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UK Water Industry Research

SINK TO RIVER – RIVER TO TAP

A REVIEW OF POTENTIAL RISKS FROM NANOPARTICLES AND MICROPLASTICS

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guardians of drinking water quality

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Contents

Attached to Report:

Glossary

Term / Acronym

ABS	acrylonitrile butadiene styrene
ATR FTIR	attenuated total reflection fourier transform infrared spectroscopy
BAFF	biological aerated flooded filter
DZR	dezincification resistant – high quality brass, suitable for drinking water
EDTA	ethylenediaminetetraacetic acid
FTIR	fourier transform infrared spectroscopy
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
HEPA filter	high efficiency particulate air filter
LOD	limit of detection
LOQ	limit of quantification
MPhunter	a dedicated FTIR particle analysis software, available on request from the originator, Jes Vollertsen at Aalborg University, Denmark
NaCl	sodium chloride
PA	polyamide
PE	polyethylene
PET	polyethylene terephthalate
PMMA	poly(methyl methacrylate)
PP	polypropylene

PS	polystyrene
PTFE	polytetrafluoroethylene
PU	polyurethane
PVC/PVC-U	polyvinylchloride, "U" stands for un-plasticized or hard, in contrast to plasticized (P) soft PVC. Given the lack of certainty in differentiating the two forms by ftir/mphunter this report will refer only to PVC
RO	reverse osmosis (water, that has been purified and de-ionised by reverse osmosis)
SDS	sodium dodecyl sulphate
SEM	scanning electron microscope
WRAS	water regulations advisory scheme
WTW	water treatment works
WwTW	wastewater treatment works
ZnCl$_2$	zinc chloride

UK WATER INDUSTRY RESEARCH LIMITED

SINK TO RIVER – RIVER TO TAP
A REVIEW OF POTENTIAL RISKS FROM NANOPARTICLES AND MICROPLASTICS

Executive Summary

Objectives and Approach

The primary objective of this study was to inform the UK and Irish water companies on the levels of microplastic particles present in raw and treated water, wastewater & treated effluent, and the sludges produced by their treatment works.

The secondary objective was to develop a robust sampling and detection methodology to allow the quantification of microplastic particles at a range of different points within the water environment and the water industry's infrastructure. It should be noted that prior to this project no standardised methods or reference materials were available.

To ensure a representative overview - samples were taken from eight water treatment works (WTW) and eight wastewater treatment works (WwTW) from different companies across Great Britain. For water, the samples included raw water, potable water, and waste sludge from WTWs. For wastewater, samples of influent, effluent and sludge cake were collected from WwTWs. The WTWs' sites were chosen to represent a range of raw water types, and the WwTWs' sites were chosen to represent a range of treatment processes. Multiple samples were taken from each site to provide information on the variability of microplastic particles.

By developing cutting-edge analytical methods, the project was able to provide accurate results, and provide a sound foundation on which to develop further research. The project devoted significant effort to understand, quantify and correct for microplastic contamination during sampling and analysis to ensure confidence in the results.

An additional objective was to re-examine a report from 2011 on the likelihood of nanoparticles entering potable water. It was considered that this report had over-estimated market growth and hence risks, but understanding is still hampered by the lack of suitable methods for routine analysis (see full report in Appendix G which is provided as a separate document).

Conclusions

A robust process for sample collection and analysis is vital to ensure accurate results, and while standard methods are not yet available, this project ensured the highest quality was maintained through the collection and laboratory analytical stages; the use of very large sample volumes for potable water (>100 litres) gave confidence in the sensitivity of the method and accuracy of the results obtained.

The results of the project found that for WATER, >99.99% of microplastic particles are removed through the treatment processes, with raw water having an average of 4.9 microplastic particles/litre and potable water having on average 0.00011 microplastic particles/litre.

For WASTEWATER, the treatment processes were able to remove 99.9% of the microplastic particles with levels of 5.1 microplastic particles/litre being found in final effluent.

As a consequence of the removal rates of microplastic particles through both water and wastewater treatment processes, these materials are present at very low levels in drinking water and in discharges to the environment.

For SLUDGE, as a consequence of the removal rates of microplastic particles through both water and wastewater treatment processes, they are present in sludge, with levels of 2,000 - 4,000 microplastic particles/g dry weight of sludge being typically found.

Recommendations

The further development of the standard processes used in this project including robust Quality Assurance (QA) systems for future sampling and analysis to enhance sensitivity, selectivity and resistance to environmental contamination during sampling and analysis.

Further and more detailed microplastics investigations should be undertaken by the water industry.

For the sludges generated as a by-product of the treatment processes used, more investigations should be undertaken on the impact of the presence of microplastic particles on the use of these materials.

Benefits

This project has confirmed the efficacy of both water and wastewater treatment processes in the removal of microplastic particles from raw water and wastewater leading to very low levels in the final products i.e. potable water and final effluent.

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Chapter 1   Introduction

1.1   Introduction to literature review

Microplastics are generally considered to be plastic particles smaller than 5 mm but larger than 1 µm or 100 nm, although a standard definition has yet to be agreed (Hartmann *et al.* 2019). These particles can derive from products to which they are added intentionally (for example sun creams and other cosmetics) or more commonly from the breakdown of larger consumer or industrial products as well as painted surfaces, tyres and synthetic fabrics. These diverse sources have led to the widespread contamination of the natural environment with microplastics (Horton and Dixon 2018). They are now the subject of considerable research efforts which have revealed they are commonly present in wastewater systems, with plastic fibres gaining particular attention (Hernandez *et al.* 2017, Ziajahromi *et al.* 2017).

Car tyres are made from a complex mix of rubber, synthetic polymers, organic molecules and metals and there has been an estimation that they represent 5-10% of the microplastics in the ocean (Kole *et al.* 2017). The chemistry of these particles becomes more complex as they adsorb materials from the road (Kreider *et al.* 2010). Currently it is not possible to routinely identify and enumerate these materials as particles, only to detect their presence. Their distinctive signature can be reported using pyrolysis GCMS (Kim *et al.* 1990) and these have been developed further using TED-GCMS (Eisentraut *et al.* 2018). Therefore, they will not be a focus in this report as their particles cannot be enumerated in an effective way.

There is some evidence to suggest that microplastics can cause harm to a range of organisms, either through physical harm such as gut blockage or internal lacerations, or via associated chemicals. However, these responses depend on the dose of microplastics given, the sensitivity of the species and particle characteristics involved. There remains scepticism that environmental levels are anywhere near effect levels (Connors *et al.* 2017). Despite the knowledge that humans are exposed to multiple types of plastics and microplastics via ingestion, inhalation and dermal contact, harmful health outcomes have not yet been identified (Wright and Kelly 2017, Prata 2018a).

In this review, the range of references and their results have been set out in a series of tables. Whilst there has been an attempt to compare and summarise the results of these different studies, this is problematical due to the lack of standardisation. The tables allow the reader to see the often very different analytical approaches and size ranges reported on.

1.2   Current knowledge of microplastics in drinking water

Understanding the presence of microplastics in potable water is a relatively recent concern. One of the first mentions of microplastics in tap water was within a report by Orb Media about the widespread contamination of tap water samples worldwide (Tyree and Morrison 2017). While not a peer-reviewed scientific publication, this report received substantial attention and increased the public's awareness of the potential for drinking water to contain microplastics. The results have since been published within the academic literature (Kosuth *et al.* 2018). Public concern was further increased with studies showing microplastics to be present in bottled water (Oßmann *et al.* 2017, Mason *et al.* 2018). However, to date there have still been few academic studies quantifying microplastics in treated drinking water

(Table 1). A review of the few published studies of microplastics in potable water has also been conducted by Eerkes-Medrano *et al. (*2018).

As far as is known, there are currently only three studies in the peer-reviewed literature on microplastics in municipal drinking water. The high variation in results (6 orders of magnitude between minimum and maximum reported values, Table 1) leaves large uncertainty as to how these should be judged. Are these genuine differences in locations, sampling methods, analytical approaches, sizes examined or simply poor quality control? There is further disagreement on what type of particles are present, for example, Pivokonsky *et al.* (2018) reported 95% particles to be between 1-10 μm, whilst Kosuth *et al.* (2018) found that 98.3% fibres in tap water (taken directly from taps within residential dwellings) were between 100-5,000 μm, even though particles down to 2.5 μm were analysed. It should be noted that according to the review of methodology by Koelmans *et al.* (2019), the Mintenig *et al.* (2019) WTW study should receive greater credence than that by Pivokonsky *et al.* (2018).

Studies on bottled water concentrations show generally higher concentrations of microplastics than found within treated potable water, although this result is extremely influenced by the one study by Zuccarello *et al.* (2019).

With respect to studying microplastics in relatively clean environments, it is vital that careful consideration is given to analysis of blanks, since contamination from the field or the laboratory could lead to misleading results. A recent review has highlighted that many of the studies in this field do not implement such control measures (Koelmans *et al.* 2019).

Table 1 Studies investigating microplastics in potable & bottled water. Where one publication is repeated within multiple rows, this refers to different sample types analysed within the same study. The ± values are the ones given in the sources, usually standard deviation.

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (μm)	Treatment type	Volume sampled (L)	Average microplastic particles/L
WTW produced potable water							
Mintenig *et al.* (2019)	Germany (6)	HCl then H_2O_2	FTIR	>20	Aeration and filtration	40,000	0.0007
Pivokonsky *et al.* (2018)	Czech Republic (1)	H_2O_2, size separation, SEM	FTIR and Raman	>1	sand filtration	27	443 ± 10
Pivokonsky *et al.* (2018)	Czech Republic (1)	H_2O_2, size separation, SEM	FTIR and Raman	>1	sedimentation + sand filtration	27	338 ± 76
Pivokonsky *et al.* (2018)	Czech Republic (1)	H_2O_2, size separation, SEM	FTIR and Raman	>1	flotation + sand filtration	27	628 ± 28

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (µm)	Treatment type	Volume sampled (L)	Average microplastic particles/L
WTW PRODUCED POTABLE WATER SUMMARY			NUMBER	MIN	MEDIAN	MEAN	MAX
			9	0.0007	0.0007	140.9	628
Tap water							
Kosuth *et al.* (2018)	World-wide	Rose Bengal stain and visual counting	None	>2.5	Tap water	79.5	5.45
Mintenig *et al.* (2014b)	Germany (6)	HCl and H_2O_2	FTIR	>20	Tap water	1500-2600	0
Bottled water							
Schymanski *et al.* (2018)	Germany	None	Raman	>5	Bottled water - single use plastic (10)	N/A	14 ± 14
Schymanski *et al.* (2018)	Germany	None	Raman	>5	Bottled water - reusable plastic (12)	N/A	118 ± 88
Schymanski *et al.* (2018)	Germany	None	Raman	>5	Bottled water – glass (9)	N/A	50 ± 52
Schymanski *et al.* (2018)	Germany	None	Raman	>5	Water carton (3)	N/A	11 ± 8
Zuccarello *et al.* (2019)	Italy	Not stated (patent)	None (SEM for visual analysis)	0.5-10	Bottled water (various) (30)	0.5	54,200,000 ± 19,500,000
Oßmann *et al.* (2018)	Germany	EDTA, SDS, Raman	Raman	>1	Bottled water - single use PET (10)	0.5-1	2,649 ± 2,857
Oßmann *et al.* (2018)	Germany	EDTA, SDS, Raman	Raman	>1	Bottled water - reusable PET (12)	0.5-1	4,889 ± 5,432
Oßmann *et al.* (2018)	Germany	EDTA, SDS, Raman	Raman	>1	Bottled water – glass (10)	0.5-0.75	6,292 ± 10,521
Mason *et al.* (2018)	Global	Nile red staining	FTIR	>6.5	Bottled water (various)	0.5-2	0-10,390*

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (µm)	Treatment type	Volume sampled (L)	Average microplastic particles/L
		and filtering					
BOTTLED WATER SUMMARY (based on different water types, across different studies)		NUMBER	MIN	MEDIAN	MEAN	MAX	
		96	11	4,889	16,939,064	54,200,000	

*No mean given therefore this study was not considered in calculations

Compared with studies of microplastics in treated drinking water, there have been fewer studies of microplastics in raw water. A thorough overview of concentrations within freshwaters can be found in Horton *et al.* (2017), however little of this research has focussed on rivers, reservoirs or ground waters abstracted for potable water treatment. It is understood that microplastics contaminate river systems worldwide, with the sources of this contamination largely linked to the proximity to population centres and industry (Wagner *et al.* 2014, Horton *et al.* 2017, Li *et al.* 2017). Concentrations within freshwaters are influenced by industrialisation, population, wastewater input, and dilution. As noted with drinking water, Mintenig *et al.* (2019) seem to be finding 6 orders of magnitude fewer microplastics in raw water than (Pivokonsky *et al.* 2018) (Table 2) but then the Czech study (Mintenig *et al.* (2019)) claims to be detecting the smallest particles.

Table 2 Studies investigating microplastics in raw water used for drinking water production. Where one publication is repeated within multiple rows, this refers to different sample types analysed within one study.

Author	Location (number of plants)	Extraction method	Polymer Analysis	Particle size [µm]	Sample type	Volume sampled (L)	Average microplastic particles/L
Raw water							
Pivokonsky *et al.* (2018)	Czech Republic (1)	H_2O_2, size separation, SEM, FTIR and Raman	FTIR and Raman	>1	Large reservoir	27	1,473±34
Pivokonsky *et al.* (2018)	Czech Republic (1)	H_2O_2, size separation, SEM, FTIR and Raman	FTIR and Raman	>1	Small reservoir	27	1,812±35
Pivokonsky *et al.* (2018)	Czech Republic (1)	H_2O_2, size separation, SEM,	FTIR and Raman	>1	River	27	3,605±497

Author	Location (number of plants)	Extraction method	Polymer Analysis	Particle size [μm]	Sample type	Volume sampled (L)	Average microplastic particles/L
		FTIR and Raman					
Mintenig *et al.* (2019)	Germany (5)	HCl, H_2O_2 then $ZnCl_2$, FPA FTIR	FTIR	>20	Ground-water	300-1,000	0.0007
RAW WATER SUMMARY			NUMBER OF WTWs	MIN	MEDIAN	MEAN	MAX
			8	0.0007	0.0007	861.25	3,605

1.3 Current knowledge of microplastics in wastewater discharges

Compared to the clean water side, microplastics in wastewater have been the subject of a fairly large number of studies, and a number of reviews have recently summarised the knowledge to date (Ziajahromi *et al.* 2016, Blair *et al.* 2017, Prata 2018b). It is known that many applications of microplastics will be washed into wastewater systems, for example, microbeads in face scrubs and glitter in cosmetics are designed to be washed off the body and down the drain (Fendall and Sewell 2009, Napper *et al.* 2015). Additionally, laundry wastewater contains fibres shed from a range of synthetic textiles including nylon, polyester and acrylic (Napper and Thompson 2016, Hernandez *et al.* 2017).

Generally most studies in wastewater have looked at microplastics >20 μm in size (Tables 3 and 4). A number of studies have reported decreasing microplastic concentrations as wastewater passes through a WwTW. For example, Murphy *et al.* (2016) found 15.7 microplastics/L in influent, 8.7 microplastics/L after grit and grease removal, 3.4 microplastics/L within primary effluent and 0.25 microplastics/L within final effluent. A similar trend was reported by Gies *et al.* (2018), with 31.1 microplastics/L in influent, 2.6 microplastics/L in primary effluent and 0.5 microplastics/L in final effluent. Dris *et al.* (2015) also saw a decrease in particle concentration throughout the treatment process, although with concentrations were 1-2 orders of magnitude higher than in the other two studies (maximum 260 microplastics/L in influent to maximum 50 microplastics/L in final effluent). This trend is also seen when considering the median data across all studies (Table 3), with influent > intermediate treatment stages > latter treatment stages > final treatment stages (25, 12.55, 0.48, 0.05). There is an enormous variability between reported influent concentrations, ranging from 1 to 15,100,000 microplastics/L (Table 3). It is hard to tell whether such differences are due to changing influent loads (therefore dilution), the effectiveness of different processes, day to day plant management, or the variation in sample processing and analytical techniques or particle size reported by the scientists.

When comparing WwTWs, there are suggestions that some processes and tertiary treatments may be particularly effective at microplastic removal. Talvitie *et al.* (2017a) compared different treatment processes, including membrane bioreactor (MBR) treatment of primary

effluent, and tertiary treatment processes of rapid sand filtration, dissolved air flotation and disc filter. A single MBR was found to be the particularly effective method for removing microplastics, with concentrations in effluent as low as 0.005 microplastics/L, corresponding to 99.9% removal. A yet lower concentration of 0.00088 particles microplastics /L in secondary effluent was reported by Carr *et al.* (2016).

Overall, the studies reviewed here imply a high removal rate (>95%) when comparing microplastics in effluent to those in influent (Magnusson and Norén 2014, Murphy *et al.* 2016, Talvitie *et al.* 2017b). However, while these particles are 'removed' from the wastewater flow they will be transferred to the sludge (Bayo *et al.* 2016, Gies *et al.* 2018, Li *et al.* 2018).

Table 3 Studies investigating microplastics in WwTW influent.

