Ministry of Environment and Food of Denmark
Environmental Protection Agency

Microplastic in Danish wastewater

Sources, occurrences and fate

Environmental Project
No. 1906

Marts 2017

Publisher: The Danish Environmental Protection Agency

Editor:
Professor Jes Vollertsen, Aalborg University and
Aviaja Anna Hansen, Krüger A/S

ISBN: 978-87-93529-44-1

Contents

Preface 6

Background of the study 6

Steering group 6

Advisory group 6

Project group 6

Conclusions and summary 7

Konklusion og sammenfatning 10

1. Introduction 13

1.1 Microplastic in wastewater and wastewater treatment plants 13

1.1.1 Fate of microplastic in wastewater treatment plants 13

1.2 Objectives of the study 14

1.2.1 Definition of microplastic in the present study 14

2. Project design 15

2.1 Occurrences, sources and fate of microplastic in Danish wastewater treatment plants 15

2.1.1 The wastewater treatment plants investigated 15

2.2 Occurrences and sources of microplastic on Danish farmlands 15

3. Methodology 16

3.1 Choosing the analytical method 16

3.2 The methodology at a glance 17

3.3 Method validation 19

4. Results and discussion 21

4.1 Microplastic in Danish wastewater treatment plants 21

4.1.1 Microplastic concentrations in raw and treated wastewater 21

4.1.2 Size distributions of microplastic particles 22

4.1.3 Microplastic concentrations in sludge 24

4.1.4 Mass balance of microplastic in wastewater treatment plants 25

4.1.5 Microplastic loads on the aquatic environment 26

4.1.6 The polymer composition of the microplastic particles 26

4.2 Occurrences and sources of microplastic on Danish farmlands 30

4.2.1 The impact of wastewater sludge on agricultural soil 31

5. Perspectives 33

Acknowledgement 35

References 36

Appendix 1. Analytical method 38

Appendix 1.1 Sampling 38

Appendix 1.2 Analysis of microplastic in wastewater and soil 39
Appendix 1.3 Spectral analysis 41
Appendix 1.4 Calculation of particle volume and mass 47

Appendix 2. Method validation, uncertainties, and detection limits 48
Appendix 2.1 Recovery of plastic particles 48
Appendix 2.2 Uncertainties 49
Appendix 2.3 Detection limits 49
Appendix 2.4 Identification of tire rubber 50
Appendix 2.5 Data overview 53

Preface

Background of the study

Microplastic in the environment was for the first time described by marine biologists in 2004 (Thompson et al, 2004) and research of microplastic has until recently mainly been driven by the field of marine biology. Microplastic emissions to the environment have in the resent years gained increasing political awareness, where also sources and reduction potentials have been on the agenda.

In Denmark a report on microplastic occurrence, effects and sources was published by the Danish Environmental Protection Agency in 2015, where wastewater treatment plants (WWTPs) were identified as potential important sources of microplastic emission to the marine waters of Denmark (Lassen et al., 2015).

To follow up on this survey the present study was initiated by the Danish Environmental Protection Agency to elucidate the role of WWTPs in the microplastic emissions to the environment.

This project was part of the Danish Government initiative to improve the understanding about sources and effects and the possibilities to reduce microplastic pollution in the environment. Funding for this activity were allocated on the Finance Act for 2015-2016.

Steering group

The steering group of the project consisted of:

- Flemming Ingerslev, Danish Environmental Protection Agency
- Linda Bagge, Danish Environmental Protection Agency
- Jes Vollertsen, Aalborg University
- Vibeke Borregaard, Krüger A/S
- Aviaja Anna Hansen, Krüger A/S

Advisory group

An advisory group with representation of various experts in the field of wastewater and microplastics has followed the project:

- Per Helmgaard, Danish Nature Agency
- Rikke Joo Vienberg, Danish Nature Agency
- Henrik Andersen, Technical University of Denmark
- Nanna Hartmann, Technical University of Denmark
- Annemette Palmquist, Roskilde University
- Hanne Løkkegaard, Danish Technological Institute
- Dines Thornberg, BIOFOS

Project group

The experimental analysis has been conducted by the research group of Professor Jes Vollertsen at Aalborg University:

- Marta Simon, Aalborg University
- Nikki Van Alst, Aalborg University
- Diana A. Stephansen, Aalborg University
- Amelia Borregaard, Aalborg University
- Kristina Borg Olesen, Aalborg University

Sampling was conducted by Anders Lund, Krüger A/S in close collaboration with the skilled operation personnel at the investigated WWTPs.

Conclusions and summary

The objectives of the present study were to evaluate the role of Danish wastewater treatment plants (WWTPs) in the emission of microplastic to the environment in terms of amounts and types of plastic polymers emitted and if possible, to evaluate which sources these plastic polymers could originate from.

Samples from 10 WWTPs (wastewater, inlet and outlet), sludge from 5 of these plants, and 10 farmlands soils (5 soils that had received sludge as fertilizer and 5 that had not) were analysed for the occurrences of microplastic in the size range 20-500 µm with the currently most advanced method available for microplastic investigations (Fourier Transformed Infrared Spectroscopy imaging applying a Focal Plane Array). This method allows both determination of the microplastic concentrations in the samples and identification of the type of plastic polymer of each microplastic particle.

The investigation was designed as a general screening study of Danish wastewater and the results are therefore an estimation of the occurrences of microplastic in average Danish wastewater, thus the results are indicative for the overall Danish wastewater, but not the distinct WWTPs.

Microplastic concentrations in wastewater and emission from WWTPs
In the raw wastewater the microplastic concentration was quantified to a median of 1.3 10^5 particles/L (size range 20-500 µm) corresponding to 5.9 mg/L, which is equivalent to one percent of the total organic matter of the raw wastewater, as it typically holds 320-740 mg COD/L. In treated wastewater the microplastic concentration was quantified to a median of 5,800 particles/L (size range 20-500 µm) corresponding to 0.02 mg/L. The variability of microplastic concentrations in raw wastewater between the 10 investigated treatment plants was quite large ranging from 13,000 to 442,000 particles/L corresponding to 0.2 to 30 mg/L.

The average emission from a Danish WWTP to the aquatic environment is from this calculated to 0.3% (with 25th and 75th percentiles of 0.0% and 0.7%) of the microplastic mass coming into the plant.

From the results obtained from the analysis of the wastewater samples it is thereby shown that the emission of microplastic from Danish WWTPs to the receiving waters is minor compared to the total load on the plants.

Microplastic concentrations in sludge
The median microplastic concentration in the wastewater sludge was quantified to 169,000 particles/g dewatered sludge corresponding to 4.5 mg/g dewatered sludge, which means that approximately 0.7% of the dewatered sludge was microplastic.

Mass balance of microplastic in wastewater treatment plants and emission to the aquatic environment
A rough mass balance can be made on the inlet and outlet mass of microplastic in wastewater. Assuming that microplastic is inert in the treatment plant, the total mass in the inlet must equal the sum of the mass in the sludge and in the discharged wastewater.

Based on the results, it is estimated that the annual load of microplastic to all Danish treatment plants is 4,000 ton/year (1,124 - 5,072 ton/y, 25th and 75th percentiles), where 11 ton/year (4.9 - 16 ton/y, 25th and 75th percentiles) is discharged with the treated wastewater and the remaining fraction measured to 3,100 ton/y (with 25th and 75th percentiles of 970 and 3,110 ton/y) is incorporated into the sludge (Figure 1). Approximately ¾ of the total microplastic mass load on the treatment plants are from the obtained concentrations accounted for. The lacking ¼ of the microplastic mass may simply be due to measurement uncertainties or other unresolved dynamics in the WWTP e.g. degradation of certain polymers.

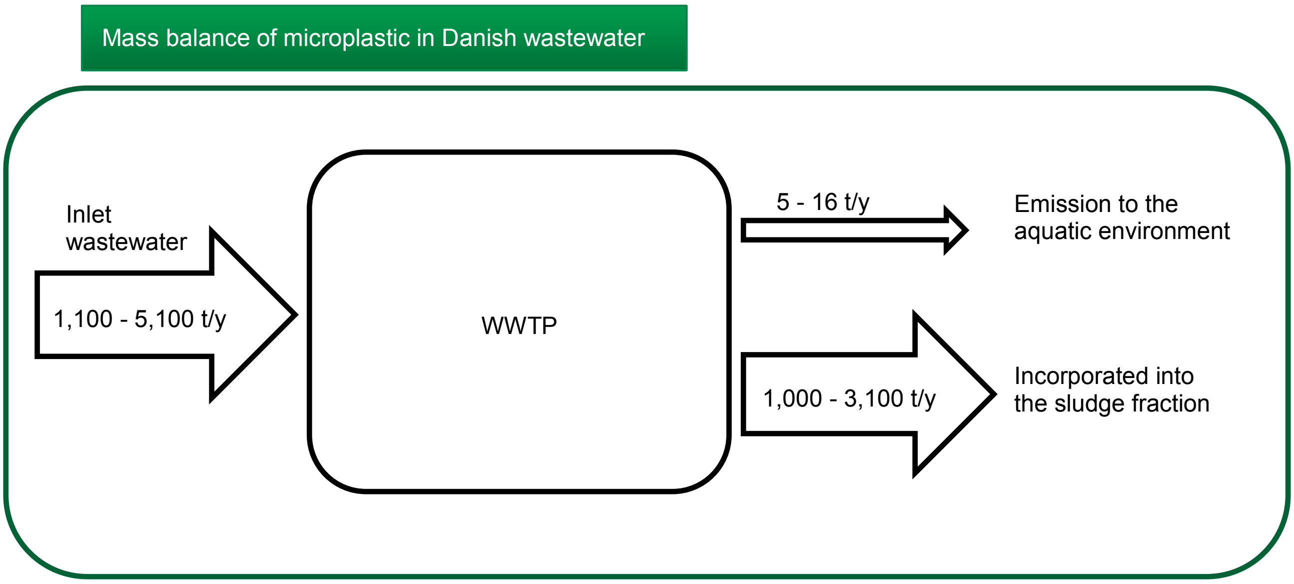


Figure 1. Mass balance of microplastic (size range 20-500 µm) in Danish wastewater. The rounded numbers of 25th and 75th percentiles are shown.

Assuming a total microplastic load to the Danish aquatic environment of 600-3,100 ton/year (size range 1µm - 5mm) as estimated by Lassen et al. (2015), the emission from the WWTPs to the aquatic environment of 5 -16 ton/y (size range 20-500 µm) represents as a worst case 3% of the total emitted microplastic to the Danish aquatic environment. It can therefore be concluded that discharge of treated wastewater from the municipal treatment plants has a minor role in terms of microplastic emission to the aquatic environment and that other sources such as stormwater runoff, combined sewer overflows, atmospheric deposition, and etcetera likely are more important sources.

Microplastic concentrations in agricultural soils

The concentration of microplastic in the soils was low and accounted between 0,0001 and 0,001% (w/w) of the soil. The median microplastic concentration in the investigated agricultural soils was 5.8 mg/kg soil (with 25th and 75th percentiles of 1.4 and 7.6 mg/kg soil), when sludge had been added as fertilizer and 12 mg/kg soil (with 25th and 75th percentiles of 4.4 and 14.9 mg/kg soil), when no sludge had been added to the soils. This means that higher concentrations of microplastic were found in soils where sludge had not been added as fertilizer. Considering the role of the sludge fertilizer in microplastic emission to agricultural soils it is estimated from the obtained results that sludge fertilization will increase the microplastic concentration of the soil by approximately 15 mg/kg soil (6.7-22 mg/kg, 25th and 75th percentiles), when assuming a tilling depth of 30 cm (i.e. the soil depth into which the sludge is mixed into). This indicates that sludge is just one of many sources of microplastic emission to the agricultural soils and further investigations are needed to understand the importance of various microplastic sources for accumulation in farmland soils e.g. windborne litter could be an important source.

The polymer types of the microplastic particles in wastewater, sludge and soil

The by far most abundant plastic material in the wastewater samples, both inlet and outlet, was polyamide/nylon, maybe originating from various forms of textiles, clothing and carpets. Polyethylene and co-polymers and zinc stearate coated particles were also detected in significant amounts, while polypropylene and PVC were detected in smaller proportions. The distribution of the different plastic polymers were more or less the same in the inlet and the outlet wastewater meaning that the treatment plant does not to any significant extent preferentially remove specific plastic polymers. Interestingly, the distribution of plastic polymers was different in the sludge-fraction, where polyethylene was the dominant plastic material followed by polyamide/nylon and polypropylene. This discrepancy between the wastewater and sludge could indicate that the anaerobic digestion process affects the plastic, either by breaking it down to particles too small to detect by the applied approach or by biological degradation. The latter is known to be possible for polyamide, but further investigations are needed to understand how and where these changes are occurring in the sludge and whether it is a matter of random variability as a consequence of the screening approach applied in the present study.

The dominant plastic material in soils that had not received sludge was polyethylene followed by polyamide/nylon and polypropylene, and it had more or less the same distribution as observed in the sludge. Polypropylene, the polymer only observed in relatively low abundances in the wastewater and sludge samples, was found to be the dominant polymer in the soils that had received sludge. This could indicate that polypropylene is more withstanding to disruption and degradation, but more samples need to be investigated to exclude random variability due to the small sample size investigated in the present screening study. Both polyethylene and polypropylene originate from a wide pallet of products, including packaging materials such as plastic bags, plastic films, plastic bottles, and so on. The study did not reveal a single rubber particle from tire abrasion (styrene butadiene co-polymers) even though tire abrasion is identified as the largest microplastic source released to the Danish environment (Lassen et al., 2015). The most likely reason is that such particles were smaller than the 20 µm, which were the lower size limit for detection in the present study.

Methods for evaluation of microplastic in wastewater, sludge and soil samples

The observations made in this study lead to the conclusion that when addressing the efficiency of wastewater treatment plants to retain microplastic, mass as the unit of measurement is significantly more reproducible than particle numbers. The number of particles is affected by physical breakdown processes, and this breakdown can result in increases in particle numbers without an increase in plastic mass. Hence, when applying only particle numbers for quantifying the efficiency of a treatment system, this system could in principle ‘produce’ microplastic because larger particles were broken down into smaller particles. On the other hand, when it comes to the impact of microplastic on aquatic fauna, the number of particles plays potentially a significant role. Hence microplastic mass should be used to assess treatment efficiencies and particle numbers should additionally be reported to support environmental impact assessment.

With the experience from the method development and optimisation of a valid approach for microplastic identification and quantification carried out in the present study, it is evident that microplastic results reported in literature should be carefully reviewed and the method used for detection of microplastic should be looked over before acknowledging the reported results. Light microscopy alone is unsuitable for investigations of microplastic in environmental samples and verification of the particle material as plastic is highly important. Therefore, we recommend that future investigations of microplastic in environmental samples should be conducted with either FT-IR or Raman spectroscopy methods. We further recommend that sampling methods and analytical methods for microplastic analysis are standardized to allow comparison between results of microplastic monitoring and investigations.

Konklusion og sammenfatning

Formålet med nærværende studie har været at evaluere danske renseanlægs rolle i udledningen af mikroplast til miljøet både i henhold til udledningsmængder og hvilke typer af plastikpolymerer, der udledes og hvis muligt, hvilke kilder disse plastikpolymerer kan stamme fra.

Prøver fra 10 renseanlæg (indløbs- og renset spildevand), slam fra 5 af disse renseanlæg og 10 landbrugsjorde (5 jorde som har fået tilført slam som gødskning og 5 jorde, som ikke har fået tilført slam) er blevet analyseret for indholdet af mikroplast i størrelsesordenen 20-500 µm med den mest avancerede metode til måling af mikroplast, der eksisterer i dag (Fourier Transformeret Infrarød Spektroskopi billedbehandling med Focal Plane Array, FT-IR). Denne metode muliggør både bestemmelsen af mikroplastkoncentrationerne i prøverne og identifikation af, hvilke plastpolymerer mikroplastpartiklerne består af.

Studiet er designet som et screeningsstudie af dansk spildevand og resultaterne er derfor et estimat af indholdet af mikroplast i dansk gennemsnits spildevand og indikationer for dansk spildevand generelt og dermed ikke spildevand fra specifikke renseanlæg.

Mikroplastkoncentrationer i spildevand og udledningen fra renseanlæg
I indløbsspildevandet blev medianen af mikroplastkoncentrationen estimeret til 1,3 10^5 partikler/L (størrelsesordenen 20-500 µm) svarende til 5,9 mg/L. Dette udgør en procent af totalindholdet af organisk materiale i indløbsspildevand, som typisk er i størrelsesordenen 320-740 mg COD/L. I renset spildevand var medianen af mikroplastkoncentrationen 5.800 partikler/L (størrelsesordenen 20-500 µm) svarende til 0,02 mg/L. Variationen i mikroplastkoncentrationen i indløbsspildevandet mellem de 10 undersøgte renseanlæg var relativ stor og lå mellem 13.000 og 442.000 partikler/L svarende til 0,2 og 30 mg/L.

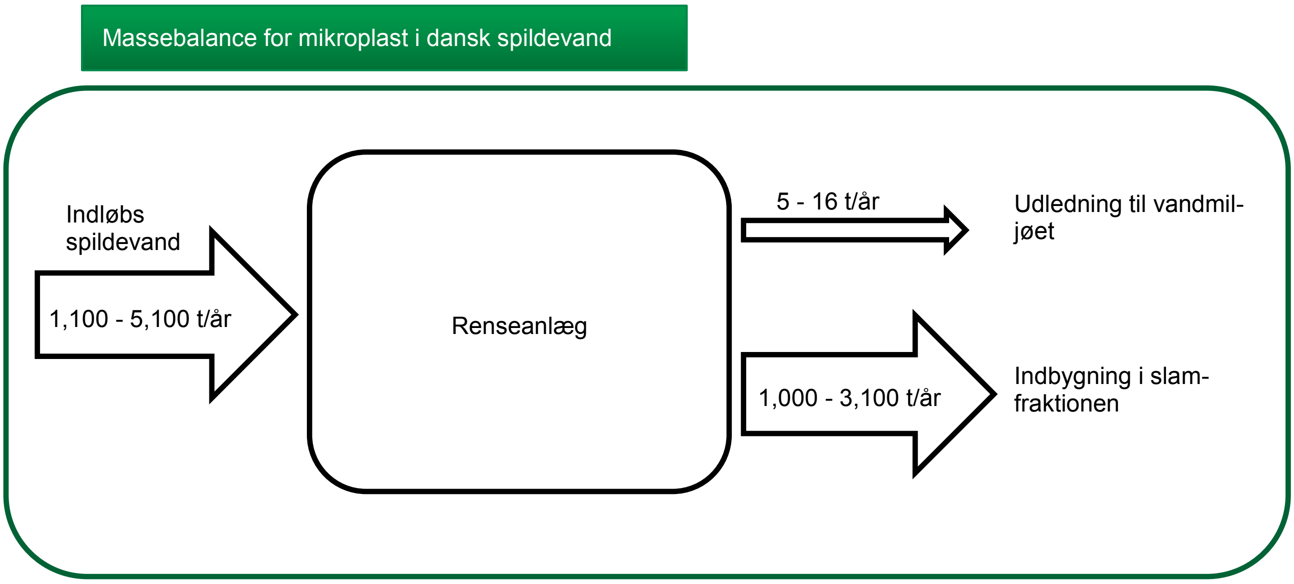
Den gennemsnitlige udledning af mikroplast til vandmiljøet fra et dansk renseanlæg er ud fra ovenstående beregnet til 0,3% (med 25 og 75% fraktiler på 0.0% og 0.7%) af massen af mikroplast, som kommer ind på renseanlægget.

