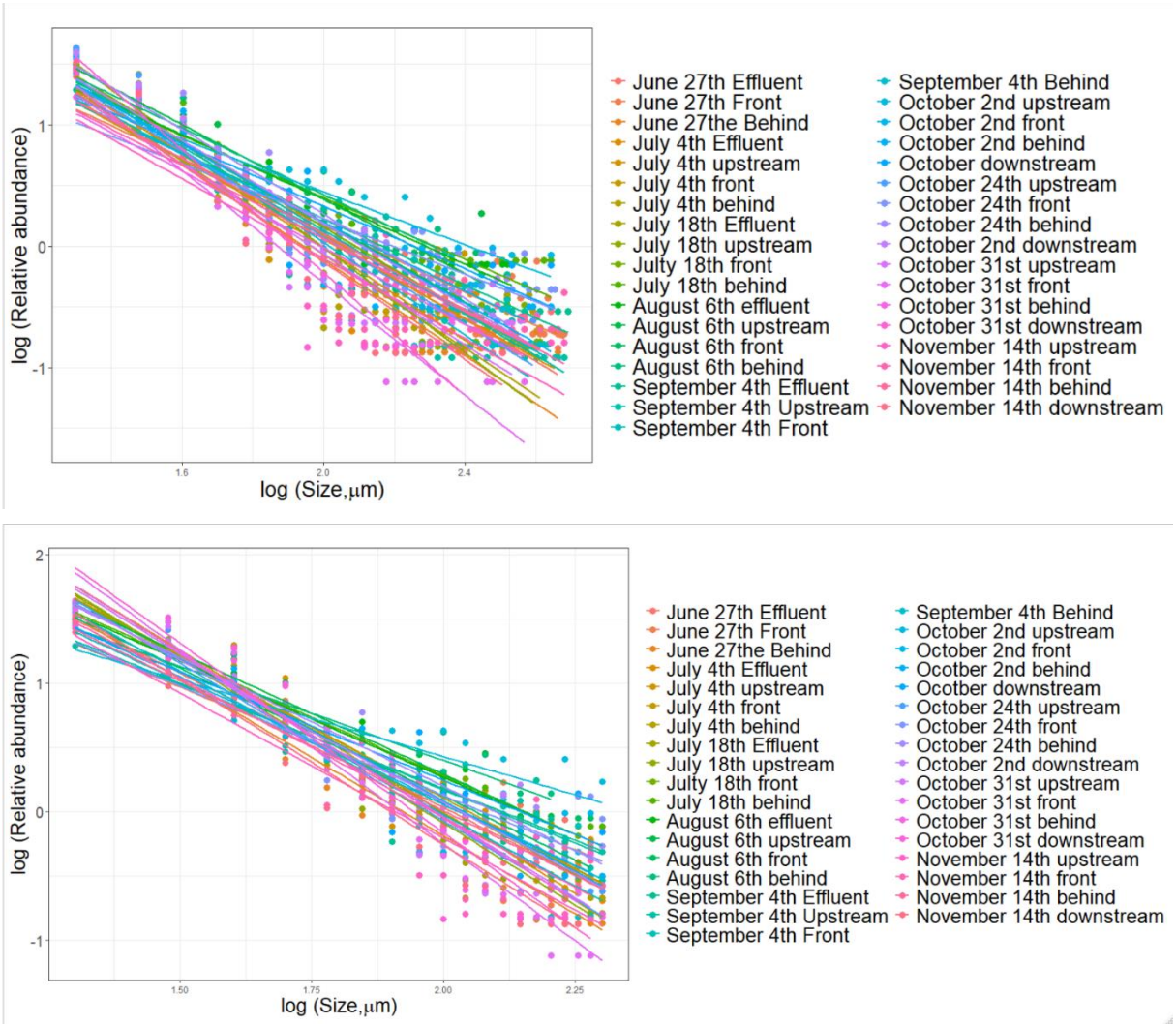


*Figure 22: Logarithmic slopes over the size range of 20 to 490 µm (top) and over the size range of 20 – 200 µm (bottom).*

*Table 10:* Statistic for Ratio between normalised LDIR and microscopy particle number.