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/L
Wastewater influent						
Blair *et al.* (2019-preprint)	Scotland, UK (1)	H_2O_2 digestion and visual sorting	ATR-FTIR	<2800	Influent and effluent	1-13*
Carr *et al.* (2016)	California, USA (1)	Sieving and visual analysis	FTIR	45-400	Influent	510
Dris *et al.* (2015)	Paris, France (1)	Visual sorting only	None	100-5,000	Influent	260-320*
Gies *et al.* (2018)	Vancouver, Canada (1)	H_2O_2 + oil extraction + visual sorting	FTIR	Not specified	Influent	31.1 ± 6.7
Gundogdu *et al.* (2018)	Seyhan, Turkey (1)	Fenton's + NaI flotation	Raman	>55	Influent for 6 d	26.56 ± 3.18
Gundogdu *et al.* (2018)	Yuregir, Turkey (1)	Fenton's + NaI flotation	Raman	>55	Influent for 6 d	23.44 ± 4.1
Lares *et al.* (2018)	Mikkeli, Finland (1)	Fenton's digestion + visual sorting	FTIR + Raman	250-5,000	Influent	57.6 ± 12.4
Leslie *et al.* (2017)	Netherlands (7)	NaCl flotation only	FTIR	10-5,000	Influent	68-910*
Liu *et al.* (2019)	Wuhan City, China (1)	Fenton's + NaCl flotation	Raman	> 47 um	Influent	79.9 ± 9.3

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/L
Magnusson and Norén (2014)	Lysekil, Sweden (1)	Visual sorting only	FTIR	Not Known	Influent	15,100,000 ± 890,000
Murphy *et al.* (2016)	Glasgow, Scotland (1)	Visual sorting only	FTIR	Not specified	Influent	15.70 ± 5.23
Talvitie *et al.(* 2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	Influent (on Wednesday)	686.7 (±155.0)
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	Influent (on Saturday)	380 (±52.2)
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	Influent (on Monday)	636.7 (±38.8)
WASTEWATER INFLUENT SUMMARY *	NUMBER of treatment plants	MIN	MEDIAN	MEAN	MAX	
	18	0	25	839,024	15,100,000	

*where a range of means were given, these were not included in the following calculations

Table 4 Microplastics reported within intermediate and final stages of wastewater treatment.

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/L
Wastewater Intermediate Stages						
Dris *et al.* (2015)	Paris, France (1)	Visual sorting only	None	100-5,000	After primary settling tank	50-120*
Liu *et al.* (2019)	Wuhan City, China (1)	Fenton's + NaCl flotation	Raman	> 47 um	After primary settling tank	47.4 ± 7.0
Murphy *et al.* (2016)	Glasgow, Scotland (1)	Visual sorting only	FTIR	Not specified	Grit and grease effluent	8.70 ± 1.56
Murphy *et al.* (2016)	Glasgow, Scotland (1)	Visual sorting only	FTIR	Not specified	Primary effluent	3.40 ± 0.28

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/L
Talvitie et al. (2015)	Helsinki, Finland (1)	Visual sorting only	None	Not specified	After primary settling tank	14.2 ± 0.7 fibres
Talvitie et al. (2015)	Helsinki, Finland (1)	Visual sorting only	None	Not specified	After primary settling tank	290.7 ± 28.2 fragments
Talvitie et al.(2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	After pre-treatment (on Monday)	14.2 (±4.0)
Talvitie et al. (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	After pre-treatment (on Wednesday)	10.9 (±2.9)
Talvitie et al. (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	After pre-treatment (on Saturday)	9.9 (±1.0)
INTERMEDIATE STAGES SUMMARY *	NUMBER	MIN	MEDIAN	MEAN	MAX	
	8	3.4	12.55	49.84	290	
Wastewater Latter Stages						
Carr et al. (2016)	California, USA (1)	Sieving and visual analysis	FTIR	45-400	Secondary effluent	0.00088
Dyachenko et al. (2017)	California, USA (1)	Wet peroxide oxidation (Fenton's digestion)	FTIR	125 – 5,000	Secondary effluent	0.2-0.17*
Gies et al. (2018)	Vancouver, Canada (1)	H_2O_2 + oil extraction protocol + visual sorting	FTIR	Not specified	Primary effluent	2.6 ± 1.4
Gundogdu et al. (2018)	Seyhan, Turkey (1)	Fenton's + NaI flotation	Raman	>55	Secondary effluent for 6 d	7 ± 7.64
Gundogdu et al. (2018)	Yuregir, Turkey (1)	Fenton's + NaI flotation	Raman	>55	Secondary effluent for 6 d	4.11 ± 3.18

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/L
Lares *et al.* (2018)	Mikkeli, Finland (1)	Fenton's digestion + visual sorting	FTIR + Raman	250-5,000	Primary effluent	0.6 ± 0.2
Leslie *et al.* (2017)	Netherlands (7)	NaCl flotation only	FTIR	10-5,000	Effluents (various)	51-81
Liu *et al.* (2019)	Wuhan City, China (1)	H_2O_2 + NaCl flotation	Raman	> 47 um	After secondary settling tank	34.1 ± 9.4
Sutton *et al.* (2016)	USA (4)	Wet peroxide oxidation (Fenton's digestion) + visual sorting	None	125->355	Secondary effluent	0.022-0.19*
Talvitie *et al.* (2015)	Helsinki, Finland (1)	Visual sorting only	None	Not specified	After secondary settling tank	13.8 ± 1.6 fibres
Talvitie *et al.* (2015)	Helsinki, Finland (1)	Visual sorting only	None	Not specified	After secondary settling tank	68.6 ± 6.3
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	After activated sludge (on Monday)	1.0 (±0.6)
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	After activated sludge (on Wednesday)	1.3 (±0.9)
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	After activated sludge (on Saturday)	2.0 (±0.2)
Ziajahromi *et al.* (2017)	Australia (3)	H_2O_2 + NaI flotation, Rose Bengal staining, visual analysis	FTIR	25-500	Primary effluent	0.28

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/L
Ziajahromi *et al.* (2017)	Australia (3)	H_2O_2 + NaI flotation, Rose Bengal staining, visual analysis	FTIR	25-500	Secondary effluent	0.48
LATTER STAGES SUMMARY *		NUMBER	MIN	MEDIAN	MEAN	MAX
		21	0.0009	0.48	6.5	69
Wastewater Final effluent						
Carr *et al.* (2016)	California, USA (7)	Sieving and visual analysis	FTIR	45-400	Tertiary effluent	0
Dris *et al.* (2015)	Paris, France (1)	Visual sorting only	None	100-5,000	Final effluent	14-50*
Gies *et al.* (2018)	Vancouver, Canada (1)	H_2O_2 + oil extraction protocol + visual sorting	FTIR	Not specified	Secondary effluent	0.5 ± 0.2
Lares *et al.* (2018)	Mikkeli, Finland (1)	Fenton's digestion + visual sorting	FTIR + Raman	250-5,000	Final effluent	1 ± 0.4
Liu *et al.* (2019)	Wuhan City, China (1)	H_2O_2 + NaCl flotation	Raman	> 47 um	After chlorination	28.4 ± 7.0
Magni *et al.* (2019)	Northern Italy (1)	NaCl flotation, H_2O_2 digestion	FTIR	Not Known	Final effluent	0.4 ± 0.1
Magnusson and Norén (2014)	Lysekil, Sweden (1)	Visual sorting only	FTIR	Not Known	Effluent	8250 ± 850
Mason *et al.* (2016)	USA (17)	Fenton's digestion, size separation, visual sorting only	None	125->355	Final effluent	0.05 ± 0.024

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/L
Mintenig *et al.* (2017)	Germany (12)	SDS, enzymes, H_2O_2, ZnCl2 flotation	FPA FTIR	10-500	Final effluent (different treatment types)	0.08-9*
Mintenig *et al.* (2017)	Germany (12)	SDS, enzymes, H_2O_2, ZnCl2 flotation	ATR FTIR	>500	Final effluent (different treatment types)	0-0.04*
Murphy *et al.* (2016)	Glasgow, Scotland (1)	Visual sorting only	FTIR	Not specified	Final effluent	0.25 ± 0.04
Sutton *et al.* (2016)	USA (4)	Wet peroxide oxidation (Fenton's digestion) + visual sorting	None	125->355	Tertiary effluent	0.047-0.13*
Talvitie *et al.* (2015)	Helsinki, Finland (1)	Visual sorting only	None	Not specified	Final effluent	4.9 ± 1.4 fibres
Talvitie *et al.* (2015)	Helsinki, Finland (1)	Visual sorting only	None	Not specified	Final effluent	8.6 ± 2.5 fragments
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	Final effluent (sampled on Monday)	3.2 (±0.7)
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	Final effluent (sampled on Wednesday)	3.2 (±0.7)
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	Final effluent (sampled on Saturday)	3.5 (±1.3)
Talvitie *et al.* (2017a)	Mikkeli, Finland (1)	Visual sorting only	FTIR	20->300	MBR	0.005
Talvitie *et al.* (2017a)	Turku, Finland (1)	Visual sorting only	FTIR	20->300	Rapid sand filter	0.02
Talvitie *et al.* (2017a)	Hämeenlinna, Finland (1)	Visual sorting only	FTIR	20->300	Dissolved air flotation	0.1

Author	Location (number of plants)	Extraction method	Polymer analysis	Particle size (μm)	Sample type	Average microplastic particles/L
Talvitie *et al.* (2017a)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	Disc filter	0.03-0.3*
Ziajahromi *et al.* (2017)	Australia (3)	H_2O_2 + NaI flotation, Rose Bengal staining, visual analysis	FTIR	25-500	Tertiary effluent	1.54
FINAL EFFLUENT SUMMARY	NUMBER	MIN	MEDIAN	MEAN	MAX	
	41	0	0.05	203	8,250	

*where a range of means were given, these were not included in the following calculations

Note: Studies investigating microplastics in WwTW intermediate treatment stages and final effluent across various treatment types. Where one publication is repeated within multiple rows, this refers to different components within one study. The results are classified in good faith but may on occasions be ascribing the actual stage incorrectly.

1.4   Microplastic presence in wastewater sludge from WwTW and WTW

Few studies have been carried out to date on the presence of microplastics within sewage sludge, and none, as far as is known, on microplastics in WTW sludge. This lack of data across sludge in general is primarily due to the methodological limitations and challenges of extracting microplastics from such a complex organic media.

Sludge is often applied to land as a soil conditioner and fertiliser, and studies have shown that microplastics within sludge are likely to accumulate within soils (Nizzetto *et al.* 2016), being retained as long as 15 years post-sludge application (Zubris and Richards 2005). Microplastics in sludge could potentially be mobilised in intense rainfall runoff events to enter surface waters.

Table 5 reports the studies that have been carried out to examine sludge for microplastics to date. Even as recently as 2018, authors have been simply visually identifying and counting microplastics from sludge without any prior digestion or flotation. This is likely to lead to misleading results: either under-reporting due to many particles being missed as they are obscured by organic matter, or over-reporting where one subjectively reports on, and multiplies up, a corner of the slide or filter where several particles were spotted.

Table 5 Microplastics reported in different forms of wastewater sludge.

Author	Location	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/g DW
WwTW Primary sludge						
Gies *et al.* (2018)	Vancouver, Canada	Water flotation + H_2O_2 + oil extraction protocol + visual sorting	FTIR	Not specified	Primary sludge	14.9 ± 6.3
WwTW Secondary sludge/biofilms						
Carr *et al.* (2016)	Los Angeles, USA (1)	Sodium hypochlorite digestion and manual sorting	FTIR	Not specified	Biosolids	1
Gies *et al.* (2018)	Vancouver, Canada (1)	Water flotation + H_2O_2 + oil extraction protocol + visual sorting	FTIR	Not specified	Secondary sludge	4.4 ± 2.8
Lares *et al.* (2018)	Mikkeli, Finland (1)	No digestion - visual sorting only	FTIR and/or Raman	Not specified	Activated sludge	23 ± 4.2
Lares *et al.* (2018)	Mikkeli, Finland (1)	No digestion – visual sorting only	FTIR and/or Raman	Not specified	Membrane bioreactor sludge	27.3 ± 4.7
Magni *et al.* (2019)	Northern Italy (1)	NaCl flotation, H_2O_2 digestion	FTIR	Not known	Recycled activated sludge	113 ± 57
SECONDARY SLUDGE/BIOFILM SUMMARY *		NUMBER	MIN	MEDIAN	MEAN	MAX
		5	1	23	33.74	113

Author	Location	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/g DW
Digested sludge						
Lares *et al.* (2018)	Mikkeli, Finland (1)	No digestion – visual sorting only	FTIR and/or Raman	Not specified	Digested sludge	170.9 ± 28.7
Leslie *et al.* (2017)	Netherlands (7)	NaCl flotation + visual sorting	None	Not specified	Sludge	5.1-7.6 (wet weight)*
Li *et al.* (2018)	China (28)	NaCl flotation, H_2O_2 digestion	FTIR	Not specified	Sludge	22.7 ± 12.1
Liu *et al.* (2019)	Wuhan City, China (1)	NaCl and NaI flotation + H_2O_2	Raman	20-5,000	Dewatered sludge	240.3 ± 31.4
Magnusson and Norén (2014)	Långeviksverket in Lysekil, Sweden (1)	Visual sorting only	None	>300	Sludge	16.7 ± 1.6
Mahon *et al.* (2017)	Ireland (7)	Elutriation (water) + $ZnCl_2$ flotation	None	<45->4,000	Sludge (various types)	4.1-15.4* across all sludge types
Mintenig *et al.* (2017)	Germany (6)	NaOH then HCl then NaCl flotation	FPA FTIR + ATR FTIR	10->500	Sludge	1-24*
Murphy *et al.* (2016)	Glasgow, UK (1)	Visual sorting only	FTIR	Not specified	Sludge cake	19
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	Excess sludge	76.3 ± 4.3
Talvitie *et al.* (2017b)	Helsinki, Finland (1)	Visual sorting only	FTIR	20->300	Dried sludge	186.7 ± 26.0
Zubris and Richards (2005)	New York, USA (1)	Water flotation and visual sorting	None	Not specified	Dewatered sludge	4
Zubris and Richards (2005)	New York, USA (1)	Water flotation and visual sorting	None	Not specified	Pelletised sludge	3

Author	Location	Extraction method	Polymer analysis	Particle size (µm)	Sample type	Average microplastic particles/g DW
Zubris and Richards (2005)	New York, USA (1)	Water flotation and visual sorting	None	Not specified	Alkaline stabilised sludge	2
Zubris and Richards (2005)	New York, USA (1)	Water flotation and visual sorting	None	Not specified	Composted sludge	2
DIGESTED SLUDGE SUMMARY *		NUMBER	MIN	MEDIAN	MEAN	MAX
		38	2	22.7	35.7	240.3

*where a range of means were given, these were not included in the following calculations

Note: Studies investigating microplastics in sewage sludge. Where one publication is repeated within multiple rows, this refers to different sample types within one study. In some instances microplastics were not quantified but were simply characterised; these studies are not included here.

1.5   Best methods to assess type, size and quantity of microplastics

There are three fundamental steps to obtain a quantified final result for microplastics within a sample. These are appropriate 1) field sampling, 2) laboratory processing, and 3) quantification and polymer analysis.

1.5.1 Sampling in the field

Sampling techniques for wastewater can be variable in both methods and volumes sampled. For example, sampling can consist simply of dropping a container such as a bucket into a flow and then processing the collected liquid – e.g. around 5-30 L (Murphy *et al.* 2016, Blair *et al.* 2019-preprint). Other studies directly filter microplastics out of the waste stream in situ (Carr *et al.* 2016, Sutton *et al.* 2016, Ziajahromi *et al.* 2017), allowing thousands of litres to be sampled. Large sample volumes (minimum hundreds of litres) are recommended wherever possible, in order to get a sufficiently representative sample, especially where the water is expected to be relatively clean. Collection of composite samples over 24 h is recommended to take into account the temporally variable input concentrations and to assess removal efficiency during treatment.

1.5.2 Sample processing

A great many different techniques and methods for processing samples have been reported. It is not within the scope of this review to cover these all in detail. For samples with a high organic content, for example - especially with wastewater influent and sludge, it is recommended to carry out an organic digestion. This can consist of acid or alkaline digestion,

for example hydrochloric acid, hydrogen peroxide or sodium hydroxide (Cole *et al.* 2014, Hurley *et al.* 2018) and/or enzymatic digestion (Cole *et al.* 2014, Löder *et al.* 2017), ideally using both in succession. More recently, Fenton's reagent has been determined to be very effective for organic digestion, with little to no damage caused to microplastic particles (Tagg *et al.* 2017, Hurley *et al.* 2018). Additionally, samples containing dense particles, such as sludge, benefit from a flotation step in a dense salt solution such as zinc chloride or sodium iodide (density > 1.6 g/cm^3 to ensure flotation of relatively dense polymers such as PVC) (Mahon *et al.* 2017, Hurley *et al.* 2018).

1.5.3 Quantification

The majority of microplastic studies to date have used visual (manual) identification of particles for analysis as a putative plastic. However, it is recognised that this method is subjective and can lead to a high level of bias, producing false positives (natural particles identified as microplastics) or false negatives (microplastics particles missed), with error rates as high as 70% (Syberg *et al.* 2015, Lusher *et al.* 2017). A recently proposed method for particle quantification (and even, to some extent, polymer analysis) is staining using Nile Red dye and then fluorescence image analysis (Erni-Cassola *et al.* 2017, Maes *et al.* 2017). This method relies on 100% removal of organic matter which is very difficult with real environmental samples. Therefore, visual methods seem insufficiently robust for real environmental matrices and examining many samples.

Methods for microplastic quantification and analysis are rapidly developing, moving from manual sorting and counting methods to automated image analysis or mapping. Currently, the most common approaches to polymer composition analysis is by RAMAN or FTIR spectroscopy. However, these methods require considerable time to run and generate very large amounts of data from one sample. Thus, the majority of studies select a sub-sample for analysis down to spectroscopic composition level, leaving the majority of particles unresolved as to polymer type. For example Lares *et al.* (2018), while recognising that particles may be visually misidentified to be plastics, only spectroscopically analysed 1.4 % all particles extracted. Additionally, it is not always made clear what criteria are applied to selecting a sub-sample, leading to possible bias in particle selection which is a concern (Koelmans *et al.* 2019).