Fra de opnåede resultater fra analysen af spildevandsprøverne er det dermed vist, at udledningen af mikroplast fra danske renseanlæg til vandmiljøet er lav i forhold til de mængder som ledes til renseanlæggene.

Mikroplastkoncentrationer i slam
Medianen af mikroplastkoncentrationen i spildevandsslammet blev kvantificeret til 169.000 partikler/g afvandet slam svarende til 4,5 mg/g afvandet slam, hvilket betyder at omkring 0,7% af det afvandede slam var mikroplast.

Massebalance for mikroplast på danske renseanlæg og udledningen til vandmiljøet
Hvis det antages, at mikroplast er inert på renseanlæg, så den totale masse af mikroplast, der kommer ind på renseanlæg er lig med summen af massen i renset spildevand og massen i spildevandsslammet. Dermed kan en grov massebalance opstilles for dansk spildevand.

Udfra de opnåede resultater er det estimeret, at den årlige tilførsel af mikroplast til alle danske renseanlæg er 4.000 tons/år (1.124 - 5.072 tons/år, 25 og 75% fraktiler), hvor 11 tons/år (4,9-16 tons/år, 25 og 75% fraktiler) bliver udledt med det rensede spildevand og den resterede fraktion målt til 3.100 tons/år (25 and 75% fraktiler på 970 og 3,110 tons/år) bliver indbygget i slamfraktionen (Figur 2). Omkring ¾ af den totale masse af mikroplast som kommer ind på renseanlæggene er dermed gjort rede for. Den resterede ¼ af massen af mikroplast kan enten skyldes måleusikkerheder eller andre uafklarede dynamikker i renseanlæggene, der påvirker mikropalsten eks. nedbrydning af specifikke polymerer.



Figur 2. Massebalance for mikroplast (størrelse 20-500 µm) i dansk spildevand. Tallene er afrundede værdier af 25 og 75% fraktiler.

Hvis det antages, at den totale udledning af mikroplast til det danske vandmiljø er i størrelsen 600-3.100 tons/år (størrelse 1µm til 5mm) som estimeret af Lassen et al. (2015), så udgør de 5-16 tons/år (størrelse 20-500 µm) udledt med renset spildevand i værste fald 3% af den totale udledning af mikroplast til dansk vandmiljø. Det kan derfor konkluderes, at renset spildevand fra renseanlæg udgør en mindre rolle i udledningen af mikroplast til det danske vandmiljø og at andre kilder som eksempelvis vejvand, overløb, atmosfærisk deponering og lignende formentlig er vigtigere kilder.

Mikroplastkoncentrationer i landbrugsjorde

Koncentrationen af mikroplast i de analyserede jorde var lav og udgjorde mellem 0,0001 og 0,001% (m/m) af jorden. Medianen af mikroplastkoncentrationen i de analyserede landbrugsjorde var 5,8 mg/kg jord (1,4-7,6 mg/kg jord, 25 og 75% fraktiler) i de jorde, som havde fået tilført slam som gødskning og 12 mg/kg jord (4,4-14,9 mg/kg jord, 25 og 75% fraktiler) i de jorde, hvor der ikke var tilført slam. Dette betyder, at der blev fundet højere koncentrationer af mikroplast i jorde, som ikke havde fået tilført slam eller andet organisk affald. Ved vurdering af slamudbringningens rolle i udledning af mikroplast til miljøet er det ud fra resultaterne estimeret, at slam på landbrugsjord vil øge jordens mikroplastkoncentration med omkring 15 mg/kg jord (6,7-22 mg/kg, 25 og 75% fraktiler), når det antages, at pløjedybden er 30 cm (den jorddybde som slammet blandes i). Dette er i samme størrelsesorden som den detekterede plastmængde i jorde uden tilført slam, hvilket indikerer, at slam kun er én af mange kilder til mikroplastudledning til landbrugsjord. Videre undersøgelser er nødvendige for at opnå forståelse for vigtigheden af forskellige mikroplastkilder i akkumuleringen af mikroplast i landbrugsjord eksempelvis er luftbåren affald måske en vigtig kilde.

Mikroplastpolymertyper i spildevand, slam og jord

Den mest udbredte plastpolymer i spildevandsprøverne, både i indløbs- og renset spildevand, var polyamid/nylon, som formentlig stammer fra forskellige typer af tekstiler, tøj og gulvtæpper. Polyetylen og co-polymerer og zink stearat belagte partikler blev også detekteret i signifikante mængder, mens polypropylen og PVC blev detekteret i mindre mængder. Fordelingen af de forskellige plastpolymerer var mere eller mindre den samme i indløbs- og renset spildevand, hvilket betyder, at renseanlæg ikke fjerner nogen polymertyper bedre end andre. Fordelingen af plastpolymerer var anderledes i slamfraktionen, hvor polyetylen var den mest udbredte polymer efterfulgt af polyamid/nylon og polypropylen. Denne forskel mellem spildevandet og slammet kunne indikere, at den anaerobe udrådning påvirker plastpolymererne enten ved at neddele dem til partikler mindre end 20µm og dermed for små til at blive detekteret i dette studie eller ved biologisk nedbrydning. Biologisk nedbrydning er vist for polyamid, men yderligere undersøgelser er nødvendige for at forstå, hvordan og hvor disse ændringer sker i slammet og om det i stedet skyldes tilfældig variation af screeningsstudiet.

Den dominerende plastpolymer i jord, som ikke havde fået tilført slam var polyetylen efterfulgt af polyamid/nylon og polypropylen. Denne type jord havde mere eller mindre den samme udbredelse af polymerer som observeret for slam. Polypropylen blev observeret i lav udbredelse i spildevand og slam, men var den dominerende plastpolymer i jord, som havde fået tilført spildevandsslam som gødskning. Dette kunne indikere, at polypropylen er mere modstandsdygtig mod neddeling og nedbrydning end de andre polymerer, men flere analyser og flere prøver er nødvendige for at udelukke tilfældig variation pga. den lille prøvestørrelse som er undersøgt i dette screeningsstudie. Både polyetylen og polypropylen indgår i mange plastprodukter, herunder emballage som plastposer, plastfilm, plastflasker ol. På trods af, at dækafslid er identificeret som den største kilde til mikroplastudledning til miljøet i Danmark (Lassen et al. 2015) er der i nærværende undersøgelse ikke fundet en eneste gummipartikel fra dækafslid (styren butadien co-polymerer). Dette skyldes højest sandsynligt, at sådanne partikler er mindre end 20µm, som var den mindste størrelse inkluderet i studiet.

Metoder til evaluering af mikroplast i spildevand, slam og jord

Det kan konkluderes fra observationerne i dette studie, at når effektiviteten af mikroplasttilbageholdelsen i renseanlæg evalueres, så er masse som enhed signifikant mere reproducerbar end partikelantal. Antallet af partikler er påvirket af fysisk neddeling og dette kan føre til flere partikler uden det øger massen af plast i renseanlægget. Derfor vil evalueringen af rensesystemer med partikelantal kunne konkludere, at der 'produceres' mikroplastpartikler i systemet fordi store partikler neddeles til små partikler. Antallet af partikler er dog potentielt vigtig ved evaluering af effekter på akvatisk fauna. Derfor skal massen af mikroplast anvendes ved evaluering af renseeffektiviteter, mens partikelantal ligeledes skal rapporteres til at understøtte evalueringen af den miljømæssige effekt.

Erfaringerne fra nærværende studies metodeudvikling og metodeoptimering til identifikation og kvantificering af mikroplast viser, at mikroplastresultater rapporteret i litteraturen skal tilgås med varsomhed og den anvendte metode brugt til mikroplastundersøgelser skal gennemgås grundigt før de opnåede resultater bør citeres. Lysmikroskopi som eneste metode er ikke tilstrækkelig til undersøgelser af mikroplast i miljøprøver og en verificering af, at partiklerne er plast er vigtigt. Vi anbefaler derfor, at fremtidige undersøgelser af mikroplast i miljøprøver anvender enten FT-IR eller Raman spektroskopi metoder. Vi anbefaler desuden, at prøvetagning og analytiske metoder til mikroplastanalyser bliver standardiserede således sammenligninger studier imellem er mulig.

1. Introduction

1.1 Microplastic in wastewater and wastewater treatment plants

Microplastic is often defined as plastic particles smaller than 5 mm. Microplastic is divided into primary microplastic and secondary microplastic, where primary microplastic is plastic particles smaller than 5 mm used in industry or commercial products e.g. in personal care products, raw materials for plastic production, rubber granules for artificial turfs etc.. Secondary microplastic is microplastic particles eroded from larger plastic objects e.g. tires, textiles, footwear, paints etc..

In connection with wastewater treatment plants (WWTPs) it is the microplastic which is of main interest, as plastic fragments larger than 5 mm are removed from the wastewater by the screens, which are the first step of the wastewater treatment.

The knowledge of microplastic in wastewater is very limited and the studies reported so far suffer from use of different methodologies, which makes the obtained results more or less incomparable (Pedersen and Winther-Nielsen, 2015). The methodological development of technologies to detect and quantify microplastic has taken a major leap the past years, which has revolutionised the research field of microplastic. However due to the method novelty, still no standardized method for investigation of microplastic exists and development of a general protocol for sample pre-treatment and for identification and quantification of microplastic in environmental samples needs to be established.

1.1.1 Fate of microplastic in wastewater treatment plants

Studies of microplastic in wastewater have shown that the majority of the microplastic entering WWTPs is ending up in the sludge fraction (90-95%; Magnusson and Wahlberg, 2014), while the fraction emitted to the aquatic environment from the WWTPs is mainly the smaller plastic particles. Magnusson and Wahlberg (2014) showed that 10-30% of plastic particles in wastewater in the sizes 20-300 µm were emitted through the outlet to the aquatic environment, while only 0-1% of the plastic particles larger than 300 µm was emitted to the receiving waters. Therefore, the flow of microplastic seems to follow the flow of sludge in a WWTP (Figure 3).

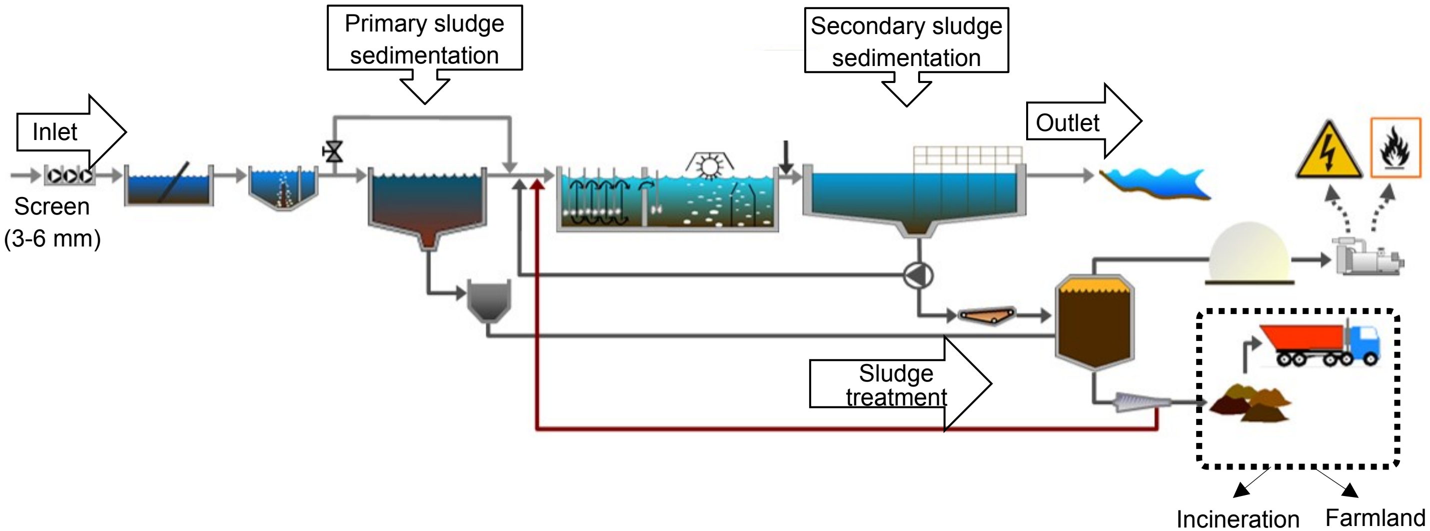


Figure 3. The flow of microplastic probably follows the flow of wastewater sludge in a wastewater treatment plant (white arrows).

In Denmark most sludge is used as fertilizer on farmland (77±5%; Sckerl, 2012), while the rest is incinerated. This means that most microplastic in wastewater is either combusted when sludge is incinerated or distributed on farm soils.

1.2 Objectives of the study

The objectives of the study are:

- To evaluate the role of Danish WWTPs in the emission of microplastic to the environment
- To evaluate the fate of microplastic entering the Danish WWTPs
- To evaluate the fate of microplastic in sludge distributed on Danish farmland
- To determine the main types of plastic polymers in the Danish wastewater and from this give an assessment of the possible sources of the microplastic in Danish wastewater

The study is designed as a screening study of Danish wastewater in general and the results are therefore an estimation of the occurrences of microplastic in average Danish wastewater meaning that the results are indicative for the overall Danish wastewater and not distinct WWTPs.

The method used for the microplastic detection is FT-IR imaging (Fourier Transformed Infrared Spectroscopy applying a Focal Plane Array), which not only allows the representation of the results in numbers of particles, but also the mass of microplastic and the determination of the plastic polymer each detected microplastic particle consists of. FT-IR imaging is currently the most advanced method available for microplastic investigations (Löder and Gerdts, 2015).

A representation of microplastic in terms of both particle quantity and mass has not previously been reported, but is necessary to allow relation of the results to other studies, for example to studies on microplastic sources and occurrence in the environment, e.g. Lassen et al. (2015). This way it becomes possible to evaluate the distribution of microplastic in the sample and to calculate mass balances, which further allows evaluation of degradation and erosion of the microplastic in the environment and in biotechnological installations such as WWTPs.

1.2.1 Definition of microplastic in the present study

There is currently no clear accepted definition of what microplastic is, but in literature most studies define microplastic as plastic fragments from 1µm to 5 mm. Microplastic is in the present study defined as polymers of a synthetic material in the size range smaller than 5 mm in all dimensions. The present screening study has investigated microplastic in the lower size range, namely 20-500 µm, and has not attempted to quantify microplastic particles above 500 µm.

2. Project design

2.1 Occurrences, sources and fate of microplastic in Danish wastewater treatment plants

A screening approach has been applied and the project was designed to evaluate as much of the Danish wastewater as possible in the given time frame. This was realised by investigating the wastewater of ten of the largest Danish WWTPs, thereby giving an estimation of the occurrence of microplastic in average Danish wastewater. The project has not been designed to give exact occurrences of microplastic at a given WWTP or to evaluate the variation of microplastic occurrences between specific WWTPs. Therefore, the obtained results are presented so that they cannot be traced back to the WWTP of their origin.

2.1.1 The wastewater treatment plants investigated

Wastewater and sludge samples were collected from ten of the largest WWTPs representing 26% of the total Danish wastewater volume (Table 1). Samples were collected at dry weather and the maximum of rain was 3 mm for 48 h before and during sampling (72 h in total). The dry weather criterion ensures that the samples are comparable. The occurrences of microplastic was analysed for the inlet and outlet wastewater from the 10 WWTP (20 samples in total) and occurrence of microplastic in sludge was analysed for 5 of these plants (five samples in total).

Table 1. Wastewater treatment plants (WWTP) included in the study and the volume of wastewater treated at each plant. Water volumes are from Miljøministeriet (2015).

WWTP	Water volume (1000 m^3/y)	Fraction of total Danish wastewater %
Lynetten	55.044	9%
Damhusåen	23.058	4%
Ejby Mølle	19.426	3%
Aalborg Vest	18.608	3%
Marselisborg	9.319	1%
Herning	9.197	1%
Vejle	9.032	1%
Kolding	8.651	1%
Fredericia	8.340	1%
Horsens	7.563	1%
Total	**168.238**	**26%**

2.2 Occurrences and sources of microplastic on Danish farmlands

The occurrences of microplastic on Danish farmlands were investigated by analysing five fields that never have received sludge as fertilizer and five fields that have received sludge as fertilizer within the past few years. From this the role of sludge in the microplastic emission to the soil environment was evaluated.

3. Methodology

Research of microplastic in the environment is an emerging field, and most of the research that has been done on this topic has been related to the marine environment. Often samples have been collected by dragging algae nets behind boats or collecting sand samples from beaches. Collected samples have in most cases been characterized visually by size and colour of the catch. This approach is reasonable for particles above, approximately 0.5 mm and especially where there are few other organic particles than plastic in the sample. However, even for such particles, visual inspection does not allow a characterization of plastic polymers.

3.1 Choosing the analytical method

The studies that have addressed smaller particle sizes and at the same time identified the plastic polymer are limited. The present study addresses the analytical quantification of 20-500 µm microplastic particles in raw wastewater, treated wastewater, wastewater sludge, and agricultural soils, and for these matrixes only one or two studies have applied comparable technologies. In order to study these sample types reliably, a method for sample preparation, sample concentration, and FT-IR imaging had to be developed and its validity assessed.

During the initial phase of method development and validation it became clear that microplastic analysis is not nearly as straight-forward as much of the literature leads to believe. This observation is in line with what a few other researchers have reported during the latter years, for example Löder and Gerdts (2015), who showed that many of the particles which by the light-microscope assisted eye might be identified as microplastic in reality are mineral particles such as quartz with a high diffraction index. A similar conclusion was made in the present study, clearly ruling out light microscopy as an analytical method for determining microplastic particles <500 µm in environmental samples. Hence, in the present study light microscopy was not a valid method for identification of particles of unknown material, size, shape, and origin. Light microscopy is, though, applicable when studying systems where distinct plastic particles, for example strongly colored or fluorescence particles, are spiked and then recovered.