| |
|---|
| Shapiro-Wilk normality test |
| |
| data: hwl.loc$Ratio |
| W = 0.93963, p-value = 0.6074 |
| |
| One Sample t-test |
| |
| data: hwl.loc$Ratio |
| t = 1.4122, df = 7, p-value = 0.2008 |
| alternative hypothesis: true mean is not equal to 1 |
| 95 percent confidence interval: |
| 0.7414196 2.0253886 |
| sample estimates: |
| mean of x |
| 1.383404 |

*Table 11: Results one sided t-test. Ratio > 1.*

| One Sample t-test | One Sample t-test |
|---|---|
| data: plot.data7$`Front/Behind KWR` | data: plot.data7$`Front/Behind HWL` |
| t = 1.1561, df = 8, p-value = 0.1405 | t = 0.32322, df = 10, p-value = 0.3766 |
| alternative hypothesis: true mean is greater than | alternative hypothesis: true mean is greater than |
| 95 percent confidence interval: | 95 percent confidence interval: |
| 0.6444883 Inf | 0.6382639 Inf |
| sample estimates: | sample estimates: |
| mean of x | mean of x |
| 1.584251 | 1.078511 |

*Table 12: Statistical analysis fort the slope comparison between front and behind (LDIR).*

| contrast | p.value |
|---|---|
| August 6th front - August 6th behind | 0.923564648 |
| Julty 18th front - July 18th behind | 0.940655245 |
| July 4th front - July 4th behind | 0.974216596 |
| June 27th Front - June 27the Behind | 0.131529144 |
| November 14th front - November 14th behind | 0.999999942 |
| October 24th front - October 24th behind | 0.999243775 |
| October 2nd front - Ocotber 2nd behind | 0.999942401 |
| October 31st front - Ocotber 31st behind | 0.980254465 |
| September 4th Front - September 4th Behind | 0.997778129 |

| | Slope | SE | lower.CL | upper.CL |
|---|---|---|---|---|
| June 27th Fron | -1.688395 | 0.1637175 | -2.010759 | -1.366031 |
| July 4th front | -1.995045 | 0.1720476 | -2.333811 | -1.656279 |
| Julty 18th front | -2.254181 | 0.1637175 | -2.576545 | -1.931817 |
| August 6th fro | -1.986778 | 0.1806548 | -2.342491 | -1.631064 |
| September 4th | -1.919114 | 0.1637175 | -2.241477 | -1.59675 |
| October 2nd fr | -1.951436 | 0.1793621 | -2.304605 | -1.598268 |
| October 24th fr | -1.700825 | 0.1670881 | -2.029826 | -1.371825 |
| October 31st fr | -2.689262 | 0.1637175 | -3.011626 | -2.366898 |
| November 14th | -2.170013 | 0.1772163 | -2.518956 | -1.821069 |
| June 27the Bel | -2.431907 | 0.1663183 | -2.759392 | -2.104422 |
| July 4th behin | -2.407007 | 0.1816096 | -2.764601 | -2.049413 |
| July 18th behin | -1.81941 | 0.1767399 | -2.167416 | -1.471405 |
| August 6th bel | -1.466438 | 0.213556 | -1.886935 | -1.045941 |
| September 4th | -2.228929 | 0.169688 | -2.563049 | -1.894809 |
| October 2nd b | -2.194745 | 0.1670881 | -2.523746 | -1.865745 |
| October 24th b | -2.006048 | 0.190687 | -2.381515 | -1.63058 |
| Ocotber 31st be | -2.28105 | 0.1951404 | -2.665286 | -1.896813 |
| November 14th | -2.01765 | 0.1671132 | -2.3467 | -1.6886 |

*Table 13: Statistical analysis fort the slope comparison between front and behind (microscopy).*