This fast pace in method development is clear within the literature when looking at studies published even just 2-3 years ago, compared to the most recent academic papers. This becomes especially evident when looking at publications by the same author(s), for example Talvitie *et al.* (2015) did not employ any spectroscopic polymer analysis, but from 2017 onwards were using this spectroscopy to analyse to polymer type in line with evolving recommendations for analytical quality (Talvitie *et al.* 2017b). However, despite recognition of the need for high-quality and unbiased analytical methods to include polymer analysis (for the purposes of eliminating false positives in addition to allowing an understanding of source), many studies continue to be published which do not employ any spectroscopic or chemical analysis of particles (Mahon *et al.* 2017, Kosuth *et al.* 2018).

In theory, it should be possible to compare results within a single study (for example, influent to effluent), but the lack of standardisation between scientists working in this field makes comparison between studies, even by the same author(s) very uncertain. In addition to possible variability in the efficiency of particle extraction between studies, this applies also

where studies report different particle sizes. For example, when comparing influent concentrations, Carr *et al.* (2016) reported 510 microplastics/L of a size greater than 45 µm, while Lares *et al.* (2018) reported 57.6 microplastics/L in influent greater than 250 µm. It is therefore uncertain whether there were in fact a number of particles 45-250 µm in size, that only Carr *et al.* (2016) were able to report. In a study of German sewage effluent (Oldenburgisch-Ostfriesischer Wasserverband 2016) the authors were able to identify microplastics down to as low as 10 µm, but also quoted numbers above 25 µm for the same samples, to allow comparisons with their previous studies (Mintenig *et al.* 2014a, Mintenig *et al.* 2017). Only 27% ±4% (standard deviation) of the total particles quantified (10-5,000 µm) were above 25 µm. Many studies do not even report the particle size that they analysed, making such inferences impossible.

1.5.4 Selection of the most suitable methods for this project

With this information, a selection was made for methods to be used based on the current state-of-the-art for microplastic sampling, processing and analysis. In short, all sample processing was carried out within clean conditions: all equipment was thoroughly washed with reverse osmosis water, filtration units were sealed before leaving the laboratory for field sampling and sample processing was conducted within a filtered-air safety cabinet to reduce any risk of airborne contamination. In terms of processing, a range of digestion methods based on the literature recommendations, including enzymatic digestion (cellulase and trypsin, all samples) and Fenton's digestion (samples with high organic content: raw water, influent, effluent and sludge), in addition to flotation (samples with dense inorganic matter: sludge) were employed. Finally, for particle quantification and polymer analysis linear array FTIR was selected based on the most up-to-date recommendations for microplastics analysis. This can currently map 92% of the filtration surface of a 13 mm filter disc and in future it is expected that it will be possible to analyse the entire filter surface, analysing all the particles from an entire sample. Particles are subsequently quantified, measured and analysed all within the same software package (MPhunter, Aalborg University, Denmark), thereby eliminating any subjective human bias. This method can identify particles as small as 6.25 µm, and therefore allows for the analysis of particles much smaller than those that could be manually manipulated and identified. The major advantage of the FTIR with MPhunter is that it takes out the human bias inherent in the usual visual selection typically associated with RAMAN approaches.

Chapter 2   Methodology

2.1   General approach to limiting microplastic contamination

Ambient microplastics in the indoor environment present a significant risk of contamination of samples during their handling in the laboratory. This potential contamination is acknowledged widely, but it is rare that specific measures taken to limit this contamination are documented in the literature (Koelmans *et al.* 2019). To minimise contamination, all processing took place in the Microflow Biological Safety Cabinet, fitted with HEPA filters that remove 99.999% of particles >0.3 µm in size. The only exception to this was the initial disaggregation, sieving and weighing of the dried sludge samples, which was performed on the benchtop in a separate laboratory room, as the dust produced during this process would contaminate the safety cabinet. Cotton lab coats were worn at all times to avoid shedding of artificial polymer fibres and subsequent contamination of the samples. Nitrile gloves were only worn when required by the COSHH assessment for specific reagents. All glassware and equipment used to handle samples was washed thoroughly immediately prior to use, as described in the general procedures (Appendix A Table 1). All rigs used in the field were also washed in this way before assembly. The rigs were assembled within the safety cabinet before being taken into the field. Filter units were only disassembled once back within the laboratory, within the safety cabinet. To avoid contamination from the reagents themselves, all reagents were filtered through a 1.2 µm glass-fibre filter before use and PTFE lined lids were used to seal glass bottles containing reagents instead of the conventional blue polypropylene lids. All stainless steel filter discs were heated in a muffle furnace at 350°C for 180 minutes to eliminate any microplastics potentially contaminating the filter. At the end of the day, all equipment used was washed thoroughly in detergent with a natural fibre scouring pad, rinsed with RO water and covered with foil. The presence of microplastic contamination was assessed through blanks that are described in more detail in section 2.5.

2.2   Sample collection

2.2.1   Collecting raw and potable water

The method requires filtering a large volume of water at the WTW site and returning the particles collected on a steel 10 µm filter to the laboratory for processing. It was modified from that used in Mintenig *et al.* (2014b), with the significant difference of using stainless steel or aluminium filter holders instead of plastic.

A schematic of the sampling equipment is shown in Figure 1 and a photo in Appendix A Figure 1. For the raw water, a large stainless steel filter holder (Spectrum Inox economic filter housing, EFH-SBR) with a woven stainless steel 10 µm cylindrical filter cartridge inside (24.77cm length, ca 500 cm^2 filter area, Wolftechnik Germany) was used. A detailed description of the materials and methods is given in Appendix A.

Figure 1 Schematic sampling setup for raw and potable water.
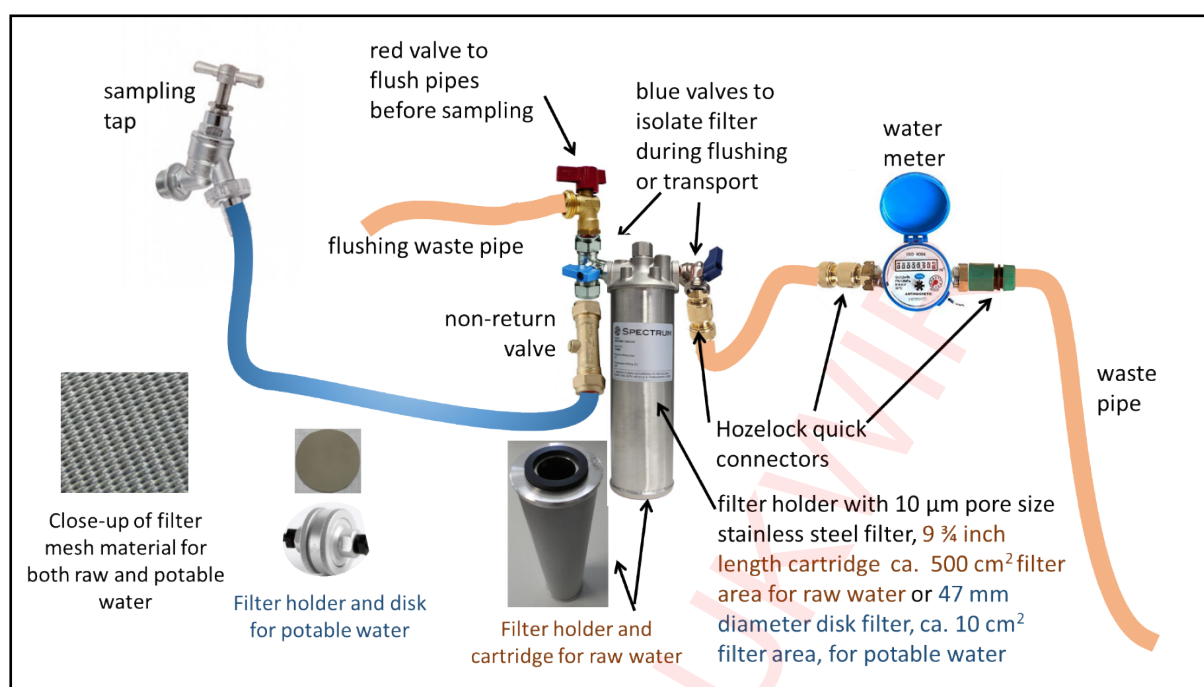
The setup for the potable water rig is the same as described above for raw water, except that a smaller filter unit was used (47 mm anodised aluminium filter holder, Pall Life Sciences, Advantec) with a woven stainless steel filter disc (same filter material as the cartridge filter above, 10 µm pore size, Wolftechnik, Germany). Given the expectation of much lower suspended solids in potable water, it was deemed more convenient to use this smaller filter set-up. This provided a filter area of about 10 cm² compared to the ca. 500 cm² of the larger units.

For each WTW, CEH staff visited to demonstrate the use of the equipment and collect the first sample. The samples were collected by attaching the sampling rigs to taps at the WTW and allowing water to flow through overnight (Appendix A Figure 1, Appendix A Figure 2). After the first visit, CEH posted the equipment and water treatment works staff carried out the sample collection and returned the filter units to CEH for processing. A detailed instruction sheet was sent along with the equipment. This was modified a few times in light of experience and the final version is shown in E. Usually several hundred to several thousand litres of water were filtered (see Table 6 and Appendix B Table 2).

2.2.2 WwTW influent and effluent sampling

To ensure the samples were representative, 24 h composite sampling was used using an ISCO Avalanche refrigerated autosampler. Both influent and effluent samples were collected at the same time. Sampling typically started around midday and was completed at midday on the following day. Composite influent samples were collected at each WwTW after the influent screen and programmed to collect 100 mL every 30 min to collect a total of 4.8 L in a 5 L Schott Duran glass bottle. This was thoroughly mixed before two sub-samples of about 0.5 L were poured into further glass bottles for storage at -18°C and later processing as described in Appendix A section A 5.

For final WwTW effluent, the same model of autosampler was employed with the sample volume set to 7.5 L every 30 min (nominally 48 * 7.5 L = 360 L). In this case, the sample was pumped through a filter cartridge setup identical to that of the raw water sampling but without the non-return valve (Figure 2). The volume put through the filter cartridge was measured by a water meter.

Figure 2 Schematic sampling setup for WwTW effluent.
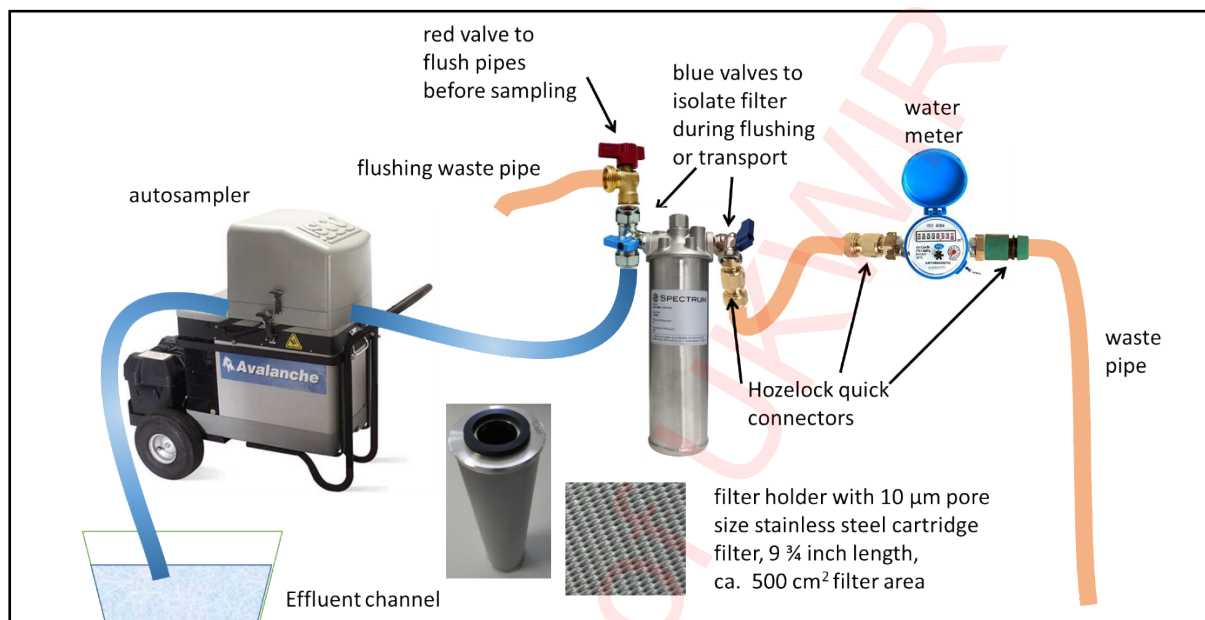
2.2.3 Sludge collection from WwTW and WTW

All sludge was collected in 1 L Kilner jars with aluminium foil between the jar and the lid to avoid contact with the rubber coating on the inside of the lids (Table 6). The sludges from three of the four WTW were liquid and could be poured into the jar, whereas those from WwTW and the fourth WTW were quite solid. In that case a metal trowel or similar was used to collect the sample. Where possible, the WwTW sludge cake sample was taken at the point just before it entered the cake pad, typically as it fell off the bench press. Where this wasn't possible it was taken directly from the fresh cake pad. The protocol is included as Appendix F. The first WTW sludge sample was collected by CEH staff and the second one by water company staff. For the WwTW sludge, five samples were collected from each site, most of those by water company staff.

Sludge samples were initially stored frozen at -18°C. The samples were then dried by replacing the metal disc in the lid with a glass fibre filter (Whatman GF/C, 1.2 µm) to provide some ventilation while keeping dust out and leaving them in an oven at 50°C until dry (about 1 week).

Table 6   WTW sampling sites: DA-dissolved air flotation, HBC-Hopper bottom clarifier, RGF-rapid gravity filter, SSF-slow sand filter, GAC-granular activated charcoal.

Code	Description	Treatment	Mean output (m3/d)	Number, type of samples and total volumes collected
LR1	Lowland river, direct abstraction	GAC, membrane, UV/H_2O_2, GAC, disinfection	20,000	5 raw (total 4.3 m^3) 5 potable (total 14.7 m^3)
LR2	Lowland river, direct abstraction	HBC, RGF, GAC, disinfection	122,000	5 raw (total 6.5 m^3) 5 potable (total 17.2 m^3) 2 sludge samples
LR3	Lowland river, direct abstraction	Disinfection, pH balancing, static mixer, clarifier with $FeCl_3$ & polyelectolyte coagulation, RGF, GAC, micro screen	55,000	4 raw (total 98.6 m^3) 4 potable (total 29.8 m^3) 2 sludge samples
LRS1	Lowland river, pumped storage	DAF or HBC, RGF, GAC, disinfection	79,000	5 raw (total 3.9 m^3) 5 potable (total 6.7 m^3) 2 sludge samples
LRS2	Lowland river, pumped storage	Reservoir with SSF, RGF, ozone, SSF, disinfection	650,000	5 raw (total 18.1 m^3) 5 potable (total 28.0 m^3)
GWC	Groundwater, chalk	Disinfection	2,500	5 potable (total 30.4 m^3)
GWS	Groundwater, greensand	Aeration and pressure, filtration, disinfection	5,100	5 potable (total 27.1 m^3)
UR	Pristine upland reservoir	$Al_2(SO_4)_3$ coagulation, RGF, disinfection, pH balancing, UV	11,000	5 raw (total 10.8 m^3) 5 potable (total 4.1 m^3) 2 sludge samples

Table 7 WwTW wastewater sampling sites.

Site	Description	PE	DWF (m³/d)	L/PE	Number and type of samples
ASTC1	activated sludge + cloth filter	264,000	56,160	213	2 influent 2 final effluent
ASTS1	activated sludge + sand filter	90,000	20,394	227	2 influent 2 final effluent
ASTS2	activated sludge + sand filter	38,000	11,476	302	2 influent 2 final effluent
ASTC2	activated sludge + cloth filter	157,000	40,300	257	2 influent 2 final effluent
TFP	plastic trickling filter	40,000	12,860	321	2 influent 2 final effluent
AS1a*	activated sludge	320,000	70,000	219	2 influent 2 final effluent
AS2b* TFSb*	activated sludge (AS) or stone trickling filter (TF)	103,348	27,500	266	2 influent 2 secondary effluent AS 2 secondary effluent TF
BAFF	biological aerated flooded filter (BAFF)	42,350, peak 54,350	9,484 av and 11,825 permitted	217-224	2 influent 2 final effluent

*Plants with the suffix 'a' or 'b' are the same. These sites are partners with sludge, influent and effluent collected at the same works

Table 8 WwTW sludge sampling sites.

Site	Description	Number and type of samples
AAD1a*	Advanced anaerobic digestion	5 sludge cake samples
AAD2	Advanced anaerobic digestion	5 sludge cake samples
LS	Limed sludge	5 sludge cake samples
ADb*	Conventional anaerobic digestion	5 sludge cake samples
AAD3	Advanced anaerobic digestion	5 sludge cake samples

*Plants with the suffix 'a' or 'b' are the same. These sites are partners with sludge, influent and effluent collected at the same works

Figure 3 Liquid sludge at LRS1 WTW, various sludge samples dried in jars with air permeable glass fibre filter lids, solid sludge at LR2 WTW and AAD1a WwTW.