In general, there is consensus that the most appropriate and effective method for identifying both size and material of microplastic particles is the use of Fourier Transform – Infrared (FT-IR) spectroscopy, preferable as an imaging system where a FT-IR spectroscope is combined with a microscope equipped with, for example, a Focal Plane Array (FPA), hereby allowing what typically is called FT-IR imaging (Vianello et al., 2013; Loder et al., 2015; Tagg et al., 2015). However, the number of such studies is very small, as the equipment is expensive and requires highly trained personnel (Rocha-Santos and Duarte, 2015). Consequently there is no standardized and generally accepted method on how an environmental sample is to be analysed for microplastic.

Another issue that became obvious through the study is that a FT-IR spectrum obtained for a particle should not simply be compared to a standard spectrum from a material database for polymer identification. While such comparison does assist the analysis, it leaves room for detection errors, i.e. there is a high risk of obtaining false positive or false negative particle material identifications. Instead, a spectral analysis has to be performed for each particle in a sample, to identify which chemical bonds are present and from this information conclude what material it is made off. Ignoring this latter step increases the risk of not detecting for example co-polymers and particles with deteriorated spectra. It also increases the risk for misinterpreting the material of the particle, for example so that natural organic particles are identified as plastic particles or that the type of plastic is misinterpreted. In this context it was our experience, that spectral analysis requires an understanding of the chemistry of polymers and personnel thoroughly trained in infrared spectral analysis.

Before analysing a sample on a FT-IR imaging system, irrelevant sample constituents must be removed and the microplastic concentrated. There are a limited number of studies that have addressed this in terms of making a sample suitable for FT-IR analysis, for example to extract microplastic from beach sand (Cauwenberghe et al., 2015) and also some studies on the preparation of wastewater samples (Mintenig et al., 2014). However, there is no clear consensus and no clearly defined method on how this should be done.

When it comes to assessing the uncertainty of a microplastic determination, similar to what is known from analytical chemistry, the knowledge is still more erratic. Most studies have simply ignored the issue of sampling and measurement uncertainty, and none have presented a systematic way to assess such uncertainty.

3.2 The methodology at a glance

The current study addresses microplastic particles in the size range 20-500 µm and all steps of the methodology are optimized towards this range. The method applied for detection of microplastic in an environmental sample is divided into 5 major steps:

1. Sample collection
   This step attempts to collect samples which are representative of the environmental system analyzed. In this study, 4 types of samples are collected: Raw wastewater, treated wastewater, wastewater sludge, and agricultural soils.

2. Sample preparation and concentration
   Ideally speaking, the purpose of this step is to remove all non-plastic particles and all other substances that might interfere with the following analytical steps. The methodology for the sample preparation differs between sample types and the sample preparation for wastewater, sludge and soil are quite different. The preparations do though all end up in similar products, namely a concentrate of particles that can be analysed on a FT-IR imaging system.

3. Sample analysis applying FT-IR imaging
   A sub-sample of the particle concentrate is transferred to a FT-IR imaging system and analysed: The sub-sample is placed on a microscope slide where it is illuminated by IR light. The spectrum of the transmitted (or reflected) IR light is analysed and a spectrum created, which is characteristic for the material of the investigated particle. The FT-IR imaging system scans the slide with a resolution of some micrometres and produces hereby a FT-IR spectral map of the scanned area. At the same time a traditional light microscope image is produced, which allows visual inspection of the same area of the slide as well as determination of the particle size. Figure 4 illustrates this principle where the upper picture is a visual map of the FT-IR scanned area shown in the picture below.

4. Interpretation of the infrared spectra applying spectral analysis techniques
   The map of FT-IR spectra is manually processed by spectral analysis. First a rough analysis is done, rejecting all particles that are of materials that cannot be plastic (for example inorganic particles). This leads to rejection of the majority of the particles as sample preparation is not able to remove all particles of natural origin. This is illustrated by the highlighted blue area of the spectrum shown in Figure 4. All plastic polymers must absorb IR light in the blue spectral range, and particles that do not absorb at this wavenumber are hence rejected up front. The possible plastic candidates then undergo manual spectral analysis to identify the nature of their chemical bonds and here through their composition.

5. Analysing results and calculating concentrations
   At the same time as the material of a particle was determined, its size was noted down. From this its volume was calculated and multiplied by the density of the plastic material, leading to the mass of the particle. Concentrations were then calculated by relating the area scanned on the FT-IR imaging system to the total volume taken into analysis.

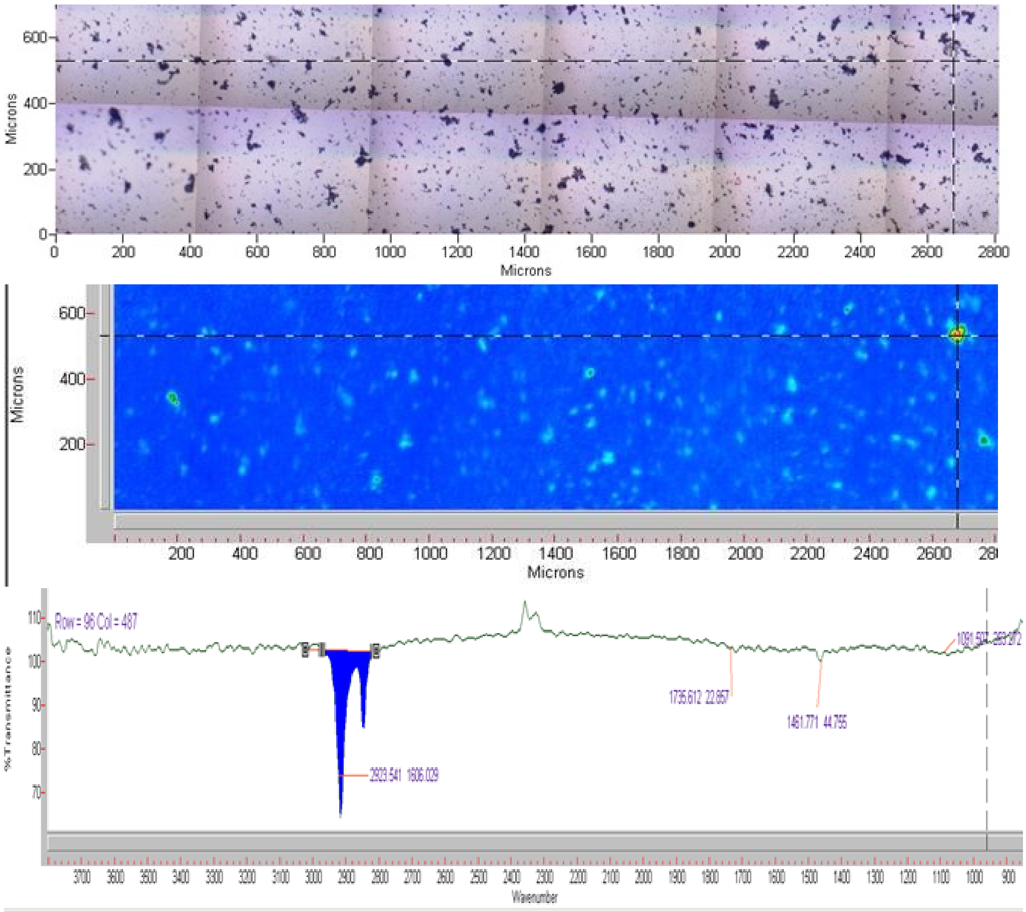


Figure 4. Searching for plastic particles on a 700x2800 µm section of an IR transmission window. The upper picture is a light microscopy image, the middle picture an IR heat map. The cross-mark of the two upper pictures shows the particle for which the spectrum in the lower picture is created.

Sampling volumes

For the raw wastewater, 1 L of sample was taken into work and pre-filtered over a 500 µm sieve. A 200 mL subsample of the filtrate was treated and the microplastic concentrated in 5 mL ethanol. For the treated wastewater, the water was filtered on site over 3 steel filters of 10 µm mesh size until these clogged. The amount of treated wastewater that could pass the filters before they clogged was between 4.1 and 81.5 L. The 3 filters where then treated and the microplastic concentrated in 5 mL of ethanol. For sludge, approx. 1 kg was collected and a subsample of 0.1 g was taken here from. The subsample was treated and the microplastic concentrated in 5 mL ethanol. For soil, cores of approx. 300 mL were collected, a sub-sample of 50 g treated and the microplastic concentrated in 5 mL ethanol. Depending on the type of sample, between 0.02 and 0.3 mL of the ethanol particle suspensions were transferred to the FT-IR imaging system for analysis.

Further details on sampling, sample preparation, FT-IR imaging, and spectral analysis are given in Appendix 1.

3.3 Method validation

Due to the nature of the targeted pollutants, methods can only be validated to a certain extent and uncertainties can only be estimated. There are several reasons for this:

- Dissimilar to analysis of a dissolved chemical compound in an environmental matrix, the targeted pollutant is not a well-defined substance. Plastic is a wide range of manmade polymers and also some natural polymers such as natural rubber. The boundary between what is plastic and what is not is a question of definition e.g. in conconnection with paint particles, composite materials, semi-synthetic fibers (such as viscose-rayon) and composite materials between such products and more traditional plastic polymers. In the present method we follow the definition by Lassen et al. (2015), namely plastics are solid materials formed from polymers of a mainly petrochemical origin, a definition that includes rubbers and paints.
- The issue that plastic is not a well-defined substance further leads to issues when applying standard analytical validation techniques, like spiking of a sample, to validate the method. What shall one spike with in order to validate methods?
- In addition to being made of a multitude of materials, plastic particles come in all shapes and sizes. This creates significant problems when validating analytical methods. So will, for example, the recovery of a plastic particle depend on all three parameters: material, size, and shape. In addition hereto, the sample preparation and concentration techniques will affect the plastic particles differently, depending again on material, size, and shape.

In a similar way, detection limits are problematic to define when identifying particles of a wide range of shapes, sizes and materials. Nevertheless, it is highly important to attempt to quantify the validity of the microplastic determination, its uncertainties, and its detection limit.

In the present study, the extraction method was validated by adding a known number of microplastic particles to raw wastewater and counting the recovered particles after sample preparation. The materials used were spherical polystyrene (PS) beads of 100 µm diameter, and high density polyethylene (HDPE) particles and styrene butadiene rubber (SBR) particles of 80-150 µm. The latter two were made by grinding down larger plastic pieces and sieving the material into appropriate sizes. The results showed recovery rates slightly below 100% for PS (78% ±17%) and HDPE (61% ±29%). For SBR it was slightly above 100% (120% ±38%) (Figure 5). The latter was deemed due to the SBR particles easily disintegrating into smaller particles. The difference between the recoveries was though not statistically significant.

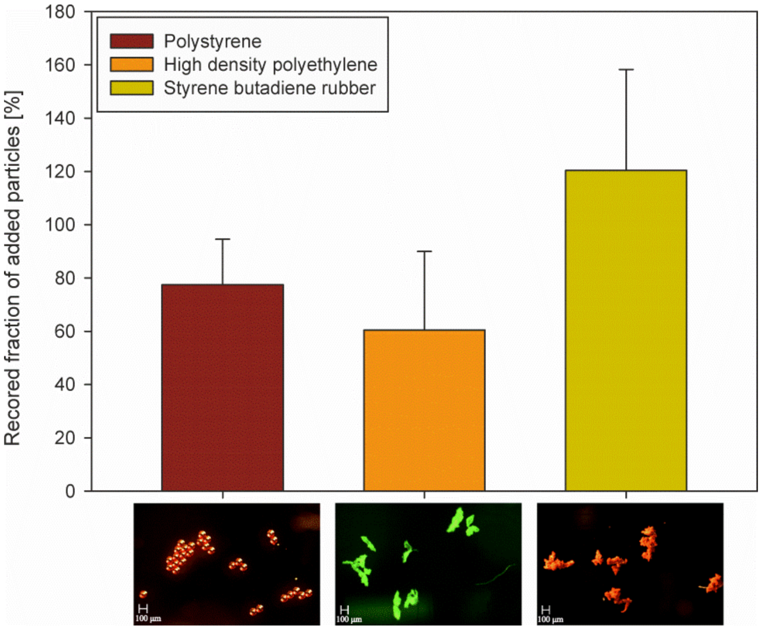


Figure 5. Recovery of microplastic particles added to raw wastewater

Detection limits

The detection limit of the method depends on the fraction of the sample scanned by the FT-IR imaging system. In the present study, we have scanned till at least 10 plastic particles had been positively identified, or till we had scanned an area of at least 4 mm^2 out of the 78 mm^2 on the microscope slide.

- For wastewater samples the detection limit was better than 4 µg/L.
- For treated wastewater the detection limit was better than 0.20 µg/L.
- For sludge the detection limit was better than 20 µg/g.
- For soil the detection limit was better than 0.04 µg/g.

Details on the method validation, detection limits and uncertainties are presented in Appendix 2.

4. Results and discussion

4.1 Microplastic in Danish wastewater treatment plants

This study addresses 10 wastewater treatment plants where microplastic in the size range 20-500 µm was analysed in the inlet and outlet wastewater. In addition, anaerobic digested sludge from 5 of these plants was analysed for microplastic in the same size range. The samples were all analysed for microplastic occurrence and which polymers the microplastic particles were made from.

4.1.1 Microplastic concentrations in raw and treated wastewater

The mass of microplastic in the raw and treated wastewater is shown in Table 2 while the corresponding numbers are shown in Table 3. Figure 6 and Figure 7 illustrate the numbers graphically. For both types of sample there is a quite large difference between the average and the median, indicating that the dataset was not normal distributed. In more concrete terms the discrepancy was caused by one treatment plant having much higher concentrations of microplastic than all the other WWTPs. It seems likely that this was caused by random variation in the sampling and the following analysis. Hence the median should be used and not the average. This issue is not observed for the numbers of particles.

The variability between the 10 treatment plants was quite large. The plant with the least microplastic in the raw wastewater had 0.22 mg/L while the one with the largest mass had 29.6 mg/L. The variability in statistical terms, i.e. 25th and 75th percentiles, is shown in Table 2. Correspondingly, the variability in number of particles was from 13,000 to 442,000 particles per litre, with the statistic variability shown in Table 3. This variability is probably due to real differences between the wastewater entering the treatment plants in combination with analytical uncertainty. A similar variability was seen for the treated wastewater. Such large variability is not uncommon for organic micropollutants where concentration ranges of several decades are often seen (Luo et al. 2014). However, the target of the present study was not to assess individual treatment plants but to assess the general median of Danish wastewater.

Table 2. Plastic mass in raw and treated wastewater. Average and median of 10 treatment plants as well as the 25th and 75th percentile of the dataset.

	Average	Median	25th percentile	75th percentile
Raw wastewater [mg/L]	8.0	5.9	2.2	10
Treated wastewater [mg/L]	0.034	0.016	0.0047	0.037

Table 3. Plastic particle numbers in raw and treated wastewater. Average and median of 10 treatment plants as well as the 25th and 75th percentile of the dataset.

	Average	Median	25th percentile	75th percentile
Raw wastewater [no./L]	127,000	86,000	70,000	130,000
Treated wastewater [no./L]	5,800	6,400	4,400	8,000

Typical wastewater holds 320-740 mg COD/L and 190-450 mg SS/L. The amount of microplastic in the size range 20-500 µm hence accounts for roughly 1% of the total organic matter of the raw wastewater and 2-3% of the total amount of suspended solids.

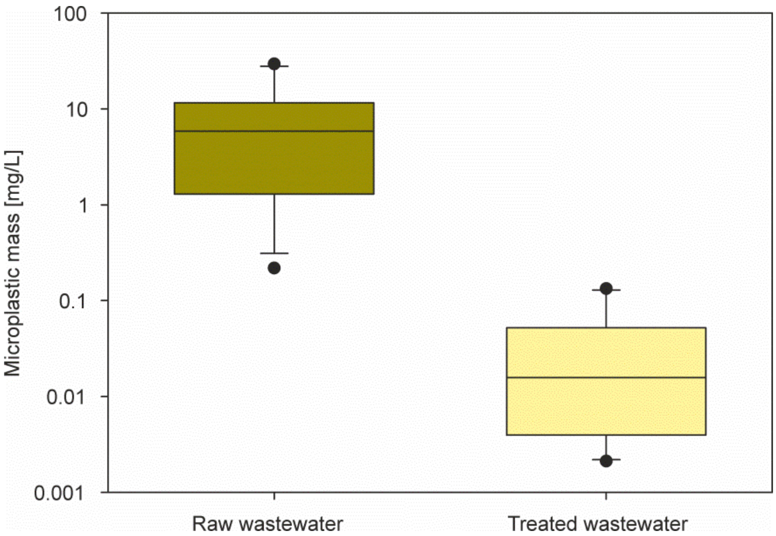


Figure 6. Microplastic mass concentrations in raw wastewater versus treated wastewater. Note the logarithmic scale on the y-axis.

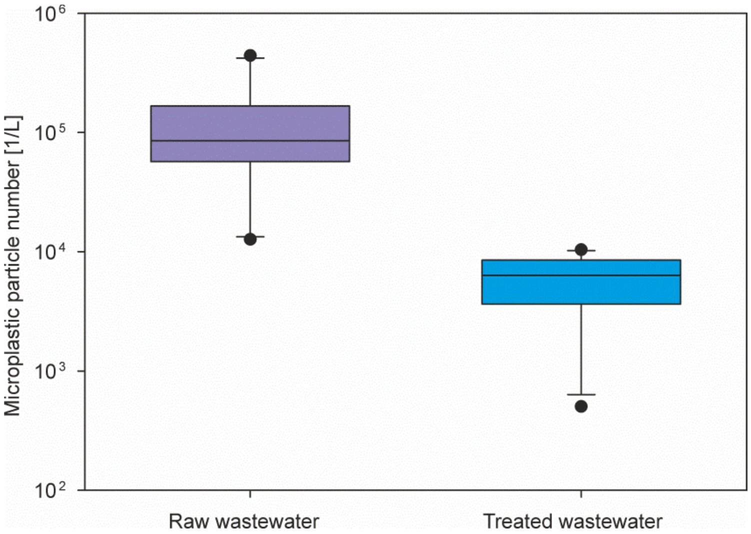


Figure 7. Microplastic particle number concentrations in raw wastewater versus treated wastewater. Note the logarithmic scale on the y-axis.