| | slope | SE | df | lower.CL | upper.CL |
|---|---|---|---|---|---|
| May 23rd front | -0.6498857 | 0.1809436 | 66 | -1.0111512 | -0.28862027 |
| June 27th front | -0.1669915 | 0.1809436 | 66 | -0.528257 | 0.19427398 |
| July 4th front | -0.2699441 | 0.1809436 | 66 | -0.6312096 | 0.09132133 |
| July 18th front | -0.4938064 | 0.1809436 | 66 | -0.8550718 | -0.1325409 |
| August 6th fron | -0.5720995 | 0.1809436 | 66 | -0.933365 | -0.21083401 |
| September 4th | -0.5690347 | 0.1809436 | 66 | -0.9303002 | -0.20776926 |
| September 26r | -0.6566134 | 0.1809436 | 66 | -1.0178788 | -0.29534788 |
| October 24th fr | -0.6977685 | 0.1809436 | 66 | -1.059034 | -0.33650306 |
| October 31st fr | -0.6957398 | 0.1809436 | 66 | -1.0570053 | -0.33447436 |
| November 14th | -0.810032 | 0.1809436 | 66 | -1.1712975 | -0.44876652 |
| November 28th | -1.2126696 | 0.1809436 | 66 | -1.573935 | -0.85140409 |
| May 23rd behir | -0.5014558 | 0.1809436 | 66 | -0.8627213 | -0.14019037 |
| June 27th behi | -0.6532772 | 0.1809436 | 66 | -1.0145426 | -0.29201169 |
| July 4th behind | -0.4167357 | 0.1809436 | 66 | -0.7780012 | -0.0554702 |
| July 18th behin | -0.5085336 | 0.1809436 | 66 | -0.8697991 | -0.14726817 |
| August 6th bel | -0.3883125 | 0.1809436 | 66 | -0.749578 | -0.02704704 |
| September 4th | -0.6349972 | 0.1809436 | 66 | -0.9962626 | -0.27373168 |
| September 26r | -0.6230512 | 0.1809436 | 66 | -0.9843167 | -0.26178575 |
| October 24th b | -0.8991452 | 0.1809436 | 66 | -1.2604107 | -0.53787977 |
| October 31st be | -0.6481526 | 0.1809436 | 66 | -1.0094181 | -0.28688717 |
| November 14th | -0.8990625 | 0.1809436 | 66 | -1.260328 | -0.53779703 |
| November 28th | -0.8920348 | 0.1809436 | 66 | -1.2533003 | -0.53076932 |

| | p.value |
|---|---|
| June 27th front - June 27th behind | 0.945978 |
| July 4th front - July 4th behind | 1 |
| July 18th front - July 18th behind | 1 |
| August 6th front - August 6th behind | 1 |
| September 4th front - September 4th behind | 1 |
| September 26nd front - September 26nd behind | 1 |
| October 24th front - October 24th behind | 1 |
| October 31st front - October 31st behind | 1 |
| November 14th front - November 14th behind | 1 |
| November 28th front - November 28th behind | 0.999637 |

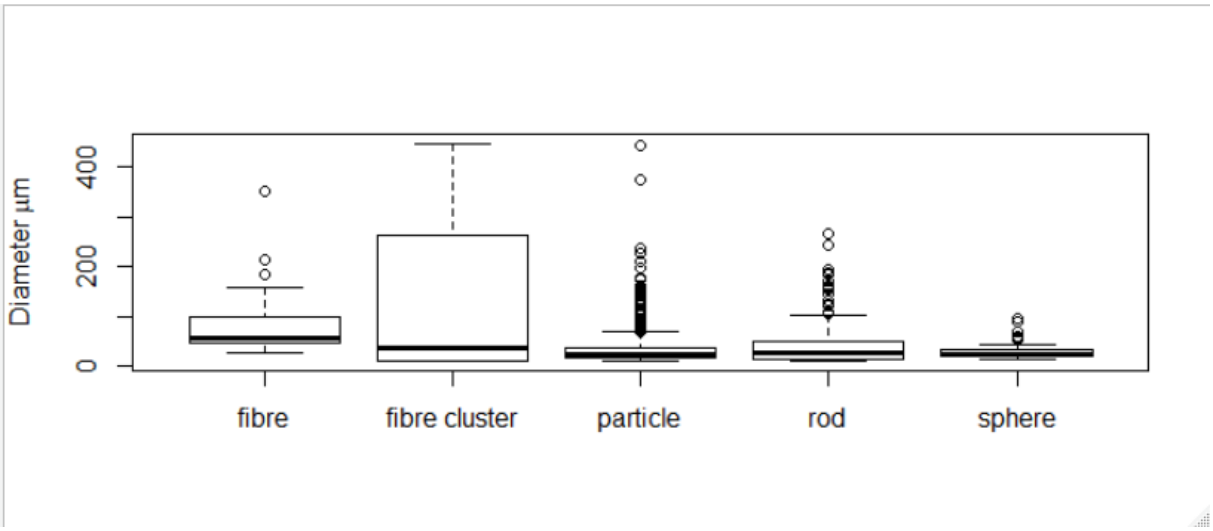


*Figure 23: Average particle size in WWTP Wervershoof per particle shape (LDIR method)*