2.3   Sample processing

2.3.1 Sample processing philosophy

The principle of the sample processing is to strip away the various organic and proteinaceous material, which may be present on the surface of the microplastic particles and so mask them from the FTIR. The methods and workflows developed for each sample type are a balance between efficacy at removing this material whilst limiting the potential for losses or degradation of microplastic material. Practical implications concerning the time required to process each sample must also be taken into account. The different sample types (e.g. potable water, raw water, sludge) required varying degrees of processing in order to extract and prepare microplastics in the sample for analysis by FTIR. Two digestion steps and one specialist separation step were employed depending on the sample type. These were: a) enzyme digestion, b) Fenton's reaction to chemically degrade general organic matter, c) density separation of microplastics through flotation in zinc chloride.

Between each processing step, it was necessary to remove the reagents from the previous step, whilst retaining the microplastics. This required filtration of the sample with a vacuum pump to retain the microplastics on a 10 µm stainless steel filter, allowing the liquid to be discarded. The particles were then dislodged from this filter and suspended in the relevant reagent for the subsequent processing step. An overview of the different procedures are shown in Table 9.

Table 9 Summary of the different processing steps required for each of the five sample types analysed in the project.

Processing step	Sample type				
	Potable water	Raw water	WWTW influent	WWTW effluent	Sludge
Subsampling	✓	✓	✓	✓	✓
Fenton's Reaction	✗	✓	✓	✓	✓
Density separation	✗	✗	✗	✗	✓
Enzyme digestion	✓	✓	✓	✓	✓
Coarse and fine fractions	✗	✗	✗	✗	✓
Storage in ethanol	✓	✓	✓	✓	✓

2.3.2 General approach to processing the field-filtered samples (raw water, potable water, wastewater effluent)

The basic approach was as follows:

1. Washing of the particles from the filter taken from the field rig and dispersion in < 1L water

2. Division of the original dispersed sample for storage or processing

3. *(This step is skipped for potable water samples:* Fenton's reaction to oxidise general organic materials)

4. Protein and complex carbohydrate removal by enzymatic digestion

5. Preservation of sample in 50% ethanol prior to analysis by FTIR.

2.3.3 General approach to processing sludge samples (WTW and WwTW)

The general workflow for processing sludge samples was as follows:

1. Drying the sludge for storage and to get a dry sample for subsequent processing

2. Sub-sampling 1 g of material following breaking up aggregated material and sieving to <1 mm

3. Fenton's reaction to oxidize organic material

4. Density separation through flotation in concentrated $ZnCl_2$ solution to remove heavier residuals

5. Protein and complex carbohydrate removal by enzymatic digestion

6. Filtering to "coarse" (>178 µm) and "fine" (<178 µm) fractions and dispersion in 50% ethanol for storage prior to analysis by FTIR

2.4 Types of plastic polymers

For this study a decision was taken to report on the following plastic polymers; acrylonitrile butadiene styrene (ABS); polyamide (PA); polyethylene (PE); polyethylene terephthalate (PET); poly(methyl methacrylate) (PMMA); polypropylene (PP); polystyrene (PS); polyvinylchloride (PVC); and polyurethane (PU). There are many 100s of plastic polymer variants. All, or almost all, are recorded by the FTIR and MPhunter combination. The selection made for reporting was based on their ubiquity and reported presence of these polymers by others in water (Koelmans *et al.* 2019).

2.5 Blank preparation and spike recoveries

2.5.1 Blanks

There is a need for a rigorous approach to correcting for microplastic contamination, particularly when looking for the presence of such particles from clean environments such as potable water. The blanks were prepared as simulating the potable, raw water / influent / effluent and sludge processing steps.

For the liquid samples, these blanks started by setting up the filter rig to run with clean water (RO water filtered to 2 µm to remove any microplastics above this size) with the 10 µm filter disc or cartridge deployed as in the field. More details of this process are given in Appendix A.

The sludge blanks followed the processing steps exactly as for a real sample, but without including the dried sludge material. Thus, five replicates for blanks were processed using the Fenton's reaction, $ZnCl_2$ flotation and enzymatic digestion. Ideally, more sludge blanks would have been run, however, there was less concern about the sludge results as it was assumed this matrix would contain high levels of plastics. This proved to be correct, as high values in

the sludge were recorded. Essentially, the blanks allowed corrections of the raw field data to be carried out to prevent 'false positives' being reported.

2.5.2 Limit of detection (LOD) and limit of quantification (LOQ)

Given that the aim of this research was to accurately quantify microplastic particles in samples, it was necessary to calculate the limit of detection (LOD) and limit of quantification (LOQ) for the analytical process for each polymer. For this study LOD was defined as the mean of the blank samples plus 3.3 x the standard deviation of the blank. The LOQ is expressed as the mean of the blank samples plus 10 x the standard deviation of the blank. This is the recommended approach of the Association of Official Agricultural Chemists (AOAC International), an internationally recognised body who recommend methods for chemicals in food and the environment (AOAC 2011). No microplastics were detected on the unused silver filters used in the FTIR analysis.

2.5.3 Approach used to correct for the blanks and report with respect to LOD and LOQ

A set of ten blanks was run for the potable water examination and eight blanks to simulate the raw water, wastewater influent and effluent processing. Five blanks were run to represent the sludge process.

In general, the results were calculated as follows:

- For each polymer, the mean blank value was subtracted from the raw count for a sample.

- If the corrected value was then above the LOD it counted as detected and if above the LOQ value it was allowed as being quantifiable.

- The resulting value was quantified by referral to the original volume (or weight in the case of sludge) of sample used for processing and the proportion of the final processed sample that was transferred to the silver disc used in the FTIR. In some cases only a 200 μL drop from the stored sample in ethanol (between 5-15 mL) was spread onto the disc because the sample contained many particles (plastic and non-plastic). This was not necessary with the potable water samples, where the whole processed sample could be transferred onto the silver filter.

- This correction to volume processed would inevitably influence the LOD and LOQ value when converted to a volumetric or weight unit. Thus, if only a small sample volume was collected in the field and only a fraction of the processed sample was placed on the silver filter for FTIR, these gave the highest (least sensitive) LOD and LOQ values.

A detailed worked example of calculations taking account of blanks and LOD/LOQ is given in Appendix D.

2.5.4 Spike recoveries

In contrast to the blanks, where the concern is with false positives, the principle behind the spike recoveries is to quantify the losses of microplastics that may occur during the processing steps. This would lead to under-reporting of the true quantity present. Some authors refer to spike recovery as 'positive controls'. In order to reduce the potential for losses, limiting the number of vacuum filtrations and processing steps required for each sample was a consideration in the initial method development. The spike recovery effort carried out in this project may only be considered preliminary as the recovery of only one polymer and one size was attempted.

Sludge samples had the greatest number of processing steps and so the highest potential for losses. Therefore, spike recoveries were performed with a real sludge sample to provide an insight into a "worst case" percentage recovery for the extraction of microplastics from the various environmental media. Spike recoveries from the sludge matrix were performed with five replicates of a single sludge sample spiked with a known concentration of polyamide (PA) particles (size range between 63-90 μm) dispersed in RO water and Tween (0.025%) and processed as a normal sample. The PA polymer particles are environmentally relevant (PA is a common polymer in synthetic fibres and this polymer is denser than water at ~1.15 g/cm^3) and have the advantage of having a low presence in the blank samples. The recoveries were calculated as a percentage of the total particles added to the sample.

2.6 Microplastic analysis by FTIR

2.6.1 Overview

The project had originally intended to use Nile Red staining followed by RAMAN analysis to quantify the microplastics. The drawbacks of the method being unable to deal with the most moderate presence of interfering organics, the high work load for the operator, and the potential for human bias led to this analytical approach being discarded.

Instead, measurement of microplastic numbers, composition and size were carried out using a Fourier Transform Infrared (FTIR) spectroscopy. After a review of different models, CEH decided to purchase and commission a Perkin Elmer Spotlight 400 FTIR for this project. FTIR is a technique used to obtain an infrared spectrum of light absorption from a material. The pattern of absorption (wavelengths and intensity) provides information about the molecular composition of a material, and this spectral 'fingerprint' can be used to identify unknown materials, providing that fingerprint is present in a database of known materials. This particular FTIR microscope has the added advantage of being able to generate a 2D spectral map of the sample by independently moving the microscope stage in small steps and collecting spectra at each step. These spectra, combined together, create a high resolution spatial map of material composition across a sample. The silver membrane filter, which these samples are filtered onto, reflects infrared light, so the light passes through the particles (if present) and is then reflected back through the particle to the FTIR detector (a mode known as transflectance). The subsequent spectral map, comprising of many hundreds of thousands of individual spectra, can then be interrogated by comparing the correlation of the component spectra with a database of target polymers. Groups of spectra that correlate with

the database are identified as particles, and the particles are sorted into polymer types and counted.

2.6.2 Resolution

Mapping was carried out using 4 accumulations (i.e. four scans per spectra) at a resolution of 25 μm per pixel, which reflects a trade-off between mapping time and spectral quality. Mapping the 11.6 x 11.6 mm area took approximately 2 h 30 minutes per sample and generated a 700 MB data file. If it had been decided to map at the finer 6.25 μm per pixel resolution and cover the entire filter disc area, this would have taken a long time. The FTIR would have to record single 1 x 1 mm squares and then be manually re-set to scan the next square. Each square would yield a 700 MB file. Each square would then be analysed independently before the whole result would be stitched together in a 70 GB file and this would take several days. Given that most efforts at quantitation in the literature are reported as particles >20 μm it was considered this resolution was sufficient given the task of the project to visit and re-visit many sites.

2.6.3 Confirmation of microplastic polymer

The software programme, MPhunter, first converts Perkin Elmer files into a format that can be read by MPhunter. Converted files are then loaded into the software along with a custom polymer database comprising reference spectra of target plastics. Due to the short time-scale of this project, it was decided to focus on a sub-set of nine common plastic polymers. There are many rare polymer types, but quantifying such additional targets in the reference database would have added considerably to the analysis time. The most common plastics in the reference database, supplied with MPhunter, were acrylonitrile butadiene styrene (ABS), polyamide (PA), polyethylene (PE), polyethylene terephthalate (PET), poly(methyl methacrylate) (PMMA), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC-U), and polyurethane (PU). Spectra were matched against this database using a threshold of 0.65 (where 1.0 is a perfect match and 0 is a complete mismatch). There is the potential to optimise the matching process by defining different thresholds for different polymers as well as sample types. The uncertainty here is related to how other macromolecules present may be coating the polymer and the degree to which weathering may alter the spectra. However, it was decided to take the conservative approach of applying the same 0.65 threshold across all polymers. There is currently no established practice for thresholds in the literature. The 0.65 threshold was chosen as a compromise between allowing for spectral modifications that occur when microplastics weather in the environment and having a reasonable confidence in the polymer assignment.

2.6.4 Reporting of results by the FTIR

The primary approach to reporting in this project was by giving the number of microplastic particles >25 μm of nine different polymers at a 0.65 confidence level as generated by the MPhunter software without any human intervention. The MPhunter software struggles to quantify long thin, fibre-like particles. It is possible to get a report on the size distribution of particles found. In theory, it might be possible to infer from the polymer and shape what the mass of the particle could be. This could then be extended to calculate the mass of all particles of all the polymers found in the sample. However, this could not be done with

confidence as there is uncertainty on this mass conversion and so was not attempted in this project.

2.7 Review of quality control

The most comprehensive review to date of studies in the field of microplastics and water used a check list to evaluate the quality of papers (Koelmans *et al.* 2019). This study evaluated quality using a scoring system. Partially met criteria had a score of 1 and fully met had a score of 2. Thus, from 9 steps a maximum score of 18 was possible. If this scoring system were applied to the methods used in the project, it would score highly (Table 10).

Table 10 Evaluation of the quality of the methods used in this study against the Koelmans *et al.* (2019) scoring approach (score of 1 is partially met and score of 2 is fully met).

Inclusion and reporting	Score
Sampling site, date and materials used fully described	2
Adequate sample size taken	2
Container cleaning reported	2
Precautions to avoid Lab contamination given	2
Clean air conditions used	2
Negative controls used (blank correction)	2
Positive controls used (spike recovery)	1
Processing/digestion step reported	2
Polymer ID	2
Total score	17

To date none of the previous studies evaluated have scored above 14 (Koelmans *et al.* 2019). The only item not fully met in this study is that of positive controls (spike recovery) and this is discussed in the report. Nevertheless, judged by the Koelmans' standard this project should be seen as meeting the highest quality standards.

Chapter 3   Results

3.1   Assessment of microplastic particles in Water Treatment Works

3.1.1 Nature of the results

In the results the reports are for:

- A limited number of plastic polymers (9).

- Only microplastic particles larger than 25 µm (this is the pixel size that was scanned within the FTIR to balance quality and time efficiency).

- Within the context of spike recovery of large PA beads from a simulated sludge processing exercise where recovery appeared to be 57 %.

It was decided not to correct the results on the basis of recovery. To do this in a fair way it would have been necessary to have a range of sized particles for each of the nine selected polymers. At this stage it is not possible to know whether the methods recover one polymer or one size more effectively than another. Thus, it cannot be certain that the recovery of 57% of large PA beads is representative for all polymers and sizes.

3.1.2 Blank results

The blanks were prepared as simulating the potable, raw water / influent / effluent and sludge processing steps.

For the liquid samples, these blanks started by setting up the filter rig to run with clean water (RO water filtered to 2 µm to remove any microplastics above this size) with the 10 µm filter disc or cartridge deployed as in the field. More details of this process are given in Appendix A.

The raw water blank contamination profile (Figure 4, Appendix C Table 6), now with the added Fenton's reaction step, was broadly similar to that found with the potable blanks (Appendix C Table 9)

It is as yet unclear whether these contaminating microplastics are due to something in the air, something from the staff, or in the reagents themselves. The laboratory, the staff and the practices followed at CEH could be considered to be representative of most well-run research establishments. In other words, it is suspected such contamination would be a common phenomenon in analytical chemistry laboratories around the world. Assessing the level of contamination should therefore be considered critical to microplastics research.

The PET contamination were slightly less than found with the potable protocol leading to a better LOD and LOQ for raw water (Figure 4). The message was that PE, PET and PP were routine if somewhat episodic contaminants, with ABS, PA and PS being minor contaminants whilst PMMA, PVC-U and PU not being found as contaminants in the blanks. As both WwTW

influent and effluent samples followed the same enzymic and Fenton's digestion steps, these raw water blanks were also used to correct their results.

Figure 4 Limits of detection and quantitation based on eight raw water blanks (corrected to 100% filter area and also used for WwTW influent and effluent).
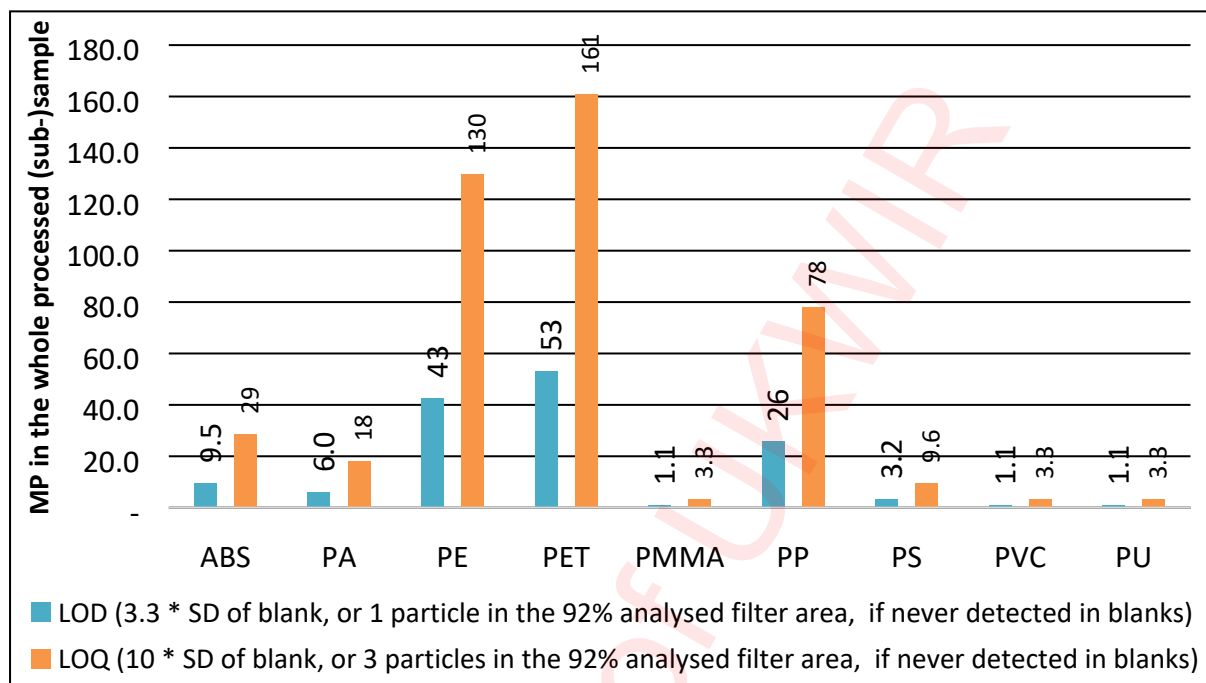
3.1.3 Raw water results

The raw water of the three WTWs that directly abstract water from a lowland rivers (LR1, LR2 and LR3) as well as the pumped storage site at LRS1 all have quantifiable microplastics present (Figure 5, Appendix C Table 8). When found, the numbers were typically around 15 microplastics/L, with the highest result of 113 PE microplastics/L at LRS1 on 29/11/2018. The microplastics were not a consistent presence in the raw water at any site except LR2. The polymers most often detected or quantified were PE, PET and PP. The polymers PMMA, PS, PU and PVC were occasionally detectable but below the LOQ. If one polymer was quantifiable on one sampling day then others would often be detected too.