Table 2 and Table 3 tell that the treatment plants released 0.3% of the mass of microplastic they received and 7.4% of the particle numbers. The variability around these numbers is though high and the results should hence only be seen as indicative. The difference in efficiency between microplastics measured as numbers compared to microplastic measured as mass might be due to the primary settling tanks of the treatment plants having a larger effect towards larger particles compared to smaller particles. Another reason might be that larger plastic particles to some degree are broken down while in the treatment plant. Nevertheless, for plastic measured in terms of both mass and numbers, the overall efficiency to remove microplastic particles was high compared to most dissolved substances that occur in municipal wastewater.

4.1.2 Size distributions of microplastic particles

The median size of microplastic particles in the treated wastewater was approx. 20% smaller than in the raw wastewater. Figure 8 and Figure 9 show the size distributions of particles in the raw and treated wastewater, respectively. The figures show the largest and smallest measured diameter of each plastic particle as well as the average of the two diameters. The median of plastic particle size in the raw wastewater was 50 µm while it was 41.5 µm in the treated wastewater. The corresponding values for the 75-percentiles (i.e. the 75% of the smallest particles) were 65 µm in the raw wastewater and 55.9 µm in the treated wastewater. Looking at the shape of individual particles, the ratio between largest and smallest diameter of a particle differed slightly between the raw and treated wastewater, namely respectively 1.22 and 1.41.

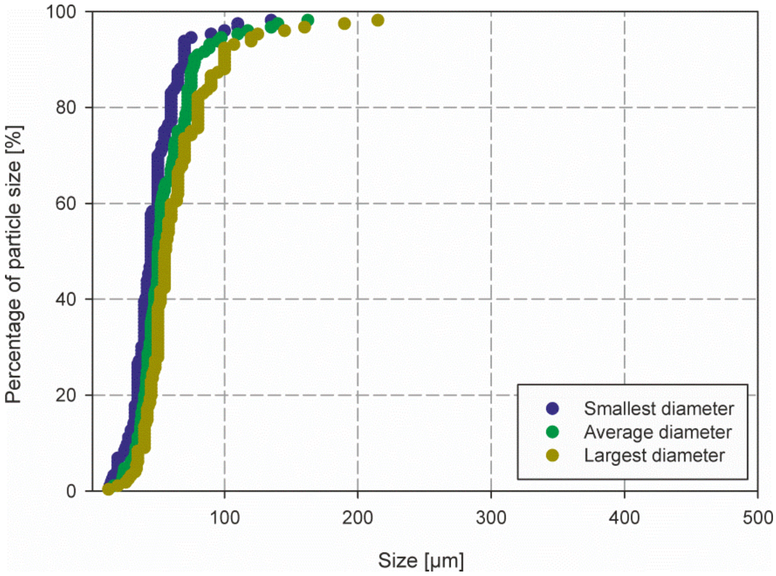


Figure 8. Size distribution of microplastic particles in the raw wastewater. Three values are given: The smallest measured diameter of a particle, the average of its two measured diameters, and its largest measured diameter.

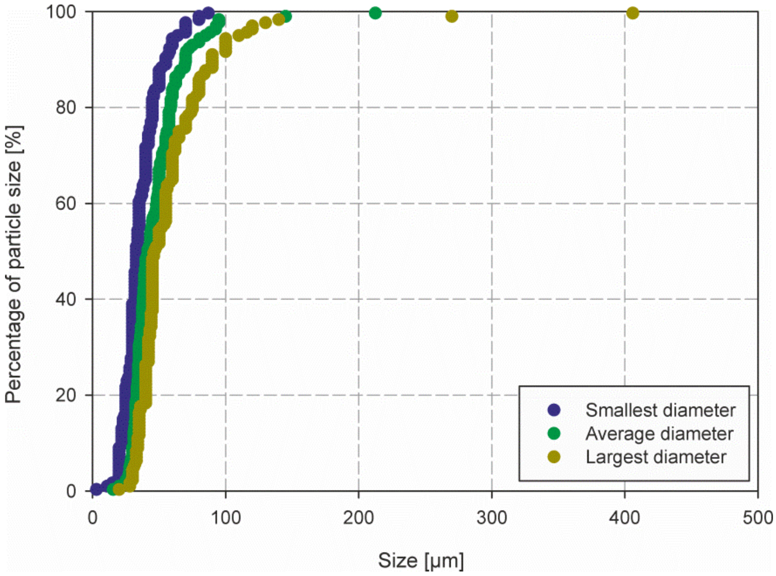


Figure 9. Size distribution of microplastic particles in the treated wastewater. Three values are given: The smallest measured diameter of a particle, the average of its two measured diameters, and its largest measured diameter.

Even though the difference in particle size was not substantial, the difference in mass is significantly more so because the volume of particles comes in the third power of their size. Figure 10 compares the particle masses for the raw and treated wastewater. The median particle mass in the raw wastewater was twice that of the treated wastewater (41 ng versus 20 ng). For particle masses less than approx. 7 ng there seems though to have been slightly more particles in the raw wastewater compared to the treated. The reason is unknown, but could relate to technical aspects of the treatment plants or to breakdown of particles that affect smaller particles more than larger ones. It could, though, also be a random artefact of analytical uncertainties.

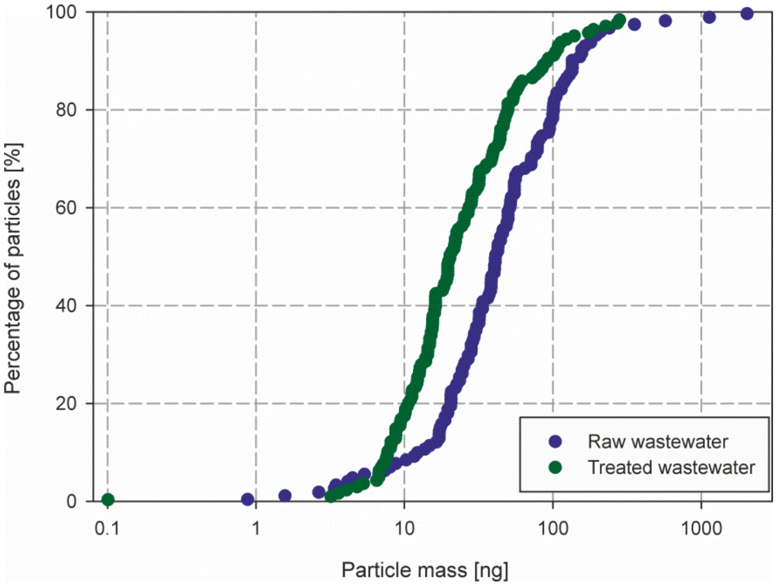


Figure 10. Mass distribution of microplastic particles in the raw and treated wastewater

The raw wastewater furthermore held some rather large particles that accounted for a significant fraction of the total microplastic mass. The 4 largest particles (Figure 10) account for 35% of all the plastic mass found in the raw wastewater samples.

The observations made in this study lead to the conclusion that when addressing the efficiency of wastewater treatment plants to retain microplastic, mass as the unit of measurement is significantly more reproducible than particle numbers. The number of particles is affected by physical breakdown processes, and this breakdown can result in increases in particle numbers without an increase in plastic mass. Hence, when applying only particle numbers for quantifying the efficiency of a treatment system, this system could in principle ‘produce’ microplastic because larger particles were broken down into smaller particles. On the other hand, when it comes to the impact of microplastic on aquatic fauna, the number of particles potentially plays a significant role. Hence microplastic mass should be used to assess treatment efficiencies and particle numbers should additionally be reported to support environmental impact assessment.

4.1.3 Microplastic concentrations in sludge

The mass and particle numbers found in digested sludge from 5 treatment plants are shown in Table 4 and Table 5. Digested wastewater sludge typically has a dry matter content of 25-30%, and the median of the measurements hence indicate that approx. 2% of the total dry matter content of the sludge was microplastic.

Table 4. Plastic mass in digested wastewater sludge. Average and median of 5 treatment plants as well as the 25th and 75th percentile of the dataset.

	Average	Median	25th percentile	75th percentile
Sludge [mg/g]	4.5	6.5	2.0	6.5

Table 5. Plastic particle numbers in digested wastewater sludge. Average and median of 5 treatment plants as well as the 25th and 75th percentile of the dataset.

	Average	Median	25th percentile	75th percentile
Sludge [no./g]	169,000	158,000	79,000	175,000

4.1.4 Mass balance of microplastic in wastewater treatment plants

A rough mass balance can be made on the inlet and outlet mass of microplastic. Assuming that microplastic is inert in the treatment plant, the total mass in the inlet must equal the sum of the mass in the sludge and in the discharged wastewater.

The total amount of wastewater entering the Danish treatment plants in the period 2012-2014 was 686 million m^3 per year (Naturstyrelsen, 2013; 2015a; 2015b). Assuming that the median concentration of dry weather microplastic concentrations in the raw wastewater are representative for the annual load on treatment plants, the corresponding microplastic load on the plants was 4,035 ton/year (25th and 75th percentiles: 1124 and 5072 ton/year). Hereof 11 ton/year (25th and 75th percentiles: 4.9 and 16 ton/year) was discharged with the treated wastewater to the receiving waters, leaving 4,024 ton/year not emitted to the aquatic environment. These numbers are subject to some uncertainty. One uncertainty relates to the samples representing dry-weather wastewater only and that concentrations during storm runoff most likely are different from those at dry weather. Another uncertainty is the analytical uncertainty of the study. The size of both these uncertainties is basically unknown.

The total amount of sludge produced in Denmark is approx. 132,600 ton-DM/year (Miljøstyrelsen, 2009). Digested sludge typically has a dry matter content of 25-30%, and the corresponding amount of dewatered sludge is hence approx. 480,000 ton-dewatered-sludge/year. Here of a median of 0.7% is microplastic. Applying the median value of the microplastic mass found in this study, this corresponds to a microplastic mass in the sludge of approx. 3,100 ton/year with 25th and 75th percentiles of 970 and 3,110 ton/year. Also this number is subject to uncertainty of an unknown magnitude. However, the mass of microplastic found in the sludge amounted to approx. ¾ of the microplastic in the inlet, which gives confidence in the number as the mass in the sludge must be equal to or less than the mass in the inlet.

Adding up the mass balance showed that approx. ¾ of the total microplastic load on the treatment plants could be accounted for. It is not known whether the lacking ¼ of the microplastic mass is simply due to measurement uncertainties, or if it is caused by degradation of the nylon particles in the digesters, as the proportion of nylon decreased significantly in the sludge-fraction (see further discussion in 4.1.6).

Lassen et al. (2015) estimated that some 2,000-5,600 ton/year of microplastic (size range 1µm - 5 mm) was discharged to sewerage and hence end up at wastewater treatment plants. Taking all the uncertainties of a literature-based assessment of microplastic loads into account, this finding is in good agreement with the values measured in the present screening study. There are though some significant discrepancies in what was predicted (not measured) by Lassen et al. (2015) in terms of polymer materials. Lassen et al. predicted that 1,600-2,500 ton/year of tire particles (styrene butadiene co-polymers) should be discharged to wastewater treatment plants. A likely explanation for these not being found in the wastewater is that they were below the size limit of the present study (20 µm). So did for example both Dall'Osto et al. (2014) and Mathissen et al. (2011) in their studies report the majority of particles from car tire abrasion between 10 and 100 nm. Nevertheless, the issue about the fate of particles created by care tire abrasion does lead to open and unsolved questions which should be addressed by future studies.

4.1.5 Microplastic loads on the aquatic environment

The literature study conducted by Lassen et al. (2015) had estimated that the total Danish emission of microplastic (size range 1µm - 5mm) to the aquatic environment is in the range 600-3,100 ton/year. Comparing this to the findings of the present study of approx. 11 ton/year (25th and 75th percentiles: 4.9 and 16 ton/year) (size range 20-500 µm) discharged with the treated wastewater, it can be concluded that treated wastewater from Danish municipal treatment plants constitute a minor fraction of the total amount of microplastic released to the aquatic environment.

4.1.6 The polymer composition of the microplastic particles

The by far most common plastic material in the raw wastewater was types of polyamide/nylon (**Fejl! Henvisningskilde ikke fundet.** and Figure 12). It seems reasonable to assume that a probable major origin for these plastics is various forms of textiles, clothing and carpets. Other types of plastic belong to the group of polyethylene and co-polymers (PE), polypropylene and co-polymers (PP), and PVC. PE is used in for example packaging like plastic bags, plastic films, plastic bottles, and so on. PP is also used in packaging as well as in for example textiles including cloth and carpets. PVC is more rigid than PE and PP and used in for example construction materials, non-food packing, and electrical cable insulations. In addition to PE, PP, and PVC, there was in a few samples found a high abundance of zinc stearate coated particles. Zinc stearate is strongly hydrophobic and used to coat a range of plastics to enhance the product’s behaviour. When a plastic particle is coated with zinc stearate it is not possible to identify the underlying plastic material but zinc stearate coating can occur on most popular household appliance plastics such as PP, PE, and PS (polystyrene).

The total distribution of plastic polymers in raw wastewater with respect to the identified mass is shown in **Fejl! Henvisningskilde ikke fundet.** while the distribution with respect to the number of particles found of each material is shown in Figure 12. Polyamide/nylon was dominant both in terms of particle numbers and particle mass. Comparing the two figures indicates that the relative distribution of polymers on particle mass and number differed somewhat for polymers other than polyamide/nylon. Especially for zinc stearate coated particles indicating that these were mainly small particles. However, the number of particles behind those fractions is comparatively small and it cannot be excluded that this variation is due to random variability.

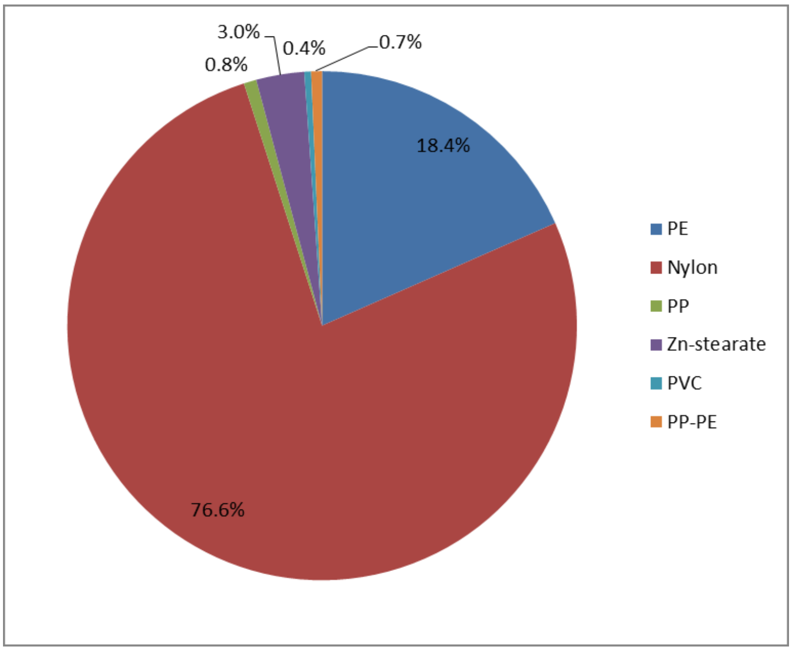


Figure 11. Distribution of the mass of plastic polymers in raw wastewater. A total of 181 plastic particles were identified with respect to their material.

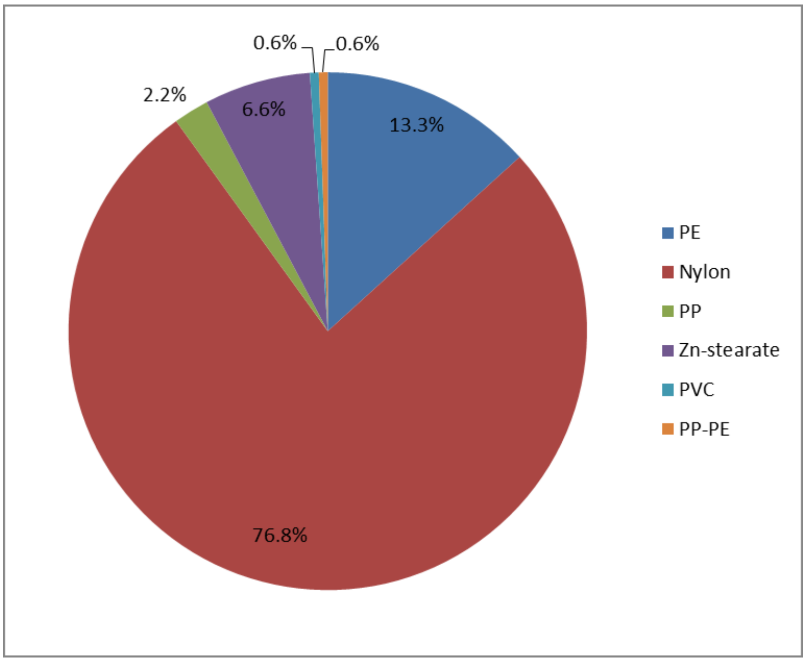


Figure 12. Distribution of the particle numbers on identified plastic polymers in raw wastewater. A total of 181 plastic particles were identified with respect to their material.

Figure 13 and Figure 14 show the similar data for the treated wastewater. Also here, it was polyamide/nylon that dominated the picture in terms of polymers, followed by PE material types. Furthermore, comparing the composition between inlet and outlet in the investigated size range of microplastic particles it is seen that the treatment plant does not to any significant extend preferentially remove specific plastic polymers, in other words, the removal efficiency for all polymers are more or less the same. However in the treated samples PVC was not found. Whether this is due to the circumstance that a limited number of outlet samples has been analysed, or due to preferential removal of particles, is unknown.

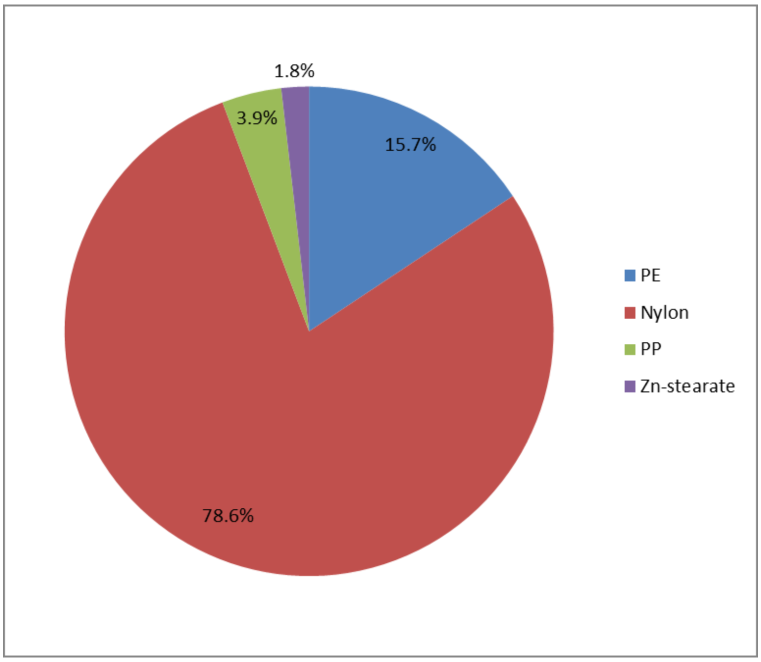


Figure 13. Distribution of the mass of plastic polymers in treated wastewater. A total of 150 plastic particles were identified with respect to their material.