The analysis of the raw water was somewhat hampered by obscuring matrix material (despite the processing) preventing full examination of the sample. In these cases only a small proportion of the sample was transferred to the silver disc for FTIR analysis. An example of this problem is the examination of the raw water from LRS1, where on each of five occasions, only 3-5% of the full sample could be examined in the FTIR (Appendix C Table 8). Whilst placing a small fraction of the processed sample on the silver disc reduced the obscuring material, this in practice meant a reduction in the LOD/LOQ. The highest microplastic numbers were usually linked to occasions when only a small amount of the sample was collected or could be analysed due to matrix effects. In other words, on these occasions there was more obscuring material present. A plausible explanation is that situations where the WTW is abstracting cloudy water, with a high contaminating matrix, are the same occasions when high microplastics are also present.

The cleanest raw water samples came from LR1, LRS2 and UR, where typically all the sample could be examined. The variable value for LOD/LOQ seen in the tables is linked to the quantity of water collected on site and the proportion of the processed sample put onto the silver disc for FTIR analysis. Thus, the analysis was at its most sensitive (lowest LOD/LOQ) when the highest quantity of water was collected (over 1 m^3) and 100% of the sample was transferred to the FTIR.

Both LRS1 and LRS2 WTWs abstract surface water but this is then stored in a reservoir before being treated (Table 6). Thus, the raw water sample was collected after the reservoir storage. Given that LRS2 is using a major river as its source water, which would be presumed to be a less than pristine environment, the virtual absence of quantifiable microplastics in the raw water is reassuring and may be a testament to effective settlement being employed here.

Although some microplastics were found occasionally in the rain fed reservoir raw water at UR, these were all below the LOQs.

Figure 5 Quantifiable microplastics in raw water, broken down by polymer type found on five repeat visits to six different WTWs having a surface water source.
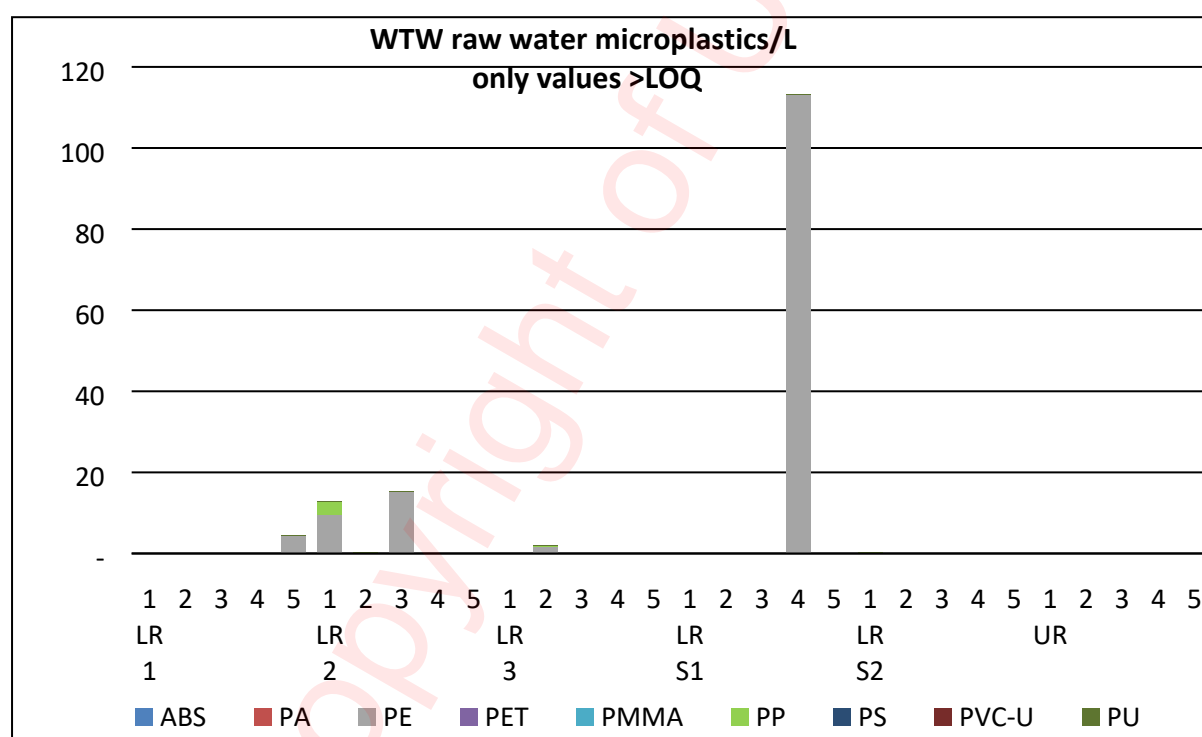
*Note: The LR sites directly treat lowland river water, the LRS sites also use lowland rivers but only following storage, whilst the UR site is an upland reservoir

3.2 Assessment of microplastic particles in potable water

3.2.1 Potable water blank results

Ten separate potable blank samples were prepared, Thompson *et al.* (2002) recommended six. These simulated capturing particles on the small 10 µm filter holders followed by only the enzymic digestion step.

The blank results are striking for the frequent presence of fairly high levels of PE, PET and PP contamination (Appendix C Table 9, Appendix C Figure 5). The net result was that this method would have low sensitivity for PE, PET and PP due to their ubiquitous nature and in some cases uneven contamination in the lab (Figure 6, Appendix C Figure 5). This approach to calculating an LOQ may appear over-precautionary to some, but an examination of the erratic contamination patterns shown in Appendix C Figure 5 should make this understandable. In other words, it cannot be certain that on another occasion, even more than 61 contaminating particles of PET as seen on 30th October 2018 may occur.

Figure 6 Limits of detection and quantitation based on ten potable water blanks (corrected to 100% filter area).
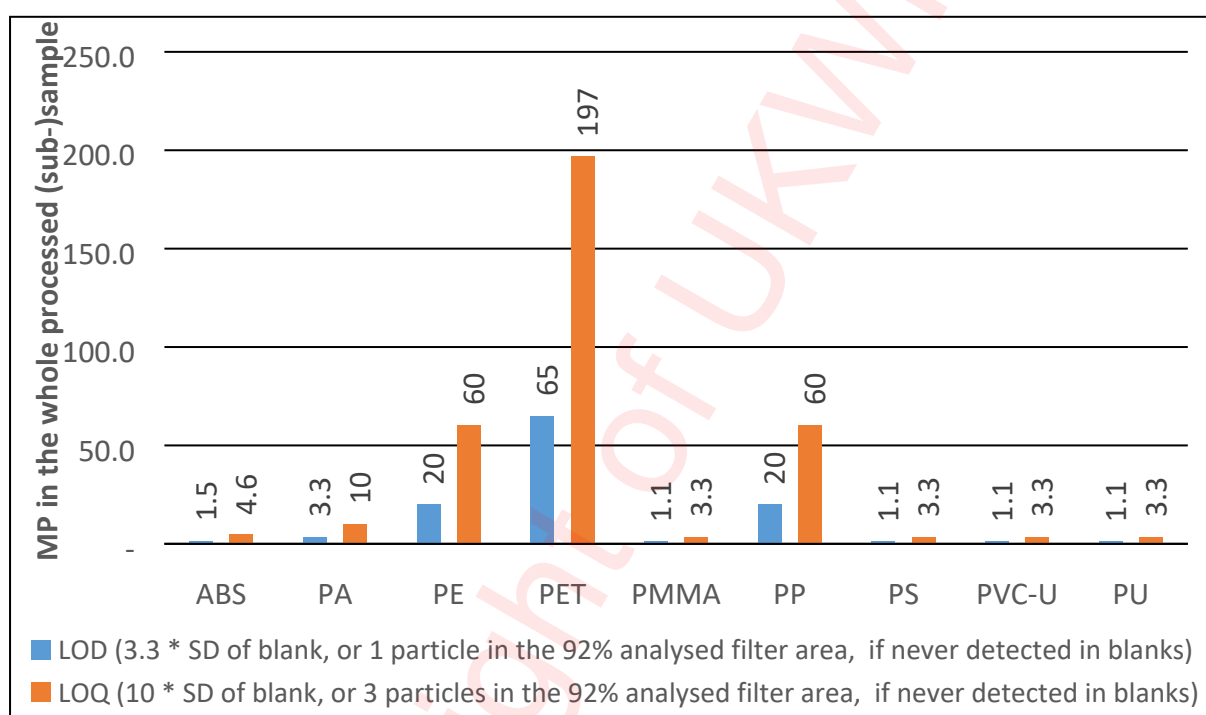
3.2.2 Potable water results

Despite obtaining 39 separate WTW samples and filtering very large quantities of potable water, it was very rare to find microplastics above the LOQ (Figure 7). It was more common to detect polymers below the LOQ but above the LOD (Figure 8). Although a Y-axis scale is shown in Figure 8, it should be stressed that these polymers can only be given as detected and not quantified.

The total volumes collected are shown in Table 6 and Appendix B Table 2. It should be noted that on occasions, low pressure and blockages reduced the volumes that could be collected. The raw data for the particles found by the FTIR and attributed to different polymer groups are shown in

Appendix C Figure 5. Whilst the numbers for PE, PET and PP may at first appear high, following the LOQ reporting protocol (see Appendix D for a worked example and Appendix C Table 9 and Figure 6 for the blanks data) these cannot be quantified. This is due to the laboratory contamination issue discussed above with the blanks.

Figure 7 Quantifiable microplastics in potable water. Results broken down by polymer type found on five repeat visits to eight different WTWs. The LR sites directly treat lowland river water, the LRS sites also use lowland rivers but only following storage, the GW sites collect and store groundwater, whilst the UR site is an upland reservoir.
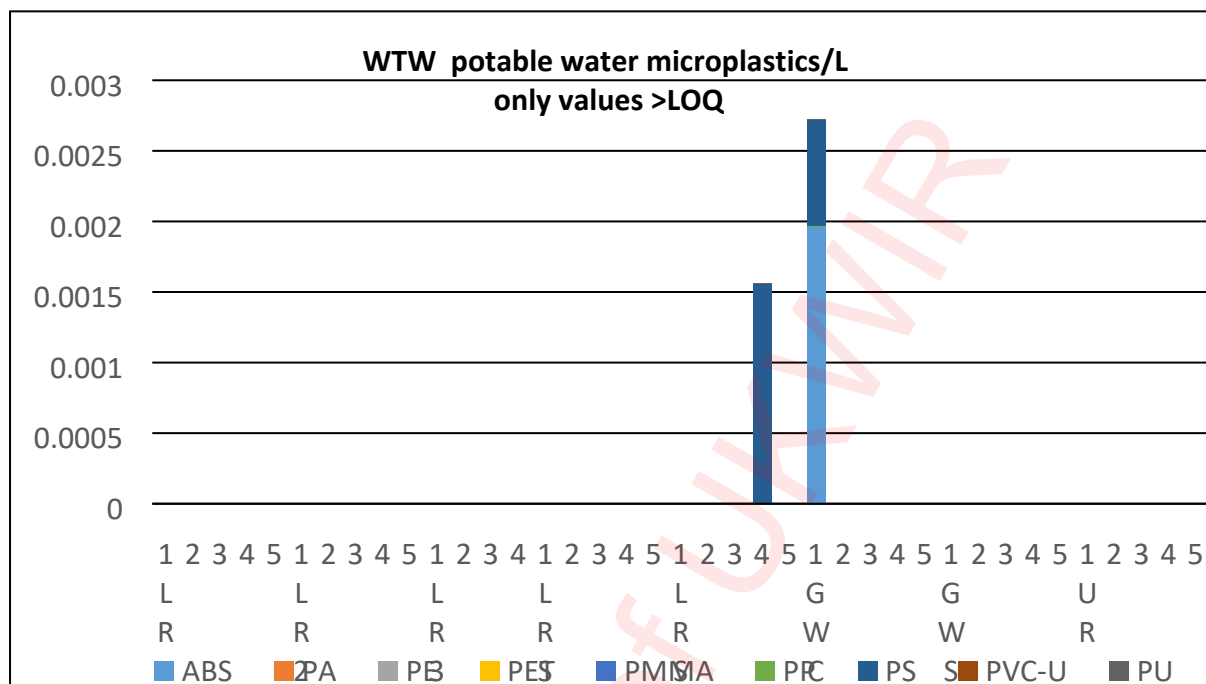
Figure 8 All detected (but below LOQ) microplastics in potable water. Results broken down by polymer type found on five repeat visits to eight different WTWs. The LR sites directly treat lowland river water, the LRS sites also use lowland rivers but only following storage, the GW sites collect and store groundwater, whilst UR site is an upland reservoir.
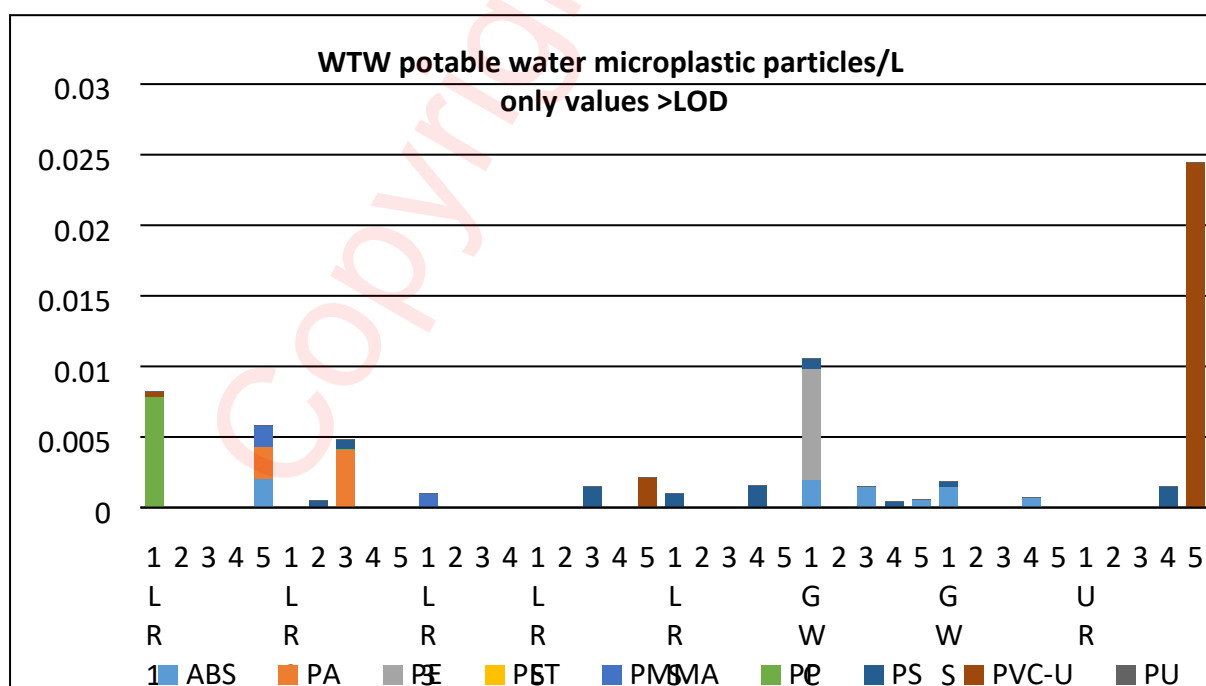
The microplastic levels found (remembering sampling typically takes 1.5 to 3 m^3) are extremely low. For example in 14.2 m^3 of all the potable water from GWC after five visits, only one particle of PS and two of ABS were quantified (Appendix C Table 11). No individual microplastic polymer has been quantified at greater than 0.002 microplastics/L. The highest value for all polymers combined in a sample was 0.003 total /L.

No one WTW was routinely different from any other. Although LR1, LR2 and LR3 directly abstract from a lowland river, their potable water product was comparable to the other WTWs.

Although GWC, GWS (groundwater) and UR (rain-fed upland reservoir) might be expected to have pristine raw water, some microplastics were occasionally detected in their potable water. The most common quantifiable polymer was PS (polystyrene) but the quantities found were very low. ABS was detected six times, five of these were at the groundwater sites (Figure 8). PS was detected nine times. It will be noted that ABS was not detected in the raw surface water of the WTWs (Appendix C Table 8). Detection is not the same as quantification.

3.2.3 Ability of WTWs to remove microplastics

These WTWs were generally extremely effective at preventing microplastics in the raw water from reaching potable water, with typically over 99.99% removal (Figure 9). The type of polymer found in the potable water is interesting as they were not necessarily found in the raw water when sampled on the same day. Examples are PS found in potable water at LRS2 on 14/11/2018, PS also at LR2 on 22/10/18 and 05/11/18, or PMMA found in potable water at LR3 on 27/09/18. Similar observations can be made for ABS (Appendix C Table 8 and Appendix C Table 11). This raises the possibility that microplastics found in potable water may have been, on occasion, generated within the WTW itself. However, given the difficulties in detection and quantification it is not a certainty that this occurred.