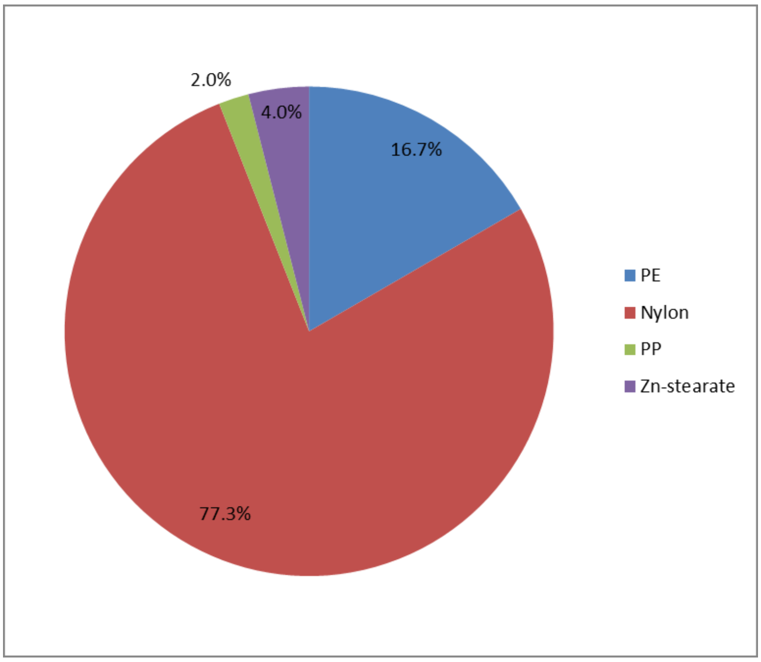


Figure 14. Distribution of the particle numbers on identified plastic polymers in treated wastewater. A total of 150 plastic particles were identified with respect to their material.

Figure 15 and Figure 16 show the distribution of plastic polymers found in the digested wastewater sludge. The total number of microplastic particles identified was comparatively low (29). This was partly due to fewer samples being analysed compared to raw and treated wastewater and partly due to time limitations of the study. Of the 29 particles, PE was the dominating material followed by nylon which was significantly lower in distribution than observed for the wastewater samples. This could indicate that the anaerobic digestion process affects the plastic, either by breaking it down to particles too small to detect by the applied approach, or by biological degradation of nylon, which decreases significantly in distribution of the plastic particles in the sludge-fraction as compared to the wastewater. Bacterial digestion of nylon has been shown in environments with sufficient nylon by bacteria producing a nylon oligomer hydrolase (Gautam et al, 2007). Whether or not this is the cause of the discrepancy between the mass distributions in the raw wastewater and the digested sludge is not known and needs further investigation. Due to a limited sample size, it cannot be excluded that the observed differences are due to random variability.

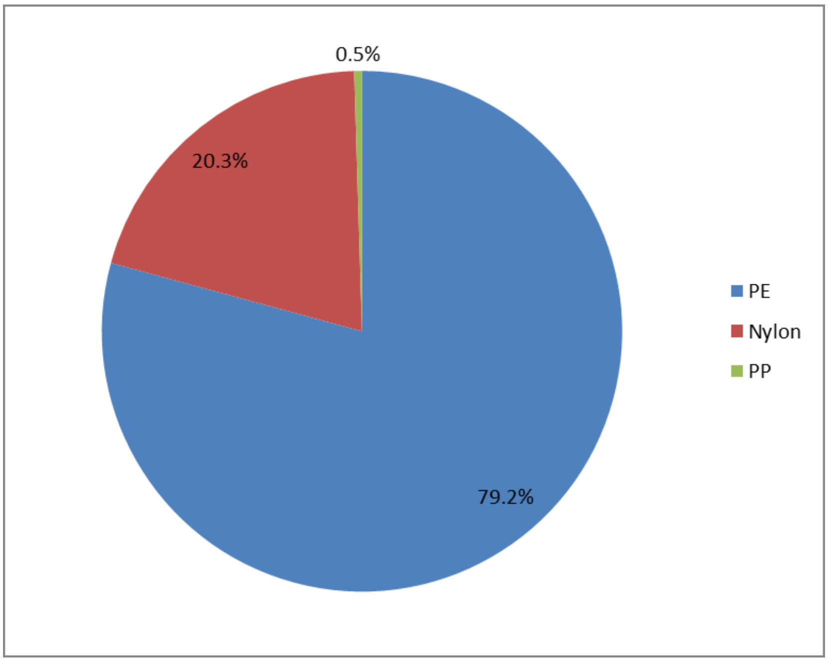


Figure 15. Distribution of the mass of plastic polymers in digested wastewater sludge. A total of 29 plastic particles were identified with respect to their material.

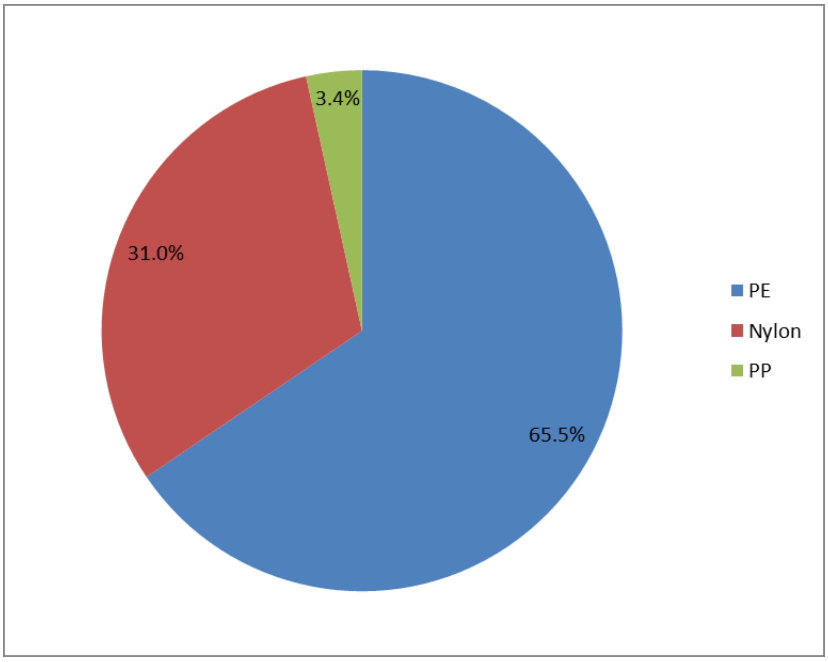


Figure 16. Distribution of the particle numbers on identified plastic polymers in digested wastewater sludge. A total of 29 plastic particles were identified with respect to their material.

It is noteworthy that the study did not reveal a single rubber particle from tire abrasion even though a literature based study on microplastic releases in Denmark had indicated tire abrasion as the largest microplastic source released to the Danish environment (Lassen et al., 2015). The most likely reason is that such particles were smaller than the 20 µm, which were the lower size limit of the present study. Mintenig et al. (2016) applied a comparable FT-IR technique on treated wastewater from German WWTPs. Comparing the identified plastic polymers to those found in that study, some significant differences were seen. For particles <500 µm, they found polyethylene (PE) to dominate the samples (40% of particle numbers), followed by polyvinyl alcohol (16%) and then polyamide (PA, what we have termed nylon or polyamide/nylon in this report) and polystyrene (PS, 8% of particle numbers each). However, similarly to the present study, Mintenig et al. (2016) did not find rubber (styrene-butadiene co-polymers) in any of their samples – neither in the smaller fraction (20-500 µm), which they measured by an FT-IR imaging technique similar to the present study, or in the larger size fractions (>500 µm), which they measured by FT-IR-ATR similar to what was done in the present study to validate spectra from car tires (Appendix 2.4).

4.2 Occurrences and sources of microplastic on Danish farmlands

Microplastic was measured in 5 samples from farmlands that had received sludge as fertilizer and 5 farmlands that had not. The latter soils have furthermore never received any other organic waste product of non-farming origin. The concentration of microplastic in these samples is shown in Table 6 and Table 7. The number of plastic particles in both types of soils was low and the soils were rather comparable with respect to the microplastic concentrations found. The soils that had not received sludge had twice the microplastic content than the soils that had not. This difference is most likely due to uncertainties in sampling and measurement as the total number of detected particles was rather low (13 and 24 particles in the soil with and without sludge, respectively).

Table 6. Plastic mass in farmland soils. Average and median of 25 soils as well as the 5th and 75th percentile of the dataset.

	Average	Median	25th percentile	75th percentile
Soils with sludge [mg/kg]	6.2	5.8	1.4	7.6
Soils without sludge [mg/kg]	51	12	4. 4	15

Table 7. Plastic particle numbers in farmland soils. Average and median of 5 soils as well as the 25th and 75th percentile of the dataset.

	Average	Median	25th percentile	75th percentile
Soils with sludge [no./kg]	82,000	71,000	29,000	143,000
Soils without sludge [no./kg]	236,000	145,000	53,000	436,000

The concentration of microplastic in the soils are deemed low, namely around 10 mg/kg. Such concentration is comparable to, for example, the background concentrations of heavy metals in Danish soils (lead, copper, zinc, chromium, nickel, or cobalt found; By- og Landskabsstyrelsen (2010)).

The composition of the plastic material found in the soils is shown in Figure 17 and Figure 18. The dominant polymers were PE and PP. Both of these polymers can origin from a wide pallet of materials, including packaging materials applied for agricultural purposes. The soils that had received sludge had both of these polymers in significant concentrations while the samples that had not received sludge only had a low concentration of PP. Nylons was present in relatively low concentration in both samples as compared with the wastewater and sludge samples. The high proportions of PP in soils with sludge may indicate that PP has a higher persistence to disruption and degradation than the other plastic polymers; this however needs further investigations and a larger sample size to further evaluate.

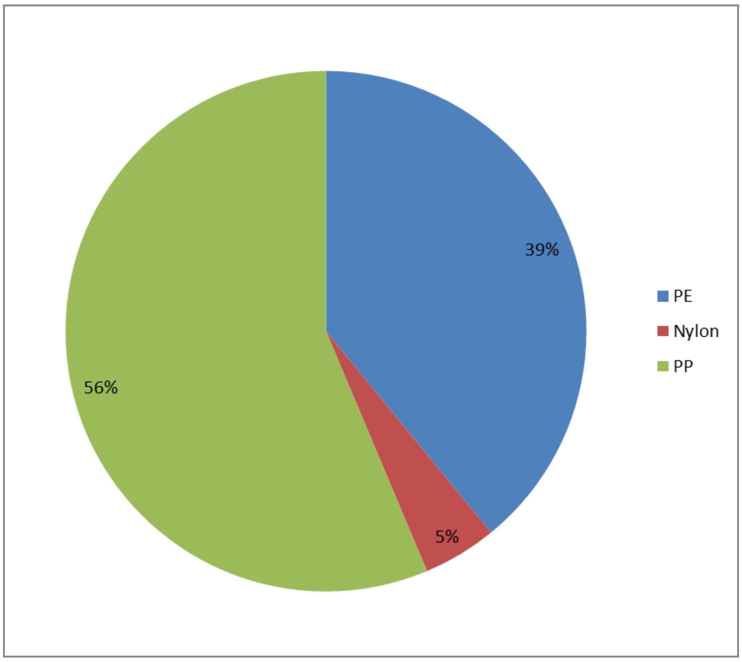


Figure 17. Distribution of the mass of plastic polymers in soils that had received sludge. A total of 13 plastic particles were identified with respect to their material.

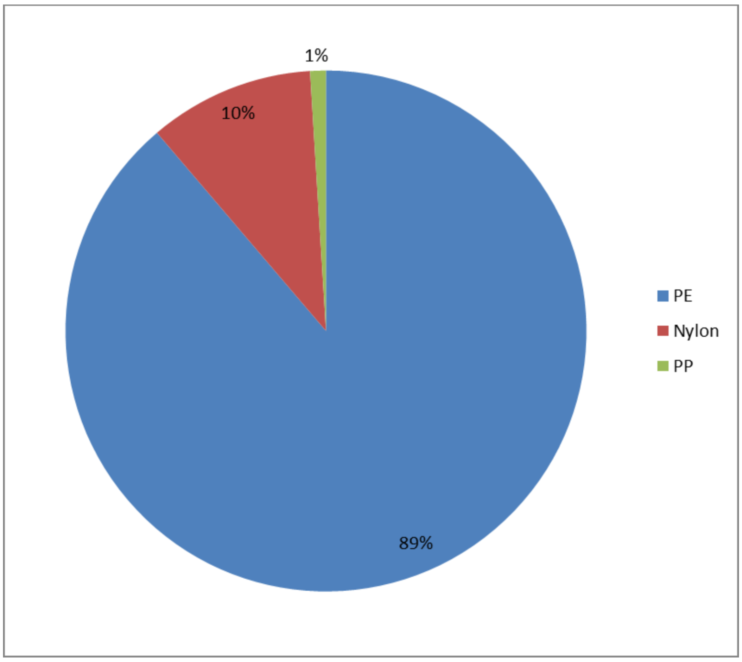


Figure 18. Distribution of the mass of plastic polymers in soils that had not received sludge. A total of 24 plastic particles were identified with respect to their material.

4.2.1 The impact of wastewater sludge on agricultural soil

The amount of wastewater sludge that can be applied on Danish agricultural soil corresponds to 90 kg of phosphorous per hectare calculated as an average over 3 years. This typically means that farmland will receive one load of sludge every 3 years. Assuming average values of dry matter content and phosphorous content as reported by Miljøstyrelsen (2013), these 90 kg of phosphorous per hectare correspond to approx. 10.4 ton of dewatered sludge per hectare. Assuming the microplastic content found in the present study (Table 4) and assuming a tilling depth of 30 cm (i.e. the soil depth into which the wastewater sludge is mixed) leads to the one batch of sludge applied every 3rd year to increase the microplastic concentration of the soil by approx. 15 mg/kg (25th and 75th percentiles: 6.7 and 22 mg/kg). In other words, one load of sludge contributes by approximately the same amount as found as background concentration in the soils that had not received sludge (Table 7). This estimate is rather rough and not a true mass balance, and there are significant uncertainties in both the estimate on microplastic occurrence in soils that have not received sludge and on microplastic in sludge. Nevertheless, the estimate indicates that while sludge application does contribute to the microplastic content of farm lands, it is not the only source of significance to microplastic in agricultural soils.

5. Perspectives

The results from the present screening study show that the emission of microplastic from Danish WWTPs to the aquatic environment is minor compared to the total load on the treatment plants. It represents at worst case approximately 3% of the total mass of microplastic released to the Danish aquatic environment. However, it has to be stressed that the results from this study is only indicative as the investigation has been a screening study and only addressing the size range 20-500 µm. Deeper sampling and analysis at each WWTP with coverage of the full range from 1 to 5000 µm is necessary to get a thorough understanding of the variation between WWTPs and to obtain statistically conclusive results.

Tertiary filter technologies are available on the market to further minimize the microplastic emission to the aquatic environment from the WWTPs. However, due to the already high removal efficiency of the wastewater treatment technologies routinely implemented in Denmark, other aspects of the urban sewerage system are likely more important with respect to discharge of microplastic into the aquatic environment. For example microplastic particles discharged via combined sewer overflows, via misconnected wastewater or via stormwater discharges.

The future focus on microplastic in wastewater is recommended to be directed to the sludge fraction as almost all microplastic mass entering the Danish wastewater treatment plants ends up in the sludge (>99%) and thus the farmlands where sludge is used as fertilizer. This study further indicates that the impact of microplastic in sludge on sludge-fertilized farmlands is low and that other sources might be of significance for the microplastic found in farmlands. There are, though, a number of open questions in this respect and future investigations are needed to further investigate other sources of microplastic in Danish soils and whether or not the plastic particles accumulate in the soil over time.

The sources of the detected plastic polymers are difficult to identify from the present study, but future investigations should look into the possible origins of polyamide/nylon, polyethylene and polypropylene, which were the most dominant polymers detected in the samples. The most abundant polymer in the wastewater was polyamide/nylon, which most probably originates from textiles, clothing and carpets, while polyethylene and polypropylene probably originates from different types of packaging or similar. The lack of detected rubber particles from tires must be further investigated. While it has never been identified in any study addressing microplastic polymers in wastewater samples, it has in mass balance based literature studies been identified as an abundant microplastic pollutant. Plastic from personal care products was not detected, which verifies previous reports that it contributes insignificantly to the total emission of microplastics in Denmark (0.2%, Lassen et al., 2015).

Some plastic polymers may be biologically degradable under the right conditions e.g. nylon and polyurethane. This agitates for development of special adapted treatment technologies of wastewater sludge, where the optimal conditions for biological or thermal degradation ensure a minimal emission of microplastic to the terrestrial environment. Further investigations should moreover evaluate the degree of plastic degradation in the terrestrial environment.

With the experience from the method development and optimisation of a valid approach for microplastic identification and quantification carried out in the present study, it is evident that microplastic results reported in literature should be carefully reviewed and the method used for detection of microplastic should be looked over before acknowledging the reported results. We have learned that light microscopy alone is unsuitable for investigations of microplastic in environmental samples and verification of the particle material as plastic is highly important. Therefore, we recommend that future investigations of microplastic in environmental samples should be realised with either FT-IR spectroscopy methods and/or possibly Raman spectroscopy methods. We further recommend that sampling methods and analytical methods for microplastic analysis is standardized to allow comparison between results of microplastic monitoring and investigations.

Acknowledgement

This investigation has only been possible due to the very friendly and collaborative personnel at the 10 investigated WWTPs, Damhusåen, Ejby Mølle, Fredericia, Herning, Horsens, Kolding, Lynetten, Marselisborg, Vejle and Aalborg West. We deeply thank their understanding and patience in connection with the sample collection.