Figure 9 Summary of data above LOQ for all eight WTWs, each with five visits and all polymer types summated. Note the GW site results are only recorded on the potable side, as these WTWs only pump water from groundwater before storage and chlorination. Number of samples >LOQ and total number of samples is also given.
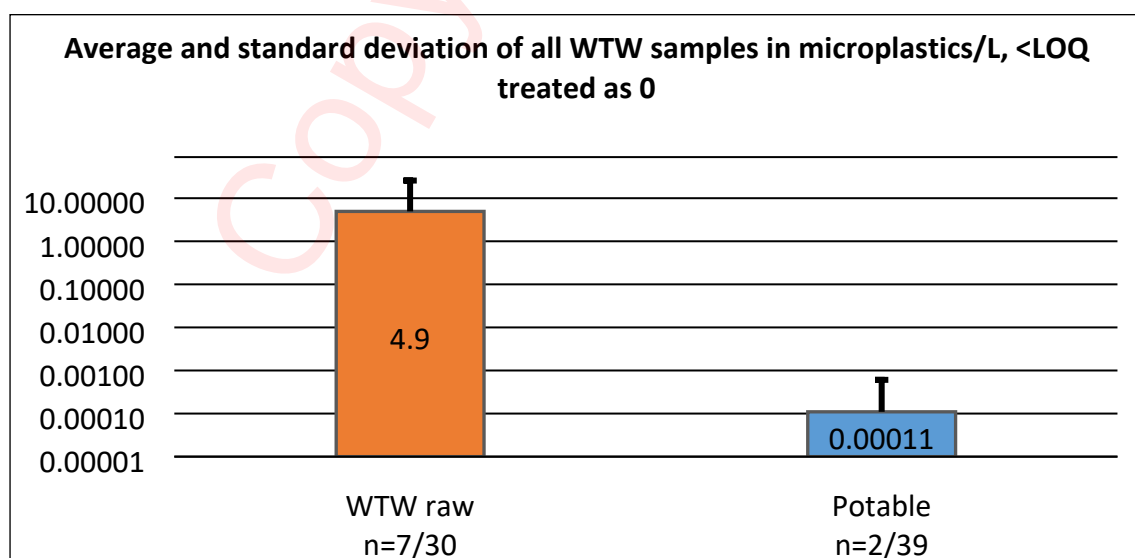
3.2.4 Presence of microplastics in WTW sludge

Not all of the WTW had a sludge collection point at which a sample could be taken. Samples were therefore taken from only four of the WTWs on two separate occasions (one sample per time). The WTW sites at LR2 and LR3 collect their raw water direct from a river. Although LR2 had quite a high microplastic presence in its raw water this did not translate to high values in its sludge other than 808 microplastics/g DW PE on the second visit (Appendix C Table 14). The other direct pumped site at LR3 had high quantifiable values for PE, PET and PP (5,000-20,000 microplastics/g DW) on the second sample. PE and PP were detected but not quantifiable at the pump and store river water site at LRS1. The rain-fed upland reservoir at UR had low but detectable PE and PP which was sufficient to be quantified at 404 microplastics/g DW of PP on one of the two occasions. It will be recalled that with the raw water, PE, PET and PP were also the most common polymer forms detected.

Figure 10 Quantifiable microplastics in WTW sludge. Results broken down by polymer type found in two repeat visits to four different WTWs. WTW sites coded as LR are direct abstraction from lowland rivers, LRS is lowland river, pumped storage. LR3 sample 2 had very high numbers of microplastic particles and therefore needed to be plotted on a different scale.
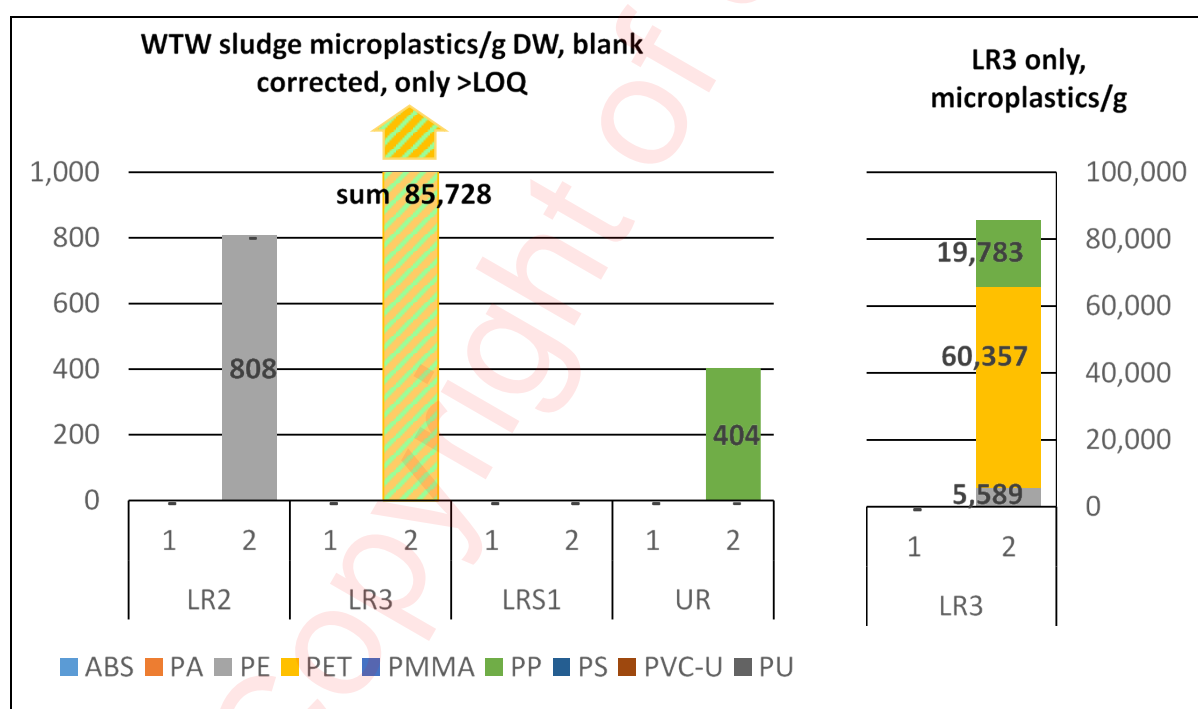
3.3 Assessment of microplastic particles in wastewater

The wastewater component of this project targeted 9 separate WwTWs/processes with two separate visits to each (Table 7). The sampling was carried out entirely by CEH staff and started in late November at ASTC1 and continued until completion on 17th February at AS2b. Influent and effluent was collected at a WwTW on the same occasion and involved setting up 24 h composite samplers. Thus, CEH staff returned on the second day to collect a liquid sample, in the case of the influent and a 10 μm filtered sample in the case of the effluent

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(Figure 2). The processing methodology replicated that used for raw water in which both Fenton's reagent and enzymatic digestion are used. Thus, blank correction used the same blank values as reported for raw water (Figure 4 and Appendix C Table 6).

3.3.1 Wastewater influent results

Microplastic particles could be detected in the influent of all of the WwTWs (Appendix C Table 16), although these could not be quantified at AS2b (Figure 11). The polymers that were detected were PE, PET and PP at levels of 1,000-17,000 microplastics/L.

Due to the high quantity of obscuring material present, only a small fraction of the original processed sample (0.6-2.3%) could be put onto the silver filter disc for FTIR to allow microplastics to be quantified. However, notwithstanding the modest amount of sample used, there were generally more than sufficient microplastic particles present to allow identification and quantification.

Figure 11 Quantifiable microplastics in wastewater influent. Results broken down by polymer type found on two repeat visits to eight different WwTWs. WwTW sites coded as AS are activated sludge, those with AST are activated sludge with a tertiary process (cloth, C or sand S), TF being trickling filter (with plastic, P or stone, S media) and BAFF as biological aerated flooded filter. At one site both AS and TF are used, therefore it has two influent codes. The suffixes a and b indicate that sludge was also collected at that site (see below).
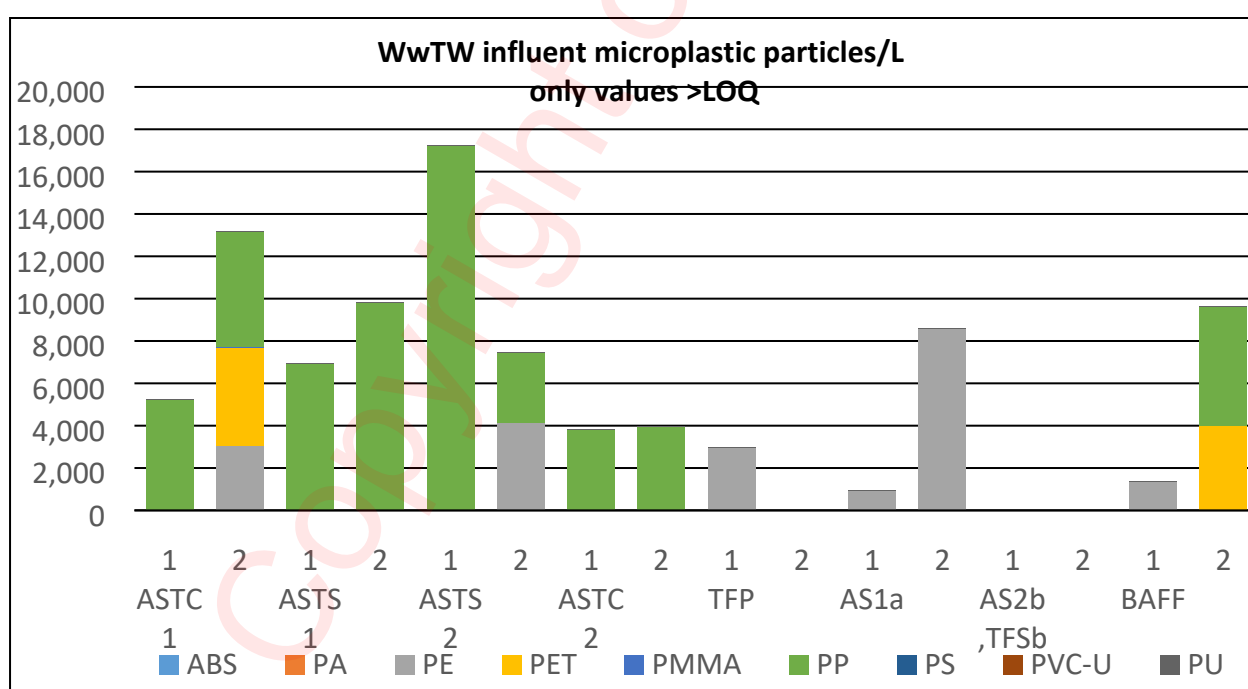
3.3.2 Wastewater effluent results

Where quantified, the numbers of microplastics were typically 2-50 microplastics/L (Figure 12). Of the 18 effluent samples, 4 had no polymers above the LOD, 7 samples had detectable polymers above the LOD but below the LOQ, whilst 7 samples had quantifiable polymers

(Appendix C Table 18). The most commonly detectable or quantified polymers were PE, PET and PP whilst ABS and PA were never detected.

The processing method of Fenton's reagent and enzymatic digestion did not eliminate all the obscuring matrix. This is considered to be due to the limited incubation time used. Other leading groups working on wastewater are known to use similar methods but to employ very prolonged digestion, often over several days (Simon *et al.* 2018). Due to the time constraints, it was not possible to employ long digestion periods in this project, although duplicate samples were retained which could be re-analysed if the opportunity arose for a more thorough digestion.

Figure 12 Quantifiable microplastics in wastewater effluent. Results broken down by polymer type found on two repeat visits to eight different WwTWs. WwTW sites coded as AS are activated sludge, those with AST are activated sludge with a tertiary process (cloth or sand), TF being trickling filter (with plastic, P or stone, S media) and BAFF as biological aerated flooded filter. The suffixes a and b indicate that sludge was also collected at that site (see below).
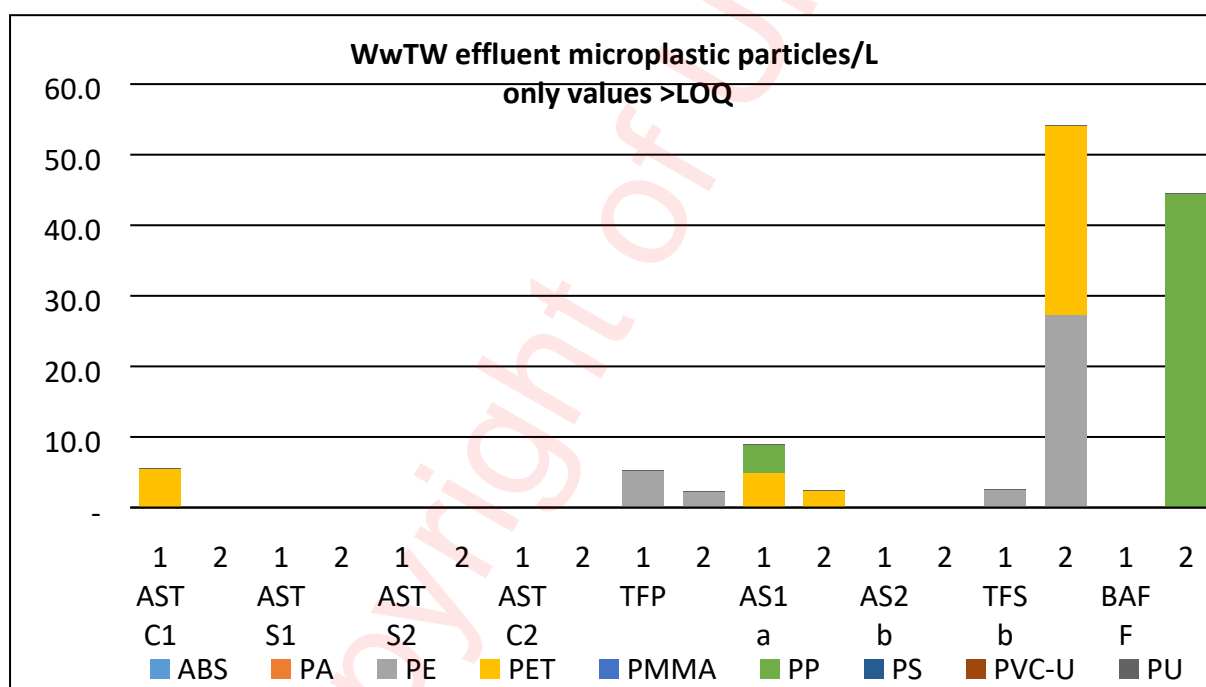
Given only two samples per plant were taken, it might be premature to speculate on the microplastic removal performance of different WwTW types. However, given the absence of quantifiable microplastics in the effluent at most ASPs with tertiary treatment (sand or cloth) (Figure 12and Appendix C Table 18) these works appear to have performed well. The trickling filters at TFSb and TFP, together with the ASP lacking tertiary treatment at AS1a could be said to be less effective due to the presence of quantifiable microplastics in their effluent.

3.3.3 Microplastic removal performance of the WwTWs

Given the average presence of PE, PET and PP microplastics in influent at levels of 5,000 microplastics/L and the average in effluent of 5 microplastics/L, this suggests an overall achievement of 99.9% removal from the effluent (Figure 13).

Figure 13 Summary of data above LOQ for all eight WwTWs, each with two visits and all polymer types summated.
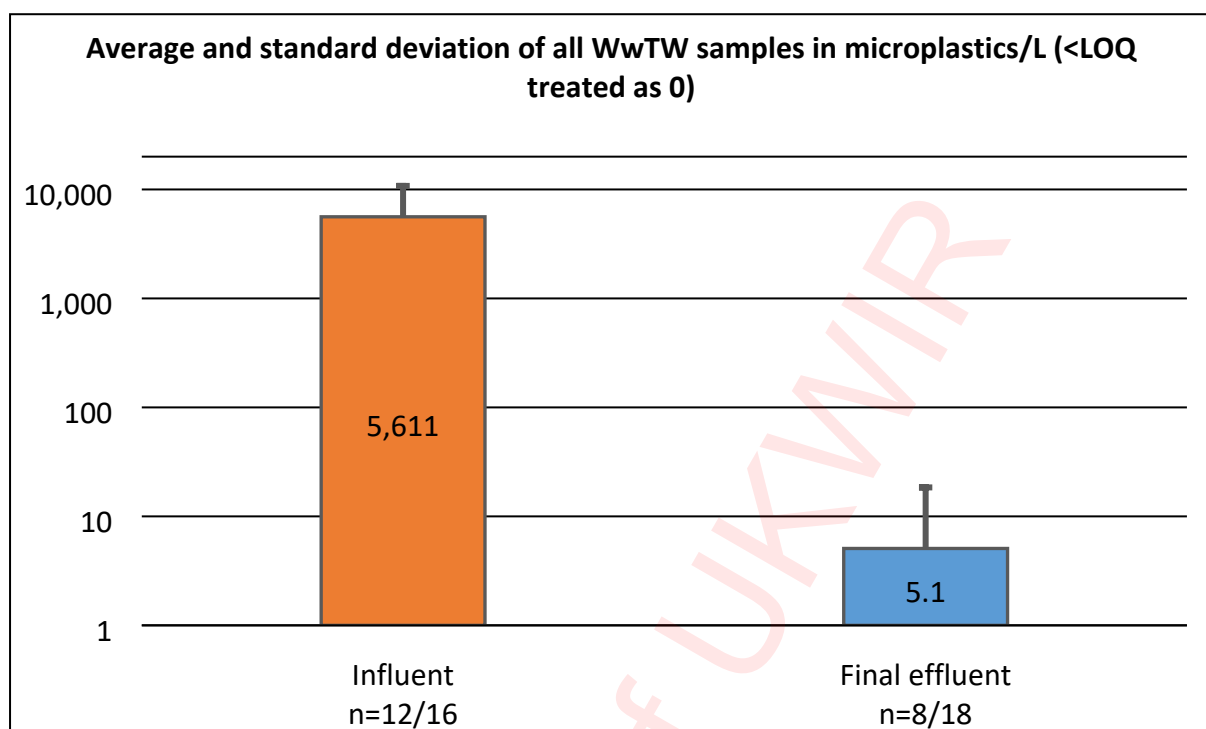
3.4   Examination of microplastics in wastewater sludge cake

3.4.1 Sludge spiked recoveries and blanks

The mean recovery of polyamide particles spiked into the sludge was 56.8% (+/- 15.2%). The deviation around this mean recovery was similar to the variation observed around the mean number of particles in the initial stock solution spiked into the sludge (19.99%). This stable recovery value gives confidence about the repeatability of the sludge processing methods. A similar recovery was attempted for PVC particles (100-150 µm), however these particles aggregated in solution and therefore it was not possible to accurately quantify these. To properly correct for recovery a more comprehensive spike recovery effort would be needed, involving a range of particle sizes and polymers. Such assessment was not possible within the period of this project and as yet there are no certificated reference materials. Thus, it would be necessary to prepare such standards within the laboratory, possibly by cryo-grinding. Without this information, it was not possible to do a recovery correction but it must be borne in mind that based on the PA result, an underestimation of microplastic particles is probable.