References

By- og Landsskabsstyrelsen (2010). Kemiske stoffer – Vurdering af stoffer i forhold til farlighed i grundvandet. By- og landskabstyrelsen, Miljøministeriet, ISBN: 978-87-92617-87-3

Dall'Osto M, Beddows DCS, Gietl JK,. Olatunbosun OA, Yang X, Harrison RM (2014). Characteristics of tyre dust in polluted air: Studies by single particle mass spectrometry (ATOFMS). Atmospheric Environment 94_ 224-230

Gautam R, Bassi AS, Yanful EK (2007). A review of biodegradation of synthetic plastic and foams. Applied Biochemistry and Biotechnology, 141(1): 85–108

Lassen C., Hansen S.F., Magnusson K., Norén F.,. Hartmann N.I.B, Jensen P.R., Nielsen T.G. and A. Brinch (2015) Microplastics – Occurrence, effects and sources of releases to the environment in Denmark. The Danish Environmental Protection Agency. Environmental project No. 1793.

Löder MGJ, Kuczera M, Mintenig S, Lorenz C, Gerdts G (2015). Focal plane array detector-based micro-Fourier-transform infrared imaging for the analysis of microplastics in environmental samples. Environmental Chemistry, 12(5): 563-581

Löder MGJ, Gerdts G (2015).Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal. Chapter 8 of Marine Anthropogenic Litter, Editors: Bergmann M, Gutow L, Klages M. ISBN 978-3-319-16509-7

Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, Liang S, Wang XC (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of the Total Environment, 473–474: 619–641

Magnusson K. and C. Wahlberg (2014) Mikroskopiska skräppartiklar i vatten från avloppsreningsverk. IVL Svenska Miljöinstitutet.

Mathissen M, Scheer V, Vogt R, Benter T (2011). Investigation on the potential generation of ultrafine particles. Atmospheric Environment 45: 6172-6179

from the tireeroad interface

Miljøstyrelsen (2009). Spildevandsslam fra kommunale og private renseanlæg i 2005. Orientering fra Miljøstyrelsen Nr. 3

Miljøstyrelsen (2013). Livscyklusvurdering og samfundsøkonomisk analyse for anvendelse af spildevandsslam. Miljøprojekt nr. 1459, 2013

Mintenig S, Int-Veen I, Löder M, Gerdts G (2014). Mikroplastik in ausgewählten Kläranlagen des Oldenburgisch- Ostfriesischen Wasserverbandes (OOWV) in Niedersachsen. Probenanalyse mittels Mikro-FT-IR Spektroskopie. Abschlussbericht. Report for Oldenburgisch-Ostfriesischer Wasserverband (OOWV) and Niedersächsischer Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz (NLWKN)

Mintenig SM, Int-Veen I, Löder MGJ, Primpke S, Gerdts G (2016). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Research, doi: 10.1016/j.watres.2016.11.015.

Murakami S. (2009). Points to Note in Rubber Analysis: Black Rubber. Tokyo: Shimadzu. Retrieved from http://www.shimadzu.com/an/FT-IR/support/tips/letter11/rubber.html

Naturstyrelsen (2013). Punktkilder 2012.

Naturstyrelsen (2015a). Punktkilder 2013.

Naturstyrelsen (2015b). Punktkilder 2014.

Pedersen B.M. and M. Winther-Nielsen (2015), Mikroplast i spildevand fra renseanlæg, litteraturindsamling. DANVA rapport Maj 2015.

Rocha-Santos T, Duarte AC (2015). A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC Trends in Analytical Chemistry, 65: 47–53

Sckerl S.Aa. (2012) Historisk stor andel af spildevandsslam til jordbrug, Teknik & Miljø, Februar 2012 p. 54-55.

Tagg AS, Sapp M, Harrison JP, Ojeda JJ (2015). Identification and Quantification of Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging. Analytical Chemistry, 87(12): 6032-6040

Thompson RC et al (2004) Science, 304, p. 838-.

Van Cauwenberghe L, Devriese L, Galgani F, Robbens J, Janssen CR (2015).Microplastics in sediments: A review of techniques, occurrence and effects. Marine Environmental Research, 111: 5-17 SI

Vianello A, Boldrin A. Guerriero P, Moschino V, Rella R. Sturaro A, Da Ros L (2013). Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuarine, Coastal and Shelf Science, 130: 54-61

Appendix 1. Analytical method

Appendix 1.1 Sampling

The risk of contamination with plastic in the sampling procedure was minimized by using glass and metal equipment. When equipment with plastic could not be avoided the contamination risk was evaluated and when possible the contaminating plastic was subtracted from the final results.

Raw wastewater

Raw wastewater was collected at three independent sampling events at each of the ten wastewater treatment plants (Table 1) giving a total of thirty raw wastewater samples. The samples were flow proportional 24 h samples collected in the inlet wastewater stream with the auto samplers of the treatment plants. The samples were collected at dry weather and the maximum of rain allowed was 3 mm 48 h before and during the sampling, 72 h in total.
The auto samplers have plastic tubing and the wastewater was collected in big plastic bottles and after 24 h, 1 litre of the collected wastewater was transferred and stored in a glass jar. The amount of contamination with microplastic during the sampling procedure was assumed to be minimal compared to the microplastic present in the raw wastewater.

Treated wastewater

Treated wastewater was collected at three independent sampling events at each of the ten wastewater treatment plants (Table 1) giving a total of thirty treated wastewater samples. The samples were collected by filtration of outlet water through 10 µm stainless steel filters until the filter clogged. To ensure enough particles for investigation three filters were clogged at each sampling event. The volume of treated wastewater necessary to filter differed from plant to plant (0.5-108 litres per filter). The samples were collected at dry weather and the maximum of rain allowed was 3 mm 48 h before sampling.
All equipment used to collect treated wastewater was of glass and metal and no risk of contamination with microplastic from the sampling procedure is expected.

Sludge

Sludge samples were collected at the final dewatering unit after the sludge digestion at the ten investigated wastewater treatment plants (Table 1). The sludge was collected at two independent sampling events giving a total of twenty sludge samples of about 1 kilogram. The period between the two sampling events were longer than the sludge age at the specific treatment plants plus the sludge retention time in the digester, thereby ensuring that the sludge samples were indeed true biological replicates.
All equipment used to collect the sludge samples was of glass and metal and no risk of contamination with microplastic from the sampling procedure is expected.

Soil from farmlands

Soil samples were collected in the early spring shortly after the frost had left the soil.
Four soil samples were taken from each of 5 fields that had received sewage sludge and another 4 soil samples were taken from each of 5 other fields that had never received sewage sludge as fertilizer. The 2x5 fields were all geographically close to each other in Northern Jutland. The 4 soil samples from each field were taken approx. 100 m apart and at least 20 m into the field. The samples were collected as columns with a planting shovel to about 15 cm depth

and 8 cm diameter. Soil from each sample was thoroughly mixed before taking a subsample for analysis.

Appendix 1.2 Analysis of microplastic in wastewater and soil

Sample preparation and concentration for micro-FT-IR image analysis

The sample preparation and concentration has the objective to create a concentrate of plastic particles suitable for the subsequent FT-IR analysis. While the sample preparation methods differ for the matrixes investigated, the endpoint is always the same, namely a concentration of particles suspended in 5 mL of ethanol.

Preparation of raw wastewater

First, 1 L of raw wastewater was pre-sieved using a 500 µm mesh size as this study only focuses on particles smaller than 500 µm. However, due to the strong adherence of plastic particles to other organic or inorganic particles, 1 mL of 150 g/L sodium dodecyl sulphate (SDS) was added to the wastewater sample before the wet pre-sieving. This was done to ensure that microplastic particles in all size ranges were detached from other particles and were sieved out into their proper size range.

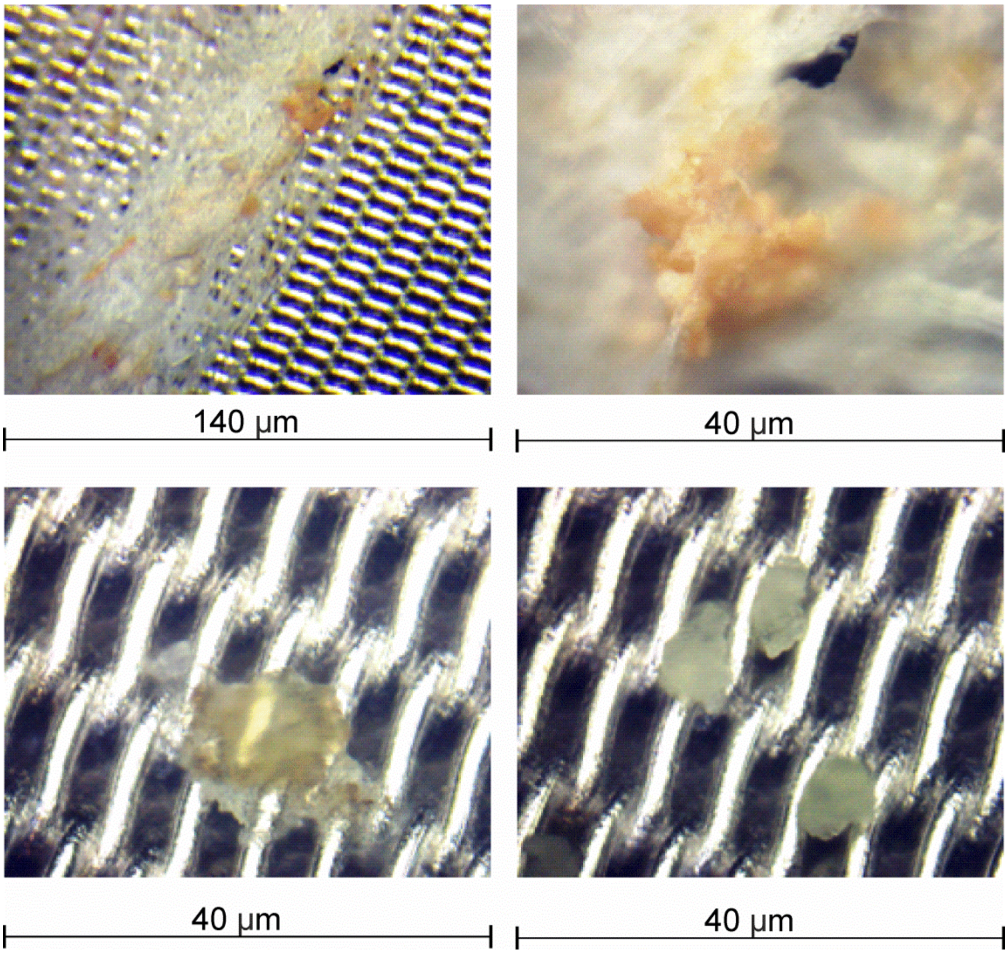


Figure 19. Material from oxidized wastewater filtered onto a 10 µm steel mesh. The top row shows the result without prior use of cellulase incubation. The bottom row shows the effect of pre-treating with cellulase.

Wastewater contains a large fraction of cellulose fibres, which cause problems in the micro-FT-IR analysis. To eliminate these fibres, a cellulose digesting enzyme (cellulase from an Aspergillus species) was added to 200 mL of the pre-sieved wastewater (Figure 19). The samples were incubated for 48 hours at 40°C. Afterwards, the hydrolysed samples were oxidised by adding 50% hydrogen peroxide (H_2O_2) to an initial concentration of 330 g/L and adding a catalyst to enhance the reaction. The oxidised samples were wet sieved into two fractions: larger than and smaller than 80 µm.

Particles were removed from the sieves by ultrasonic treatment and scraping and gathered into filtered demineralised water (filter size 0.8 µm) containing 0.15 g/L SDS. The liquids of each fraction were filtered separately through a 10 µm stainless steel filter mesh (a mesh as shown in Figure 19). After filtration the filters were immediately put into ethanol, sonicated and scraped. The liquid was transferred into a glass vial and the beaker was flushed 3 times with ethanol. After the final flushing, ethanol was completely evaporated from the vial. Then 5 mL ethanol was added and the vial was sonicated. Particles stick very much to the glass when they are dry. Therefore, they need to be removed from the glass walls and into the ethanol. The ethanol containing the particles was evaporated to 5 mL in volume, stored, and at a later time transferred to a Zinc Selenide transmission window or MirrIR slide as described in Appendix 1.3.

Preparation of treated wastewater samples
As described previously, the sampling of treated wastewater resulted in 3 Ø47 mm steel filter meshes of 10 µm pore size. These filter where hydrolysed and oxidized similar to the raw wastewater samples, resulting in two fractions of particles: larger than and smaller than 80 µm suspended in 5 mL of ethanol.

Preparation of sludge samples
Sludge samples were prepared by taking 0.1 g dry matter and suspending it in 50 mL of milliQ water. The sample was then treated similar to the raw wastewater, but for correspondingly modified sample volumes.

Preparation of soil samples
Soil contains a large inorganic fraction (grit, sand, silt and clay) which has to be separated from the organic fraction. However, due to the strong tendency of microplastics to adhere to other particles, samples were first treated with a surfactant (SDS) solution. A soil sample of 50 g was suspended into approx. 1 L filtered demineralised water with 0.15 g/L of SDS. In order to force the microplastic to separate from the soil, it was slightly agitated before the suspension was wet-sieved with several litres of SDS solution into fractions 10-80 µm and 80-500 µm. From here on, each fraction was treated identical, but kept separated for later FT-IR analysis. The particles were retrieved from the sieves in the same manner as described for the wastewater treatments, except that the filters were sonicated into zinc chloride solution and not into ethanol.

Each soil fraction was transferred to a straight glass funnel with stopcock, for separation of the inorganic fraction (Figure 20). This was done by gravimetrical separation using a 1.7 kg L^{-1} zinc chloride solution made by dissolution in 0.8 µm filtered demineralised water. The soil sample suspended in the zinc chloride solution was agitated for approximately 15 minutes by aeration from below. After the aeration was turned off, organic material sticking to the inside of the glass was flushed into the bulk liquid with fresh zinc chloride solution. The column was left for sedimentation/floatation and the approx. top 8 cm of liquid column was drained (approx. 100 mL) through a side port. The zinc chloride solution was resupplied and the floatation sequence was repeated twice. For the first two flotations, the column was left for sedimentation/floatation for 2 hours, while it for the third floatation was left over night.

The mixed liquid obtained from the floatation sequence was then filtered over a 10 µm steel mesh filter. Here upon the material from the filters were oxidised as described for treated wastewater. For soil samples, the material was not treated with cellulose prior to oxidation. After removing the organic matter, the sample was again filtered over a 10 µm steel mesh and the particles transferred from the filter into ethanol using a combination of washing, scraping and ultrasonic treatment. The ethanol containing the particles was evaporated to 5 mL, stored, and at a later time transferred to the appropriate FT-IR windows.

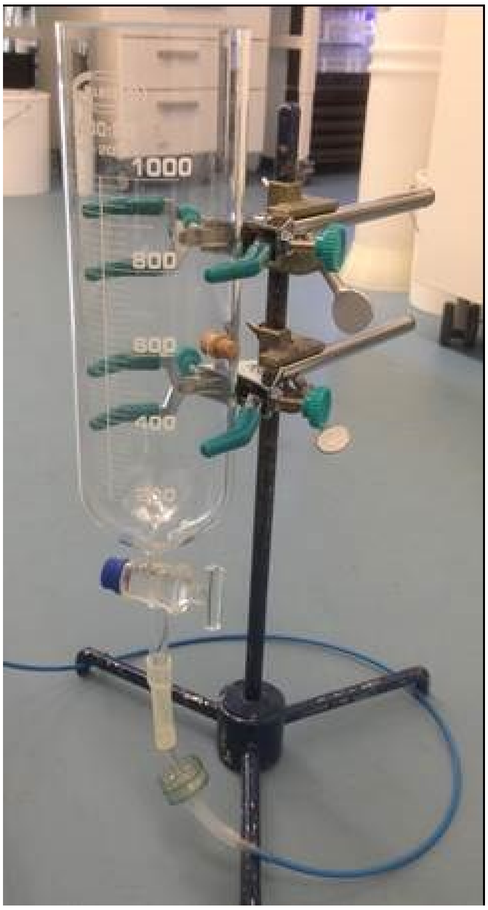


Figure 20. Gravimetrical separation funnel. The blue line supplied the compressed air (CA) for aeration. Between the tubing connecting the glass funnel to the CA, an air filter was placed to reduce contamination. At the 600 mL mark there was a side port with a cork stop, to allow for draining the fluid containing low density particles

Micro-FT-IR spectroscopy for sample imaging

The equipment used to identify and quantify microplastic was a Fourier Transform Infrared (FT-IR) system, consisting of an Agilent Cary 620 FT-IR microscope combined with an Agilent Cary 670 FT-IR spectrometer (Figure 21). The microscope has a 128 x 128 pixel Focal Plane Array (FPA). The equipment can operate in reflection, transmission and ATR (Attenuated Total Reflectance) mode. The equipment has 4, 15 and 25x objectives, allowing pixel sizes on the FPA (in transmission and reflection mode) of 20.6, 5.5, and 3.3 µm, respectively. It can furthermore operate in a 'high magnification mode' that allows for a 5 times finer pixel resolution, i.e. the lowest pixel resolution with which the equipment can operate is 0.66 µm. In ATR mode the equipment can operate at 1.1 µm pixel resolution.

Appendix 1.3 Spectral analysis

The spectral analysis is done by interpreting at which wavenumbers various chemical bonds absorb energy. An example of such interpretation is given below for 3 different particles: A natural cellulose particle, a polyamide/nylon particle and a polyethylene particle (Table 8).

Table 8. Examples of analysis of spectra from particles

Discernible Peaks	Peak Assignment	Particle Definition	Confidence
3360	O-H stretch	Cellulose	Probably
2915 & 2849 split peak	Aliphatic C-H stretch. Asym & symm methylene stretch		
1746	C=O stretch		
1635 with shoulder at 1604 & 1539	C=O stretch of Amide I & C-N stretch & N-H bend of Amide II		
1428 with shoulder at 1459	Asymm deformation methyl		
1370 + 1336 + 1317 peak with shoulder at 1397	Methyl symm deformation + other peaks		
1281 + 1250	Poss. C-O stretch		
1205			
1052 & 1031 split peak shoulders at 1160 + 897 + 863			
3295	O-H stretch with clear N-H stretch	Polyamide or Nylon	High
2921 & 2851 with shoulder at 2954 & 2868	Aliphatic C-H stretch. Asym & symm stretch methylene + asym stretch methyl		
1742	C=O stretch		
1648 & 1547 with split at 1537	C=O stretch of Amide I & C-N stretch & N-H bend of Amide II		
1464 & 1445 split peak	Asymm & symm deformation of methyl + methylene		
1400 with 3 additional peaks	C-H deformation		
1245	C-O stretch		
1108			
1015			
2913 & 2847 split peak	Aliphatic C-H stretch. Asym & symm C-H stretch of methylene	Polyethylene	High
1462	Asym C-H deformation of methyl groups		

Figure 21. Micro-FT-IR spectrometer and imaging system

The micro-FT-IR spectrometer and imaging system creates a visual image and infrared (IR) image. The images are made up of tiles depending on the chosen resolution of the imaging system, and the tiles can be combined to a mosaic. The IR tiles consist of a full IR spectrum per pixel and is visualised by focusing on one IR wavelength at a time. An example of such a combined view is shows in Figure 22. On the top left hand side the visual image is shown; the top right shows the IR data image of the same area, showing pixels that transmitted less IR light at the chosen wavenumber peak at 2326 cm^{-1}. The lower hand image is the full IR spectrum of the pixel indicated by the cross on the upper images. For identification of the particle's material, its full IR spectrum is interpreted by spectral analysis.

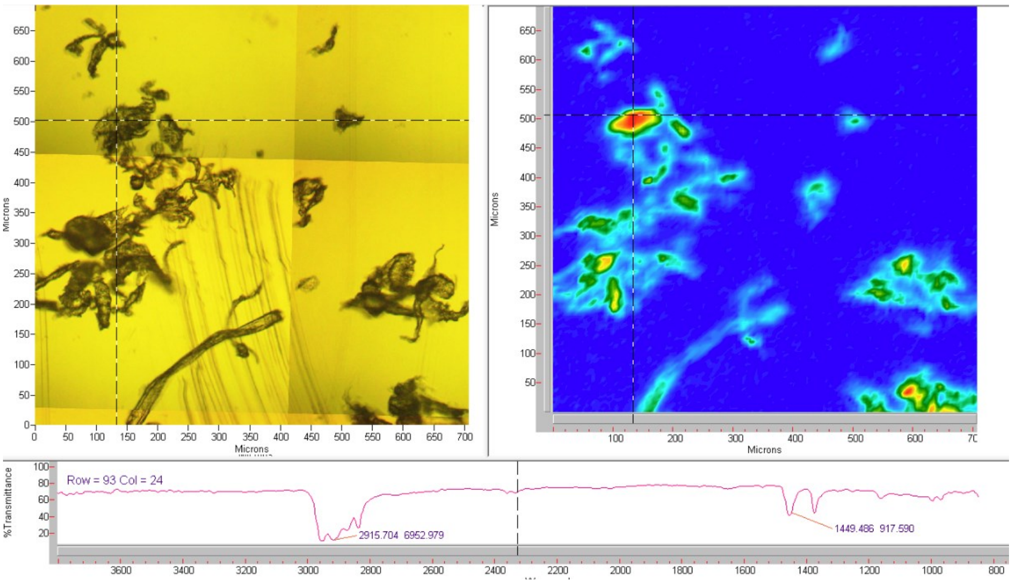


Figure 22. Scrapes of plastic particles including material from blue-cab bottles. The particles are placed on a gold reflection window

In some ways, the simplest FT-IR operation mode is reflection. The sample is transferred to a Kevley MirrIR microscope slide, made especially for IR reflection scanning. The IR light is reflected off the particles on the slide and as the particles only reflect part of the wavelengths transmitted, their material can be identified from the resulting reflection spectrum. However, due to scattering during the reflection, this mode has a low signal strength compared to the signal strength in other modes (transmission, ATR). Furthermore, small particles amplify this effect, meaning that reflection mode is only an adequate option for comparatively large microplastic particles. For the equipment used in the present study and for the type of sample analysed, reflection mode is used for particles from 80 µm and up. Two size fractions of samples are hence scanned: 10-80 µm and 80-500 µm.

For smaller particle sizes, transmission mode or ATR mode is required in order to acquire better IR spectra. Of these methods, transmission mode allows more freedom in scanning. For transmission mode, the particles are transferred to an IR transparent microscope window (Zinc Selenide) and the transmission of IR light through the particles is measured. This method demands that the particles are sufficiently thin to allow IR transmission with good signal strength. This means that transmission mode is good for smaller particle sizes, but not always adequate for larger plastic particles. In both transmission and reflection mode, the system is able to automatically create mosaics of larger sample areas, by stitching together adjacent tiles.

ATR is generally considered the most sensitive of the methods and works by bringing an ATR crystal into physical contact with the sample. While this method does not demand transferring the sample to a transmission or reflection window, it has the drawback that when pulling up the ATR crystal from the sample, some particles tend to stick to the crystal. As a result, scans can only be taken once and the crystal has to be cleaned between each scan. Because of this ATR mode cannot be used to create an automated mosaic of a larger sample area.

One important issue when operating a micro-FT-IR spectroscopy system is interference with IR-absorbing constituents of the atmosphere. While this is not an issue when applying ATR (as the ATR crystal is brought in contact with the sample and the IR light hence does not have to penetrate through atmospheric air), it is a significant issue when operating in transmission or reflection mode. Here the modus operandi is that first one takes a background spectrum of the interfering air. Then one takes a spectrum of the sample, from which the background then is subtracted. The main constituents interfering with the measurement are CO_2 and H_2O gases. Especially the latter absorbs IR light in a region of the spectrum that is critical for the identification of plastic polymers. It is hence critical to ensure a dry and especially highly stable atmosphere between the objective and the sample window. Not doing so creates spectra that cannot be reliably interpreted. It hence drastically increases the risk of obtaining false positives or false negatives in the spectral analysis as peaks from water vapour interference can be interpreted as peaks from plastic materials.

For analysis in reflection mode, a MirrIR microscope window has been prepared by fixing a steel washer with an inner diameter of 10 mm on the window. A well-defined amount of sample solution (particles suspended in ethanol) is then transferred to the restricted area. After the ethanol has evaporated, the window is checked in a traditional light microscope to ensure that the window is adequately populated by particles. If the slide is insufficiently populated, additional particle suspension is added till the window is deemed sufficiently populated. Here after the slide is placed the microscope stage.

For transmission mode, pre-treated samples are transferred to a transmission window. The windows used are Ø13 mm Zinc Selenide (ZnSe) windows mounted in a compression cell (Figure 23). The population of the window is ensured similar to the reflection windows. The compression cell has a clear aperture of 10 mm, in which the sample is deposited. The sample window is taken out of the compression cell, placed on a metal disk which is then placed on the microscope stage and analysed.

The area of the window scanned (i.e. the number of tiles scanned) depends on how rich the sample is on plastic particles. Based on experience for the various types of samples, the number of tiles scanned is set for each sample type. The number of actually analysed tiles differs with population density of plastics on the windows.

The micro-FT-IR system applied in the study is equipped with a 128x128 pixel FPA (focal plane array); however, due to technical problems with the equipment, the majority of the scanning had to be done using only a quarter of this size, namely 64x64 pixel FPA. I.e. to achieve the same area operating in 64x64 FPA mode, 4 times as many tiles are needed to cover the same area (and unfortunately also 4 times the scanning time). Hence when analysing raw wastewater, treated wastewater, sludge, and soil samples the predefined number of tiles scanned were:

- Transmission mode, 80-500 µm
  Raw wastewater: 64x64 pixel FPA: 16 tiles; 128x128 FPA: 4 tiles
  Treated wastewater: 64x64 pixel FPA: 32 tiles; 128x128 FPA: 8 tiles
  Sludge: 64x64 pixel FPA: 16 tiles; 128x128 FPA: 4 tiles
  Soil: 64x64 pixel FPA: 32 tiles; 128x128 FPA: 8 tiles
- Reflection mode, 10-80 µm
  Raw wastewater: 64x64 pixel FPA: 32 tiles; 128x128 FPA: 8 tiles
  Treated wastewater: 64x64 pixel FPA: 32 tiles; 128x128 FPA: 8 tiles
  Sludge: 64x64 pixel FPA: 32 tiles; 128x128 FPA: 8 tiles
  Soil: 64x64 pixel FPA: 32 tiles; 128x128 FPA: 8 tiles

The scan time for one tile depends on the actual sample, but is between 5 and 8 minutes. For 32 tiles the resulting total scan times including startup and finalization of the scans is hence 3-5 hours per sample.

Figure 23. Compression cell for transmission mode imaging. The cell is only used for evaporating the sample onto a well-defined area of the transmission window, and, despite of its name, never actually used to compress two windows.

Spectral analysis of particles

As discussed previously, one should not simply compare measured particle spectra to reference spectra as this will lead to an increased risk of both false positives and false negatives. A better approach is to perform an initial screening of spectra for materials which could be plastic – i.e. rapidly rejecting for example mineral particles, which in most samples constitute the majority of the particles on a window. The candidate particles are then, one by one, analysed for the material composition by identifying all assignable peaks in their spectra. Figure 24 illustrates this principle.

First all particles that absorb IR light at the wavelengths marked by blue in the lower image are highlighted in the image analysis software. Then particles showing significant absorption are selected, for example the one indicated in the two upper images. The analyst goes through all the relevant data starting with the first collected tile and moving right and down. The peaks in the spectra of the highlighted particles are analysed, providing an indication of the material type (in this case PE). In some cases a spectrum contains peaks characteristic to for example polyethylene, while also containing other peaks. These are all noted down, and when a determination is made, it receives a confidence scale which rates from Maybe to Possible, Likely and High. Maybe is a relatively low quality spectra but peaks are still attributable, Possible relates to not all peaks being found or a few too many (for example from interference from scattering). Likely means all peaks are present and the spectral quality is OK, some interference peaks may be present or uncertainty given due to the large permutations of possible copolymers. High is only attributed when a spectrum is of such good quality and clarity that the analyst could not possibly assign it as anything else. When the analyst is in doubt, a second opinion is asked.

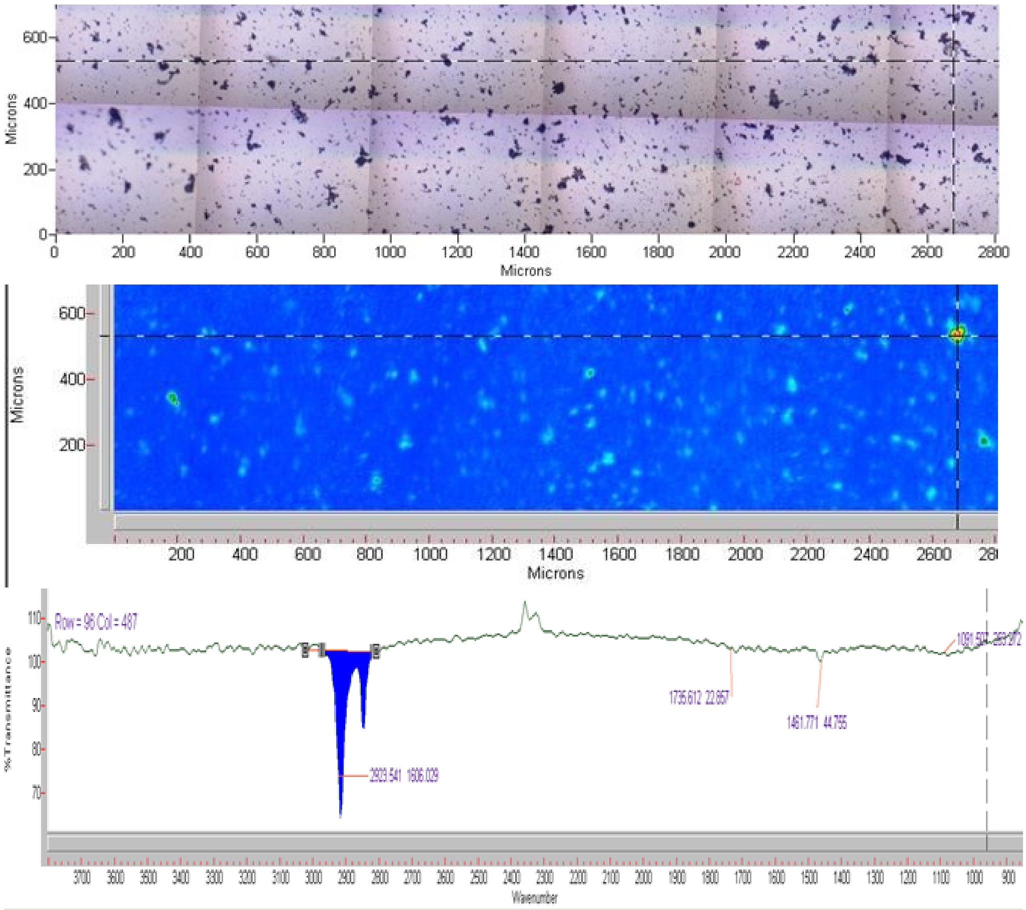


Figure 24. Searching for plastic particles on a 700x2800 µm section of an IR transmission window

A zoom on the particle shows its shape and size as 40x45 µm (Figure 25). The image is of course only 2-dimensional, and the thickness of the particle must hence be estimated. In the present study, it is as a general rule assumed that the thickness of a particle is 60% of its shortest axis. This assumption is not substantiated by evidence, and hence a source of uncertainty. It does, though, seem reasonable to assume that particles orient themselves on the window with the smallest dimension pointing upwards.

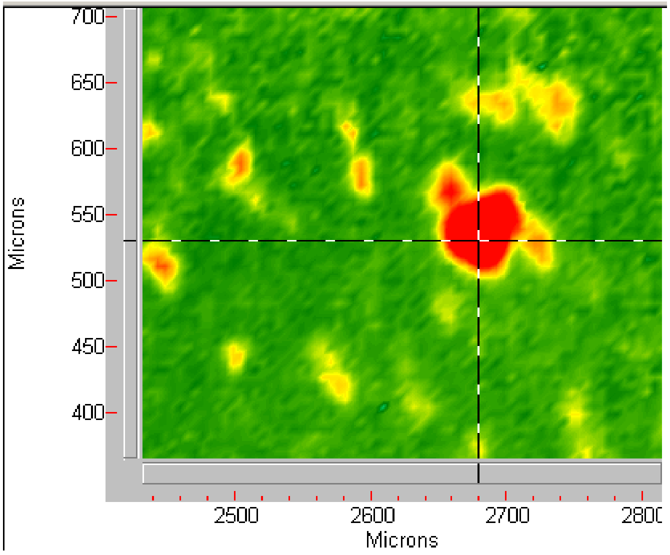


Figure 25. A zoom on the PE particle identified in Figure 24

Appendix 1.4 Calculation of particle volume and mass

For each particle the longest and shortest diameter was noted down from the light microscopy image. For calculation of the particle volume it was assumed that particles were ellipsoidal and the volume calculated by applying the two determined diameters as two of the ellipsoid diameters. The third diameter of the ellipsoid was set to 60% of the smallest determined diameter. The plastic mass was found as this volume multiplied by reference densities of the plastic material (Table 9). With the above discussed assumptions and limitations, the identified particle of Figure 25 is estimated to have a mass of 22 ng.

Table 9. Plastic materials and their densities. Sources: British Plastics Federation (bpf.co.uk) and Prospector (ulprospector.com).

Material	Abbreviation	Density range [g/cm^3]	Density average [g/cm^3]
PAM/Nylons		1.13-1.41	1.27
PP		0.905	0.905
PE	HDPE	0.944-0.965	0.94
	LDPE	0.917-0.930	0.929
	PTFE	2.14-2.19	2.155
Polyesters			1.37
Polystyrene	GPPS	1.05	1.05
	HIPS	1.03-1.06	1.045
Polyvinyl Chloride		1.38	1.38
Polyurethane	TPU foam	0.4-1.0	
(very variable in density)	Generic PUR	0.94-1.11	1.025
	PUR-Ester	1.15-1.27	1.21
	Generic PUR (MDI/TDI)	0.0451 - 1.25	
	PUR-Glasfiber	1.26-1.67	
SBR	ABS	1.03 - 1.05	1.04
	ASA	1.08	
Zinc Stearate coated particles*		1.069	

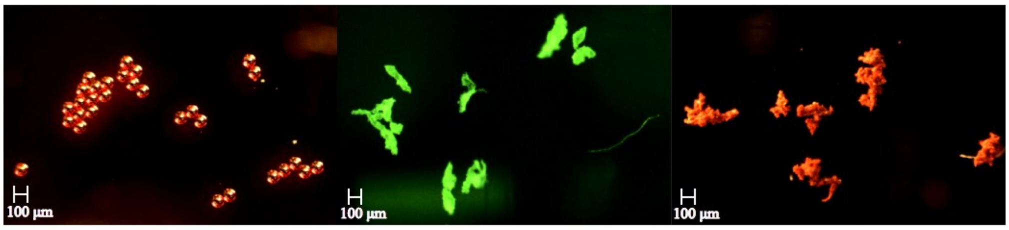
* Assuming that Zinc stearate is coated on most popular household appliance plastics such as PP, PE & PS

Appendix 2. Method validation, uncertainties, and detection limits

Appendix 2.1 Recovery of plastic particles

In the present study we have approached the validity of the sample preparation method by recovery of microplastic particles. We spiked known plastic particles to raw wastewater and quantified the recovery in terms of numbers of particles. The validation was done by spiking the raw wastewater with 3 types of plastic particles before any pretreatment: Red 100 µm spherical polystyrene particles (Sigma Aldrich, product no. 56969), light-green high-density polyethylene (HDPE) particles, size 80-150 µm, made from a HDPE water bottle, and red styrene butadiene rubber (SBR) particles made from a SBR sheet (Figure 26).

Instead of analysing only part of a sample for microplastic applying FT-IR, the complete sample was analysed by light-microscopy. This was made possible by the highly characteristic colours and shapes of the materials used for spiking. The number of plastic particles added to wastewater samples was quantified by counting under a light microscope. A known number of particles were then added to 200 mL of raw wastewater, upon which the sample underwent the same preparation steps as presented in Appendix 1.2. The complete sample was then counted by means of light-microscopy.



(a) *Polystyrene particles.* (b) *High-density polyethylene particles.* (c) *Styrene butadiene rubber particles.*

Figure 26. Microscope images of the plastic particles used in this study for method validation.

The obtained recoveries when sonicating the 10 µm filters once (i.e. the transfer of particles from 10 µm filter meshes to ethanol) are shown in Figure 27. On average the recovery for polystyrene was approx. 85% while HDPE particles were somewhat lower. The recovery of SBR particles were above 100% which most likely was due to breakup of particles during sample preparation.