The process used to prepare the sludge samples run as blanks for the FTIR revealed conventional levels of polymer contamination although this was high for PET (Figure 14), thus reducing the sensitivity for this polymer.

Figure 14 Limits of detection and quantitation based on five sludge blanks (corrected to 100% filter area and proportion of the sample added to the silver filter in the FTIR).
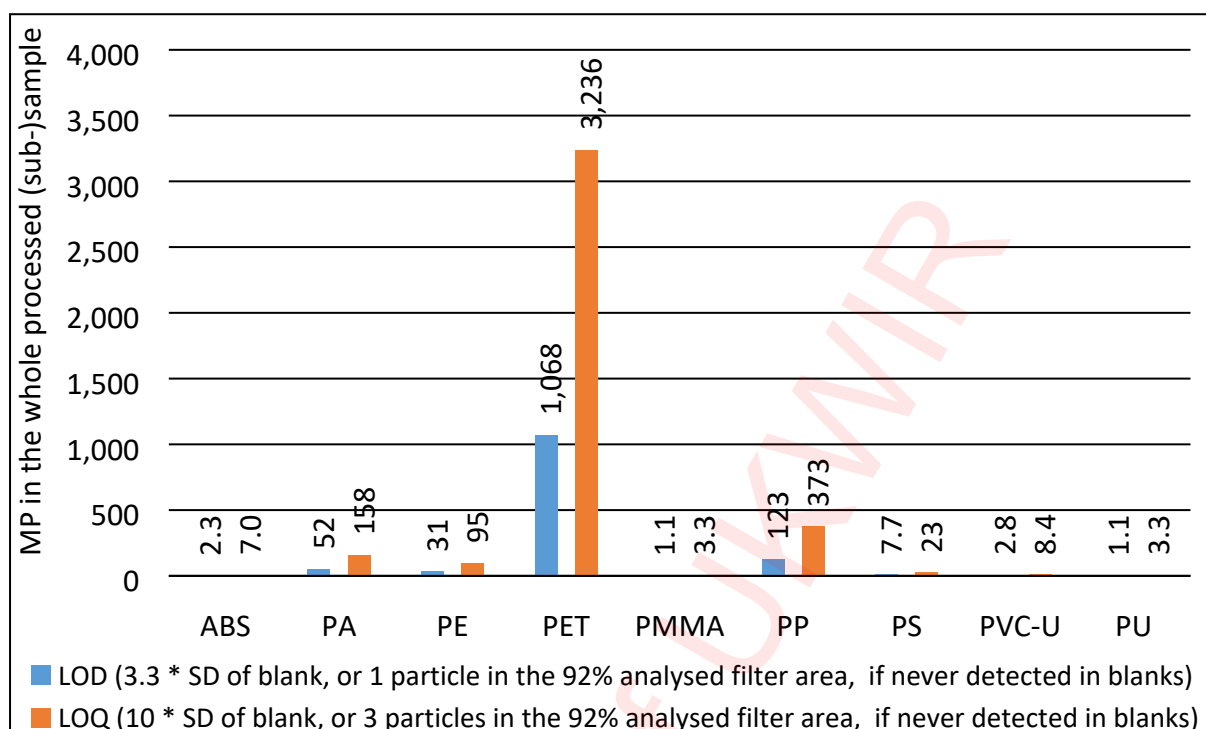
3.4.2 Wastewater sludge results

In the WwTW sludge study, 5 different WwTW with sludge treatment facilities were involved, each tasked with collecting a series of 5 separate samples over the autumn and winter of 2018 (Figure 15, Table 8).

Given the limited abilities of the processing method to clean up a sample sufficiently to permit FTIR analysis, only 1 g of dried sludge was examined on each occasion. Thus, there was a concern that results may be erratic and not representative given the small quantity of sludge examined. To test this, from the sludge sample from AAD3 collected on 26th July 2018, following the normal mixing and homogenization process, four separate 1 g samples were taken for analysis. It was reassuring to find that these four replicate samples from AAD3 gave remarkably similar numbers, above the LOQ, for the main polymers of PE and PP (Appendix C Table 21).

Whilst, the actual amount of material of the original 1 g that could be transferred to the silver disc for FTIR was typically only 0.2-3.7%, this did not prevent very consistent results being obtained across the different WwTWs and dates (Appendix C Table 20). The outstanding feature of the data is the similarity of PE and PP presence at 500-4,000 microplastics/g DW throughout the WwTWs and different sampling dates. The raw counts for these polymers were typically 20 to 30 on the silver filter discs and so are within a range that are reliable. The exceptions were AAD2, an advanced anaerobic digestion site, which tended to have non-detects or a maximum of only 500 microplastics/g DW, and the limed sludge (LS) with no PP detected. PE, PET and PP were the polymers most frequently detected or quantified in wastewater effluent and PE and PP were also common in sludge, PET could not be detected

with the surprising exception of AAD1a (another advanced anaerobic digester site). Overall, WwTW sludge had a wide range of detectable polymers, these included ABS, PA, PMMA, PS and PVC more than any of the other sample types, nevertheless, the dominance of PE and PP here was striking.

Figure 15 Quantifiable microplastics in wastewater sludge, broken down by polymer type found in five repeat visits to five different WwTWs.
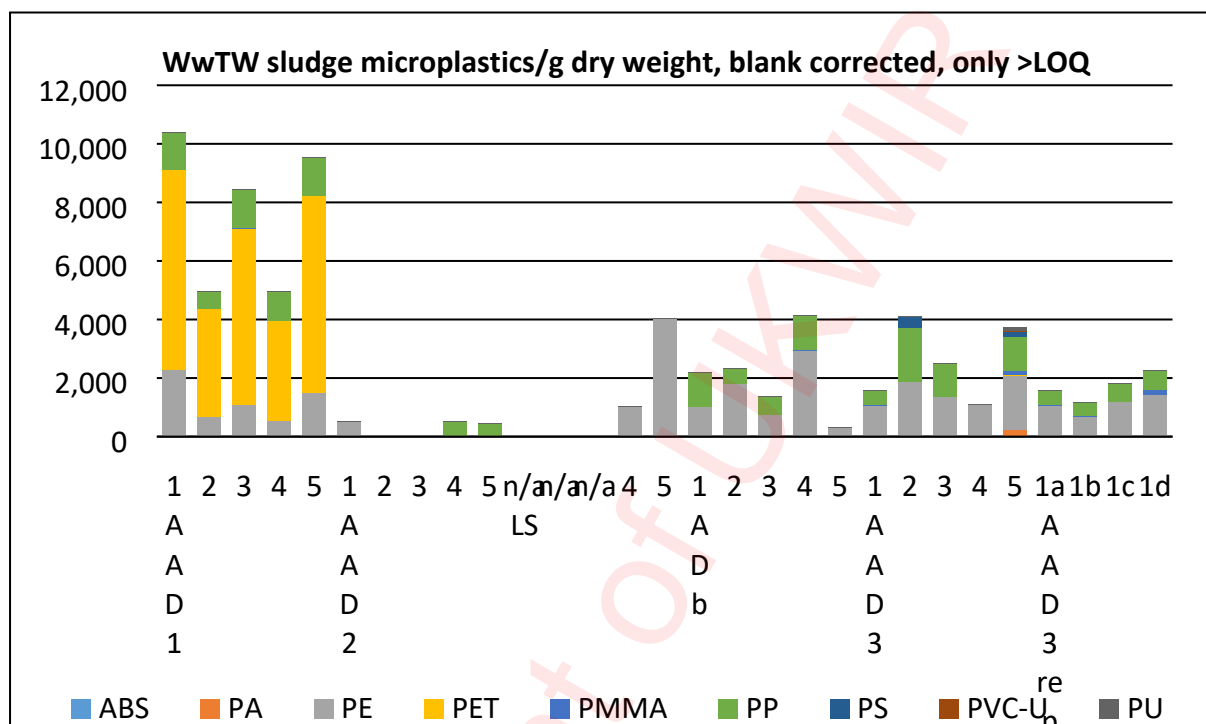
*WwTW sites coded as AD have conventional anaerobic digestion, those with AAD have different varieties of advanced anaerobic digestion and LS is for limed sludge. Note, to test the within sample variability, the sample from the first visit to AAD3 was divided into four (1a, 1b etc).

These values, on per unit of weight basis, are higher than those found in any of the liquid samples and reflects the ability of a WwTW to remove a high proportion of microplastics from wastewater. These sludge numbers for microplastics are higher than reported in most of the literature (Table 5) but many previous methods relied on visual sorting and looking only at large particles, the disadvantages of which have been discussed previously. It will be recalled that the spike recovery effort with the PA polymer revealed a 58% recovery. In theory, if more complete spike recovery data had been available it would be possible to adjust upwards the final result. But in the absence of a full suite of spike recovery data, it was not possible to make such an adjustment here.

3.5 Size distribution

It will be recalled that there is as yet no agreed definition of a microplastic. In this project a 10 µm filter was used, which was generally successful with avoiding clogging, however, it will be appreciated that finer filter sizes bring with them more practical challenges in dirty matrices. With the FTIR, 25 µm resolution was chosen as the most suitable pragmatic choice between being able to survey the majority of a silver filter disc, generate a dataset within the

700 MB file size machine limit and to give a 2.5 h run time per sample. The MPhunter software is able to provide an output of the size distribution of plastic particles found. When reviewing this output, it is clear that whilst a few larger particles exist, a logarithmic distribution is apparent, with numbers vastly increasing the smaller you go (Figure 16). This distribution was the same for the contamination in the blanks as for the field samples.

Figure 16 Size distribution of the microplastics found in the different sample types.
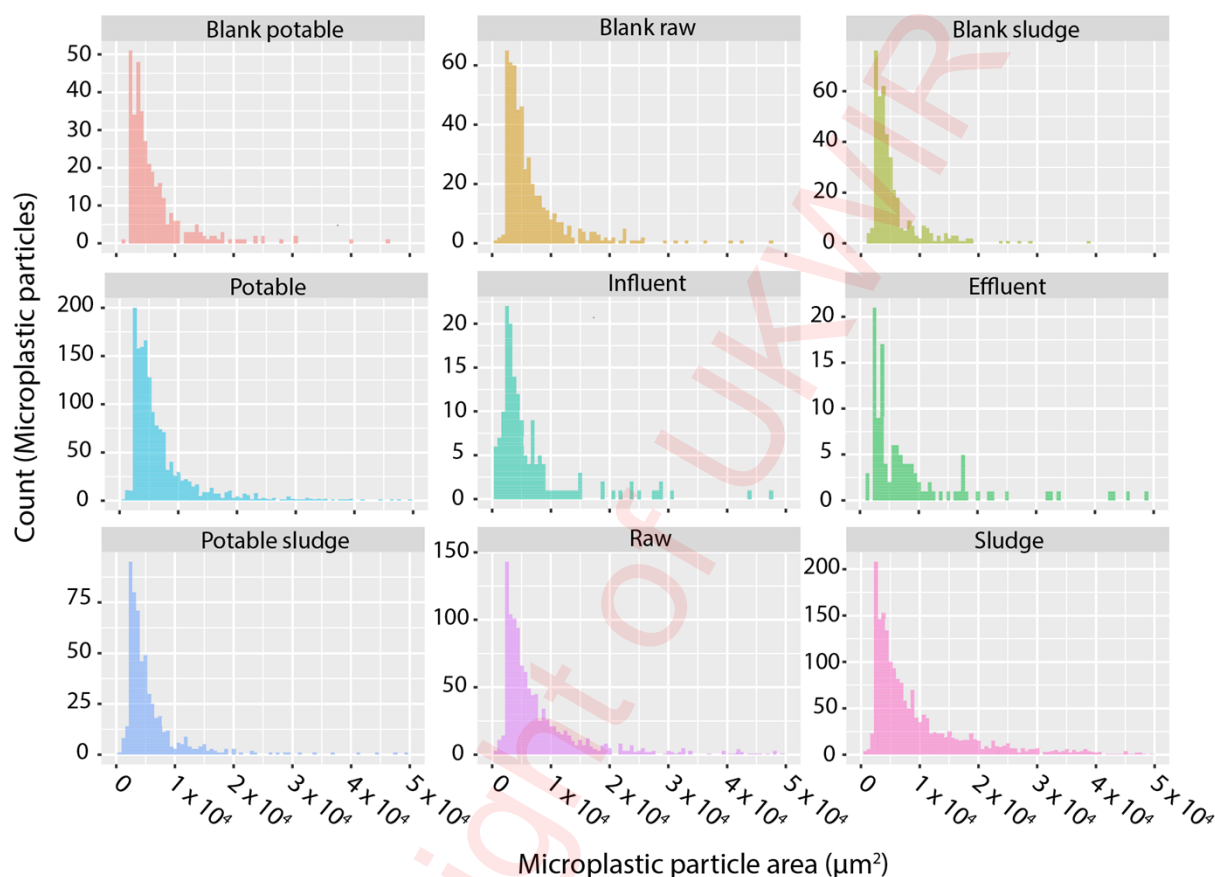
The implication of these size distribution graphs is that it is very likely that large numbers of microplastics, less than 25 μm, were present but have not been quantified. It is technically possible for the FTIR to identify and enumerate down to 6.25 μm. Examination at the finer resolution would require much greater machine effort. It is predicted that to examine one of the 13 mm silver filter discs to this resolution would take several days.

The analytical approach was not able to specifically distinguish microfibres from other non-microfibre plastic particles. Although FTIR images are generated of the particles for each polymer type, currently no reliable method of distinguishing particles based only on shape is available. However, it is feasible that an analytical approach to categorise particles and fibres could be developed in future based on image analysis. Given that the lower limit of the FTIR resolution was 25 μm, it is possible that thin fibres were under-represented in the final dataset if they had a width below 25 μm. Further work is needed to better understand the lower limits of detection of particles in terms of size, and how this interacts with polymer shape, material and age.

Chapter 4   Discussion

An issue that continues to confuse is 'how do you define a microplastic'? Most of the studies carried out when the subject began relied on microplastics being spotted by eye down a microscope. Thus, many early studies are reporting on particles of 0.1 mm and above and here microfibre particles were relatively easy to spot and gained a lot of attention. Using the RAMAN approach, a beam could be manually targeted at the putative microplastic and its polymer type revealed. Thus, many studies focused on large particles and relied on the skill (and bias) of the operator to find and identify them. In theory, microplastics have no lower limit in size. What limits the ability to quantify smaller and smaller particles are practical and analytical constraints. Thus, for this project, microplastics were defined as particles >25 µm that had been captured on 10 µm filters.

Obtaining reliable data on the presence of microplastic particles in the water infrastructure was the central mission of this project. This was of particular importance in the case of the potable water product from WTWs. Very little literature information exists on drinking water, and what exists is contradictory. Over the course of the project it became clear that better analytical equipment than RAMAN was needed to reliably and consistently enumerate microplastics. This led to a purchase and switch to FTIR analysis. Secondly, it became clear that understanding and correcting for microplastic contamination in the laboratory was key to generating the reliable data the Water Industry needed. This led to running many more blanks than had been originally envisaged. It was accepted that following the protocol commonly used in 'wet' analytical chemistry to generate LODs and LOQs was a sensible way forward to manage the uncertainty aspect of laboratory contamination. It may be possible over time to reduce or eliminate this laboratory contamination and so lower the LODs and LOQs, but it may have to be accepted that microplastics are ubiquitous contaminants of any laboratory.

The values for microplastics found in potable water are extremely low (typically less than 0.002 microplastics/L where quantifiable) and are not dissimilar from those found in German potable water sourced from groundwater (Mintenig *et al.* 2019). Given the range of WTWs examined, the five-time repeat sampling, the large volumes examined, and the success of the processing step to provide clear images for the FTIR, confidence can be placed in the robustness of this result.

For the raw water, the processing was less successful in providing clear, non-plastic contaminant-free images. However, notwithstanding the methodological limitations, it would appear that where challenged, the WTW are succeeding in eliminating over 99.99% of microplastics from their source water. The most frequent presence of microplastics in raw water was at LR2 which has direct river abstraction, but the highest value of 113 microplastics/L was at LRS1 (pump storage).

Similar to the raw water samples, the processing method struggled to eliminate all the non-plastic contamination from the WwTW influent and effluent samples, leading to a lower proportion of the original sample having to be put through the FTIR. Where quantifiable, the results show PE, PET and PP microplastics present in the effluent of the trickling filter and ASP treatments without tertiary stages of 2-27 microplastics /L. An important message is that very high loads of microplastics are arriving in the influent (1,000s microplastics/L) at all the

WwTWs, but that the treatment is removing 99.9% from the effluent. The size distribution of microplastics appears to indicate more smaller particles are present than larger ones. Thus, researchers who focus on larger particles will underestimate the numbers present.