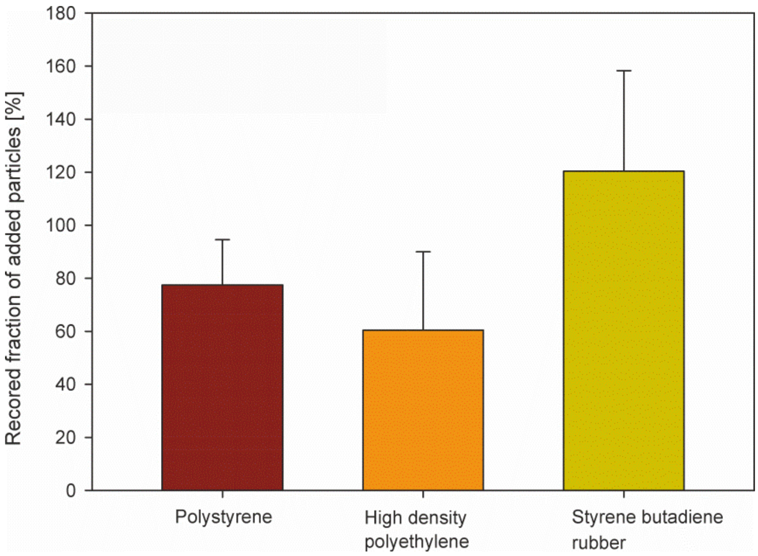


Figure 27. Average recovery for each type of plastic from wastewater samples when sonicating filters once.

The recovery presented in Figure 27 led to a scrutinizing of the method and it turned out that even better recovery could be obtained by repeating the sonication (i.e. the transfer of particles from the filters to the ethanol). Hereby a close to 100% recovery could be obtained. The procedures for sample preparation were hence adjusted accordingly.

Appendix 2.2 Uncertainties

The above presented tests on recovery show some of the issues of quantifying microplastic in environmental samples: Not all plastic particles behave the same, and plastic particles might actually break down during the sample preparation. Nevertheless, the uncertainty of the sample preparation method seems to be rather good – at least for the plastic particles used to spike the natural wastewater samples.

Sample preparation is, though, not the only uncertainty to be faced. A second uncertainty lies in the fact that not all of a sample can be analysed by micro-FT-IR imaging. To do so would require unrealistic scanning and interpretation times (weeks of scanning). The uncertainty of selecting a sub-area of slide compared to the whole slide has not been assessed in the present study, but is believed not to introduce a systematic error and hence equal out when analysing many samples for determining average microplastic contents.

A third uncertainty lies in the spectral analysis of the FT-IR images. The spectra of particles from natural samples seldom look like the reference spectra of the pure materials. There hence lies uncertainty in analysing the spectra, and the analysis furthermore becomes to some degree subjective. A similar issue is well-known from biological analysis, for example when identifying and counting algae in a water body. Again similar hereto, this uncertainty can be minimized by increasing the time used to analyse and understand each and every spectrum. Quantifying this uncertainty is, though, not straight forward.

Appendix 2.3 Detection limits

Defining detection limits for microplastic particles is not straight forward, and one has to consider whether detection limits are to be defined in terms of number of particles or in terms of particle mass. In the present study we have somewhat circumvented the issue of detection limits as we simply have identified plastic particles until at least 10 plastic particles within a certain size range were found. We have, though, limited ourselves to a maximum number of tiles analyzed to keep the scanning and interpretation times within limits. Detection limits were hence variable.

The detection limit of the method depends on the fraction of the sample scanned by the FT-IR imaging system. We have scanned till at least 10 plastic particles had been positively identified, or till we had scanned an area of at least 4 mm^2 on the microscope slide. The microscope slide was circular with a radius of 5 mm and hence had an area of 78 mm^2.

- For wastewater samples, particles from 0.2 L were concentrated into 5 mL of ethanol. Of this, at least 0.2 mL was deposited on the scanned slide. Assuming that microplastic particles are of size 20 µm, this corresponds to a detection limit of better than 4 µg/L.
- For treated wastewater, particles from between 4.1 and 81.5 L were concentrated into 5 mL of ethanol. Of this, at least 0.2 mL was deposited on the scanned slide. Assuming that microplastic particles are of size 20 µm, this corresponds to a detection limit of better than 0.20 µg/L.
- For sludge, particles from 0.1 g were concentrated into 5 mL of ethanol. Of this, at least 0.1 mL was deposited on the scanned slide. Assuming that microplastic particles are of size 20 µm, this corresponds to a detection limit of better than 20 µg/g.
- For soil, particles from 50 g were concentrated 5 mL of ethanol. Of this, at least 0.1 mL was deposited on the scanned slide. Assuming that microplastic particles are of size 20 µm, this corresponds to a detection limit of better than 0.04 µg/g

Appendix 2.4 Identification of tire rubber

During the course of the project there have been voiced concerns whether rubber from car tires can be identified by the applied FT-IR technique and spectral analysis. The concern was that the carbon black content of car tires would absorb basically all IR light and render spectral analysis impossible. To test this issue, a series of tests were conducted on car tire rubber.

Car tires are an amalgam of several compounds, whereof the main components are copolymer rubbers and fillers. The rubbers used in car tires are often a combination of natural rubber (polyisoprene), styrene rubber and butadiene rubber. The exact composition changes with the tire producer and the wished use for the tire. Typically, the amount of synthetic rubber will decrease with an increasing need for heat resistance, therefore tires for busses, trucks and aircrafts will contain higher natural rubber contents than those of passenger cars. In general, the rubber in a tire will consist of 40-60% of its total mass, while the next large contribution will come from reinforcing fillers (20-50%). The most common reinforcing fillers are carbon black and amorphous silica. Due to low cost, carbon black was the most common filler in the automotive industry for a long time, but slowly amorphous silica becomes more widely used as technological advances are made.

The different components of rubber copolymers have specific infrared absorbance spectra, as they all absorb some of the infrared light. This means that a spectrum taken from car tire fragments will also show a combination of these components. This spectrum will naturally not look like only that of the rubber but will provide a type of fingerprint for each car tire dependent on its composition. In Figure 28 four spectra are displayed which were acquired from four different car tire brands, and of which two are summer tires and two winter tires. As can be seen, even though the exact composition may be different, the spectra are regardless very similar to each other, since most tires are made from the same type of styrene and butadiene copolymer (SBR) and similar reinforcing fillers.

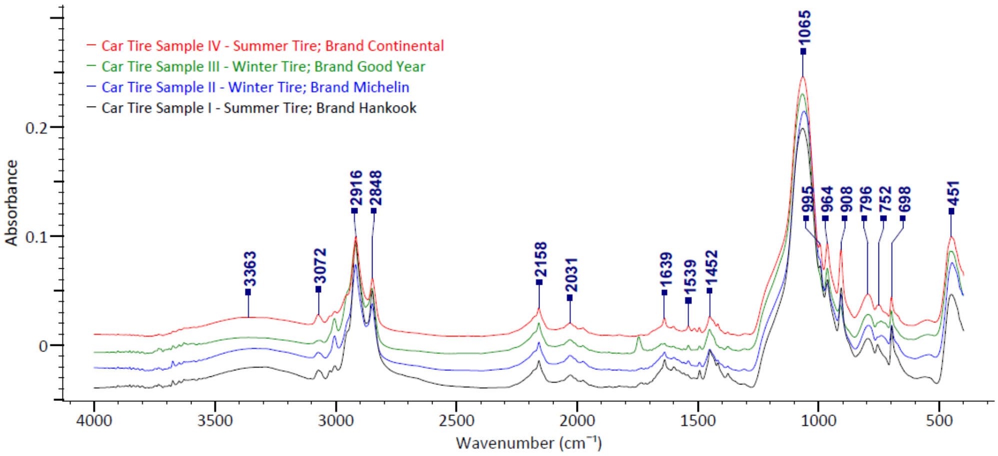


Figure 28. FT-IR-ATR spectra taken from 4 different brands of car tires, of which 2 were winter and 2 were summer tires. The spectra are slightly offset from each other to be able to distinguish between them.

Car tires do contain carbon black and pure carbon black does indeed absorb all light at all wavelengths, and a pure carbon black sample can hence not be analysed by the applied technique. However, samples having carbon black as one constituent among others can be measured using FT-IR as shown also by other researchers (Murakami, 2009). The carbon black will have an impact on the provided spectra, but the change has been shown to be a predictable one, and can be accounted for in spectral analysis and interpretation. Dissimilarly to what was seen in the Shimadzu study, the tire samples analysed in the present study did not show problems related to carbon black.

In ensuring that identification of car tire fragments is possible, even after treatment, an analysis has been made of fragments treated with possible affecting agents used in the sample preparation method. The two processes considered possibly detrimental to the FT-IR spectral outcome are the oxidation and ultra-sonication processes. Therefore, a small triplicate study was done of one of the car tires for which spectra were acquired, after having undergone treatment. Figure 29 shows these respective spectra before treatment, after oxidation only and after oxidation and ultra-sonication. It seems that especially the ultra-sonication may result in some slight alteration of the car tire spectra. However, it is mainly in absorbance intensity, which is already variable due to differences in composition between different tire brands. The spectral analysis as such was hence not affected by the treatment methods. Hence there is no reason to believe that the treatment method of the present study affected identification of car tire particles in itself.

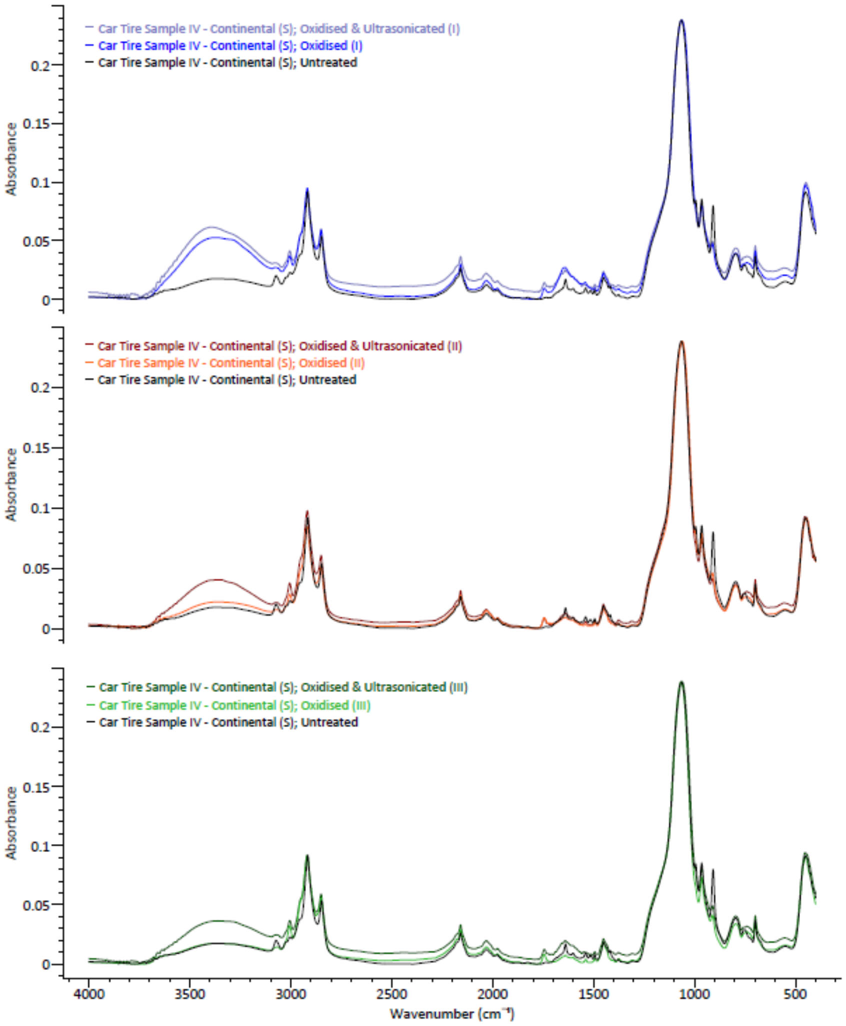


Figure 29. Spectra collected from a triplicate treatment test to assess the impact of sample treatment on the infrared spectra of a given car tire rubber composite.

Appendix 2.5 Data overview

Overview of raw data. Samples for wastewater: WW, treated wastewater: TW, Sludge: Sludge. The following number indicates the plant number. Soils are indicated by Soil with sludge and Soil without sludge. The analysed volume refers to the corresponding volume analysed at the FT-IR imaging system. Number of particles describes the plastic particles identified. Min and max dimensions are the smallest and largest dimensions of any particle.

Plant number	Analyzed volume	Number of particles	Mass of particles	Min dimension	Max dimension	No concentration	Mass concentration	Plastic types
	[mL]		[mg]	[µm]	[µm]	[no/L]	[mg/g]	
WW1	5.93E-01	4	7.18E-05	20	64	12711	0.22	PE-co-polymer, PA/NYlon
WW 2	3.66E-01	16	8.00E-04	20	100	98443	4.86	PA/NYlon
WW 3	8.58E-01	8	3.94E-04	33	120	19148	1.14	PE-co-polymer, PA/NYlon
WW 4	2.46E-01	17	9.92E-04	26	107	237704	13.13	PE-co-polymer, PA/NYlon, LDPE
WW 4, only particles below 80 µm	9.16E-02	20	3.36E-04	12	75	760903	6.05	PE, PA/NYlon, LDPE, PVC, PP
WW 5	1.01E+00	11	3.72E-03	36	215	85837	11.13	PA/NYlon
WW 5, only particles below 80 µm	2.75E-01	7	2.36E-03	27	60	52273	15.18	PE-co-polymer, PA/NYlon
WW 6	7.91E-01	13	4.19E-04	13	100	69624	1.35	PE-co-polymer, PA/NYlon
WW 7	5.30E-01	18	2.08E-03	15	190	70092	6.80	PE-co-polymer, PA/NYlon, Zn-stearate coated particle
WW 8	3.53E-01	11	8.88E-04	27	125	85177	6.29	PE, PA/NYlon
WW 8, only particles below 80 µm	1.37E-01	9	2.62E-04	13	83	141962	2.99	PE, PE-co-polymer, PA/NYlon
WW 9, only particles below 80 µm	3.03E-01	5	4.37E-04	40	80	16507	1.44	PA/NYlon
WW 9	2.90E-01	11	3.80E-04	17	80	144063	5.47	PA/NYlon
WW 10	2.40E-01	28	1.83E-03	25	100	442393	29.55	PA/NYlon, PE co-polymer, PP co-polymer, Zn-stearate coated particle

Plant number	Analyzed volume	Number of particles	Mass of particles	Min dimension	Max dimension	No concentration	Mass concentration	Plastic types
	[mL]		[mg]	[µm]	[µm]	[no/L]	[mg/g]	
TW1	3.52E+01	25	1.54E-03	23	120	1801	0.1336	PE, PA/NYlon, PE co-polymer
TW2	1.44E+01	19	3.98E-04	3	100	4254	0.0930	PP, PA/NYlon, Zn-stearate coated particle
TW3	1.26E+02	7	3.30E-04	30	70	6933	0.0056	PA/NYlon, PE-co-polymer
TW4	6.76E+01	17	5.76E-04	22	90	503	0.0170	PA/NYlon, PE-co-polymer, PE
TW5	2.49E+01	15	3.79E-04	20	270	6053	0.0325	PA/NYlon, PE-co-polymer, PE

TW6	2.28E+01	11	2.71E-04	20	120	10389	0.0384	PA/NYlon, PP
TW7	1.71E+02	13	3.74E-04	20	140	6650	0.0043	PA/NYlon, PE co-polymer
TW8	2.78E+02	14	3.66E-04	15	90	8716	0.0028	PA/NYlon, PE
TW9	1.65E+02	12	2.39E-04	20	90	4695	0.0021	PA/NYlon, PE co-polymer
TW10	1.37E+02	17	9.94E-04	19	406	8419	0.0145	PA/NYlon, PP

Plant number	Analyzed volume	Number of particles	Mass of particles	Min dimension	Max dimension	No concentration	Mass concentration	Plastic types
	[mg]		[mg]	[µm]	[µm]	[no/g]	[mg/g]	
Sludge 1	1.70E-01	5	4.13E-04	23	90	79235	7.23	PE-co-polymer, PP
Sludge 2	1.34E-01	10	2.74E-04	20	95	413780	6.45	PE-co-polymer, PA/NYlon
Sludge 3	1.26E-01	9	3.27E-04	11	80	158469	6.48	PE-co-polymer, PA/NYlon
Sludge 4	2.84E-01	4	9.00E-05	28	65	17608	0.40	PE, PA/NYlon
Sludge 5	1.34E-01	4	8.17E-05	20	60	174976	2.02	PE-co-polymer, PA/NYlon

Plant number	Analyzed volume	Number of particles	Mass of particles	Min dimension	Max dimension	No concentration	Mass concentration	Plastic types
	[mg]		[mg]	[µm]	[µm]	[no/g]	[mg/g]	
Soil with sludge 1	5.28E+01	0	0.00E+00	0	0	0	0.0000	5.28E+01
Soil with sludge 2	5.60E+01	4	1.62E-04	40	110	143	0.0058	PP, PE co-polymer, PA/Nylon
Soil with sludge 3	3.03E+02	9	7.26E-04	10	130	165	0.0165	PE co-polymer, PA/Nylon
Soil with sludge 4	1.14E+02	4	4.43E-04	22	120	71	0.0076	PE co-polymer, PA/Nylon, PE
Soil with sludge 5	1.70E+02	5	2.41E-04	22	85	29	0.0014	PE co-polymer
Soil without sludge 1	2.27E+01	6	1.69E-04	20	77	528	0.0149	PE co-polymer, PE, PA/Nylon
Soil without sludge 2	8.20E+01	7	5.73E-04	27	150	145	0.0117	PE co-polymer, PE
Soil without sludge 3	1.53E+02	6	4.14E-04	20	120	53	0.0044	PE co-polymer, PP
Soil without sludge 4	3.53E+01	5	2.29E-03	30	245	436	0.2243	PE co-polymer, PA/NYlon
Soil without sludge 5	8.52E+01	1	4.83E-06	22	25	53	0.0001	PA/NYlon

Microplastic in Danish wastewater – Sources, occurrences and fate

The objectives of the present study were to evaluate the role of Danish wastewater treatment plants (WWTPs) in the emission of microplastic to the environment in terms of amounts and types of plastic polymers emitted and if possible, to evaluate which sources these plastic polymers could originate from.

Samples from 10 WWTPs (wastewater, inlet and outlet), sludge from 5 of these plants, and 10 farmlands soils (5 soils that had received sludge as fertilizer and 5 that had not) were analysed for the occurrences of microplastic with the currently most advanced method available for microplastic investigations (Fourier Transformed Infrared Spectroscopy imaging applying a Focal Plane Array). This method allows both determination of the microplastic concentrations in the samples and identification of the type of plastic polymer of each microplastic particle.

Environmental
Protection Agency
Strandgade 29
DK-1401 København K

www.mst.dk