Although it was necessary to limit the amount of processed sample transferred onto the silver filter disc for FTIR, the results for WwTW sludge showed remarkable consistency. It was found that PE and PP polymer based microplastics were routinely present. Levels were typically around 1,000-4,000 microplastics/g DW in wastewater sludge. The advanced anaerobic digestion set-up at AAD2 was more capable than the other WwTWs at reducing microplastics, particularly in removing PE.

It is likely that there are many microplastic particles present in the environment which are smaller than 25 μm (Figure 16). Whilst this ultra-small particle fraction may be numerous, its contribution to the total mass is likely to be trivial. Thus, if the question was 'how successful are the Water Industry at removing microplastics >25 μm in size from raw water or crude sewage?' the answer would appear to be very successful. It would be more difficult to give an answer as to how successful the Water Industry is at removing all microplastic particles including the ultra-small varieties. The current methodology puts severe constraints on the ability to quantify such small <25 μm particles.

Chapter 5   Conclusions

5.1   Lessons from the practical side

The capability of any effort to enumerate microplastics in the environment is hampered by the routine presence of microplastic contamination in the laboratory. This study has gone to considerable lengths to ensure a thorough blank correction process was used so that the microplastic values reported truly reflected the original sample. If and when it becomes possible to identify and eliminate these sources of contamination, lower detection and quantification limits may be available in the future.

For practical reasons the resolution used for the FTIR analysis was 25 µm and above. Thus, particles approximately >10 µm (the filter size) but <25 µm were not reported on. This practical limitation is common to research in this field.

5.2   The story from the drinking water side

The potable water investigation revealed that the nine common polymers, which the study focused on, are rarely present above the LOD or LOQ. In the five return visits for any WTW, at worst, microplastics were only quantifiable on two separate occasions. The three values above LOQ were 0.0008 to 0.002 microplastics/L. The most common plastic polymers found in potable water were PS and ABS.

Due to residual (non-plastic) material causing interference, enumeration in raw water, wastewater influent & effluent proved to be more challenging. Nevertheless, microplastics in raw water could be present at relatively high levels for WTWs using direct pumped river water, especially at LR2 site where PE is prevalent. This tended to be much less for pumped storage WTWs, except for one episode at LRS1, and not quantifiable for a rain-fed upland reservoir.

Where a WTW is challenged by high microplastics in the raw water, such as from a river, the WTW is extremely effective at removal (99.99%). This was reflected in the relatively high numbers of microplastics found in WTW sludge.

The detection of very small quantities of PS and ABS in the potable water, but not in the raw water, may need further consideration. The inability to detect and quantify these polymers in the raw water may be due to poor sensitivity in this matrix but there is a possibility these were released during the treatment process or from the pipes within the WTWs themselves.

5.3   The situation in wastewater and sludge

Enumeration was challenging with wastewater influent and effluent due to the presence of interfering non-plastic material. However, all WwTWs appear to be receiving 1,000s of microplastics/L in influent, largely of the PE, PET and PP polymers, of which they are removing around 99.9% from the effluent. In effluent, where quantified, the numbers were typically in the 2-27 microplastics/L range. The most common plastic polymers found in wastewater effluent were again PE, PET and PP.

A consistent and steady signal of PE and PP polymer microplastics present at around 1,500 microplastics/g DW could be found in most wastewater sludge samples.

Chapter 6   Recommendations

6.1 Further methodological work

- In order to assess whether, how frequently and at what stage microplastics can be found in the water infrastructure, it is necessary to employ a robust method that can be trusted by all stakeholders. If more data is required by the Water Industry to make management decisions, or indeed if there is a need to quantify microplastics 'in house', then further effort will be needed in method development and QA/QC protocols. It is considered that the methods used in this project were suitable for potable water but would benefit from further development for more dirty matrices such as sewage sludge. A defendable approach to blank correction was employed, but more effort is needed in the field of spike recovery also known as 'positive controls' to better understand the losses that may occur during sample processing.

- These results are restricted to reporting on 9 common polymers, it may be a wise precaution to spread the net wider for some samples and to examine whether more diverse 'exotic' polymers might also have been present. This would also require LODs and LOQs to be derived from the existing blanks.

- Whilst the method used is efficient at quantifying microplastic particles >25 μm size it is less suitable at quantifying either fibres (which are large i.e., long yet narrow) or very small sub-25 μm particles. It would be possible to re-examine a small sub-set of the project samples down to 6.25 μm resolution if more information on these smaller microplastics was considered desirable. To go to even smaller sizes would need a re-think on capture, processing and analysis.

It should be noted that all the original samples from the field campaign have been retained due to their being sub-divided on reception. These stored samples would be available to re-examine with a refined processing method if required.

6.2 Further WTW research questions

- Given the use of plastics in pipes, containers and valves associated with the delivery of potable water from the WTW to the consumer, it would be worth examining whether the presence of microplastics in tap water is comparable or greater than those present within the potable water sampled directly from the treatment plant.

- It is not clear to what extent microplastic particles less than 25 μm in size will be considered a concern. As discussed, quantification of such ultra-small particles brings many challenges, with the size of sample capable of being examined the major limitation. But it is possible that ultra-small particles are being deposited from the air and so it may need consideration with respect to protecting open water reservoirs.

- On the WTW side, there may be a wish to explore whether some works, such as the groundwater sites appear to be releasing some ABS and PS polymers into potable water from their own pipes/equipment?

- At some locations and on some days the raw water had a higher microplastic content. It might be informative to go back and review these situations to help understand the source of the microplastics.

6.3 Further WwTW research questions

- Further confirmation might be required that trickling filters or ASPs without tertiary treatment release the largest quantities of microplastics in effluent?

- On the sludge side, would it be worth confirming whether some anaerobic or advanced anaerobic digesters really are more or less successful at eliminating microplastics? What conditions led to success?

- With concerns over the use of sludge in agriculture introducing microplastics, and given the numbers found within this study, it would be valuable to explore the relative concentrations in sludge amended and non-amended soils. Is there a significant difference?

Chapter 7   References

AOAC (2011) "Standard Format and Guidance for AOAC Standard Method Performance Requirement (SMPR) Documents." http://www.aoac.org/aoac_prod_imis/ AOAC_Docs/ISPAM/3.5SMPRGuidelinev12.1.pdf (accessed 26/7/2019)

Bayo, J., Olmos, S., López-Castellanos, J. and Alcolea, A. (2016). "Microplastics and microfibers in the sludge of a municipal wastewater treatment plant." International Journal of Sustainable Development and Planning 11(5): 812-821.

Blair, R.M., Waldron, S. and Gauchotte-Lindsay, C. (2019-preprint). "Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period." (DOI: 10.31223/osf.io/ez5gm).

Blair, R.M., Waldron, S., Phoenix, V. and Gauchotte-Lindsay, C. (2017). "Micro- and Nanoplastic Pollution of Freshwater and Wastewater Treatment Systems." Springer Science Reviews 5(1-2): 19-30.

Carr, S.A., Liu, J. and Tesoro, A.G. (2016). "Transport and fate of microplastic particles in wastewater treatment plants." Water Res 91: 174-182.

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C. and Galloway, T.S. (2014). "Isolation of microplastics in biota-rich seawater samples and marine organisms." Sci Rep 4: 4528.

Connors, K.A., Dyer, S.D. and Belanger, S.E. (2017). "Advancing the quality of environmental microplastic research." Environ Toxicol Chem 36(7): 1697-1703.

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N. and Tassin, B. (2015). "Microplastic contamination in an urban area: a case study in Greater Paris." Environmental Chemistry 12(5): 592.

Dyachenko, A., Mitchell, J. and Arsem, N. (2017). "Extraction and identification of microplastic particles from secondary wastewater treatment plant (WWTP) effluent." Analytical Methods 9(9): 1412-1418.

Eerkes-Medrano, D., Leslie, H.A. and Quinn, B. (2018). "Microplastics in drinking water: A review and assessment of an emerging concern." Current Opinion in Environmental Science & Health.

Eisentraut, P., Dumichen, E., Ruhl, A.S., Jekel, M., Albrecht, M., Gehde, M. and Braun, U. (2018). "Two Birds with One Stone-Fast and Simultaneous Analysis of Microplastics: Microparticles Derived from Thermoplastics and Tire Wear." Environmental Science & Technology Letters 5(10): 608-613.

Erni-Cassola, G., Gibson, M.I., Thompson, R.C. and Christie-Oleza, J.A. (2017). "Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 μm) in Environmental Samples." Environmental Science & Technology 51(23): 13641-13648.

Fendall, L.S. and Sewell, M.A. (2009). "Contributing to marine pollution by washing your face: microplastics in facial cleansers." Mar Pollut Bull 58(8): 1225-1228.

Gies, E.A., LeNoble, J.L., Noël, M., Etemadifar, A., Bishay, F., Hall, E.R. and Ross, P.S. (2018). "Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada." Marine Pollution Bulletin 133: 553-561.

Gundogdu, S., Cevik, C., Guzel, E. and Kilercioglu, S. (2018). "Microplastics in municipal wastewater treatment plants in Turkey: a comparison of the influent and secondary effluent concentrations." Environmental Monitoring and Assessment 190(11): 10.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L. and Wagner, M. (2019). "Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris." Environmental Science & Technology 53(3): 1039-1047.

Hernandez, E., Nowack, B. and Mitrano, D.M. (2017). "Polyester Textiles as a Source of Microplastics from Households: A Mechanistic Study to Understand Microfiber Release During Washing." Environ Sci Technol 51(12): 7036-7046.

Horton, A.A. and Dixon, S.J. (2018). "Microplastics: An introduction to environmental transport processes." Wiley Interdisciplinary Reviews: Water 5(2): e1268.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E. and Svendsen, C. (2017). "Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities." Science of The Total Environment 586: 127-141.

Hurley, R.R., Lusher, A.L., Olsen, M. and Nizzetto, L. (2018). "Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices." Environmental Science & Technology.

Kim, M.G., Yagawa, K., Inoue, H., Lee, Y.K. and Shirai, T. (1990). "Measurement of tire tread in urban air by pyrolysis-gas chromatography with flame photometric detection." Atmospheric Environment Part a-General Topics 24(6): 1417-1422.

Koelmans, A.A., Mohamed Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M. and De France, J. (2019). "Microplastics in Freshwaters and Drinking Water: Critical Review and Assessment of Data Quality." Water Research.

Kole, P.J., Lohr, A.J., Van Belleghem, F. and Ragas, A.M.J. (2017). "Wear and Tear of Tyres: A Stealthy Source of Microplastics in the Environment." International Journal of Environmental Research and Public Health 14(10): 31.

Kosuth, M., Mason, S.A. and Wattenberg, E.V. (2018). "Anthropogenic contamination of tap water, beer, and sea salt." PLoS One 13(4): e0194970.

Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, L.I. and Finley, B.L. (2010). "Physical and chemical characterization of tire-related particles: Comparison of particles generated using different methodologies." Science of the Total Environment 408(3): 652-659.

Lares, M., Ncibi, M.C., Sillanpää, M. and Sillanpää, M. (2018). "Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology." Water Research 133: 236-246.

Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M. and Vethaak, A.D. (2017). "Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota." Environment International 101: 133-142.

Li, J., Liu, H. and Chen, J.P. (2017). "Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection." Water Research.

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G. and Zeng, E.Y. (2018). "Microplastics in sewage sludge from the wastewater treatment plants in China." Water research 142: 75-85.

Liu, X., Yuan, W., Di, M., Li, Z. and Wang, J. (2019). "Transfer and fate of microplastics during the conventional activated sludge process in one wastewater treatment plant of China." Chemical Engineering Journal 362: 176-182.

Löder, M.G., Imhof, H.K., Ladehoff, M., Löschel, L.A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I. and Laforsch, C. (2017). "Enzymatic purification of microplastics in environmental samples." Environmental science & technology 51(24): 14283-14292.

Lusher, A.L., Welden, N.A., Sobral, P. and Cole, M. (2017). "Sampling, isolating and identifying microplastics ingested by fish and invertebrates." Anal. Methods 9(9): 1346-1360.

Maes, T., Jessop, R., Wellner, N., Haupt, K. and Mayes, A.G. (2017). "A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red." Sci Rep 7: 44501.

Magni, S., Binelli, A., Pittura, L., Avio, C.G., Della Torre, C., Parenti, C.C., Gorbi, S. and Regoli,

F. (2019). "The fate of microplastics in an Italian Wastewater Treatment Plant." Science of the Total Environment 652: 602-610.

Magnusson, K. and Norén, F. (2014). Screening of microplastic particles in and down-stream a wastewater treatment plant, IVL Swedish Environmental Research Institute.

Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R. and Morrison, L. (2017). "Microplastics in Sewage Sludge: Effects of Treatment." Environmental Science & Technology 51(2): 810-818.

Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D. and Rogers, D.L. (2016). "Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent." Environ Pollut 218: 1045-1054.

Mason, S.A., Welch, V.G. and Neratko, J. (2018). "Synthetic Polymer Contamination in Bottled Water." Front Chem 6: 407.

Mintenig, S.M., Int-Veen, I. and Löder, M.G. (2014a). Mikroplastik in ausgewählten Kläranlagen des Oldenburgisch- Ostfriesischen Wasserverbandes (OOWV) in Niedersachsen, Oldenburgisch- Ostfriesischer Wasserverband (OOWV).

Mintenig, S.M., Int-Veen, I., Loder, M.G., Primpke, S. and Gerdts, G. (2017). "Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging." Water Res 108: 365-372.

Mintenig, S.M., Löder, M.G.J. and Gerdts, G. (2014b). Mikroplastik in Trinkwasser - Untersuchung im Trinkwasserversorgungsgebiet des Oldenburgisch - Ostfriesischen Wasserverbandes (OOWV) in Niedersachsen. Probenanalyse mittels Mikro-FTIR Spektroskopie, Oldenburgisch- Ostfriesischer Wasserverband (OOWV).

Mintenig, S.M., Löder, M.G.J., Primpke, S. and Gerdts, G. (2019). "Low numbers of microplastics detected in drinking water from ground water sources." Sci Total Environ 648: 631-635.

Murphy, F., Ewins, C., Carbonnier, F. and Quinn, B. (2016). "Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment." Environ Sci Technol 50(11): 5800-5808.

Napper, I.E., Bakir, A., Rowland, S.J. and Thompson, R.C. (2015). "Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics." Mar Pollut Bull 99(1-2): 178-185.

Napper, I.E. and Thompson, R.C. (2016). "Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions." Marine pollution bulletin 112(1): 39-45.

Nizzetto, L., Futter, M. and Langaas, S. (2016). Are Agricultural Soils Dumps for Microplastics of Urban Origin?, ACS Publications. 50: 10777-10779.

Oldenburgisch-Ostfriesischer Wasserverband (2016). Mikroplastik in den Kläranlagen Holdorf und Oldenburg.

Oßmann, B.E., Sarau, G., Holtmannspotter, H., Pischetsrieder, M., Christiansen, S.H. and Dicke, W. (2018). "Small-sized microplastics and pigmented particles in bottled mineral water." Water Res 141: 307-316.

Oßmann, B.E., Sarau, G., Schmitt, S.W., Holtmannspotter, H., Christiansen, S.H. and Dicke, W. (2017). "Development of an optimal filter substrate for the identification of small microplastic particles in food by micro-Raman spectroscopy." Anal Bioanal Chem 409(16): 4099-4109.

Pivokonsky, M., Cermakova, L., Novotna, K., Peer, P., Cajthaml, T. and Janda, V. (2018). "Occurrence of microplastics in raw and treated drinking water." Sci Total Environ 643: 1644-1651.

Prata, J.C. (2018a). "Airborne microplastics: consequences to human health?" Environmental pollution 234: 115-126.

Prata, J.C. (2018b). "Microplastics in wastewater: state of the knowledge on sources, fate and solutions." Marine pollution bulletin 129(1): 262-265.

Schymanski, D., Goldbeck, C., Humpf, H.-U. and Fürst, P. (2018). "Analysis of microplastics in water by micro-Raman spectroscopy: release of plastic particles from different packaging into mineral water." Water research 129: 154-162.

Simon, M., van Alst, N. and Vollertsen, J. (2018). "Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging." Water Research 142: 1-9.

Sutton, R., Mason, S.A., Stanek, S.K., Willis-Norton, E., Wren, I.F. and Box, C. (2016). "Microplastic contamination in the San Francisco Bay, California, USA." Marine pollution bulletin 109(1): 230-235.

Syberg, K., Khan, F.R., Selck, H., Palmqvist, A., Banta, G.T., Daley, J., Sano, L. and Duhaime, M.B. (2015). "Microplastics: addressing ecological risk through lessons learned." Environ Toxicol Chem 34(5): 945-953.

Tagg, A., Harrison, J., Ju-Nam, Y., Sapp, M., Bradley, E., Sinclair, C. and Ojeda, J. (2017). "Fenton's reagent for the rapid and efficient isolation of microplastics from wastewater." Chemical Communications 53(2): 372-375.

Talvitie, J., Heinonen, M., Pääkkönen, J.-P., Vahtera, E., Mikola, A., Setälä, O. and Vahala, R. (2015). "Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea." Water Sci Technol 72(9): 1495-1504.

Talvitie, J., Mikola, A., Koistinen, A. and Setälä, O. (2017a). "Solutions to microplastic pollution - Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies." Water Res 123: 401-407.

Talvitie, J., Mikola, A., Setälä, O., Heinonen, M. and Koistinen, A. (2017b). "How well is microlitter purified from wastewater? - A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant." Water Res 109: 164-172.

Thompson, M., Ellison, S.L.R. and Wood, R. (2002). "Harmonized guidelines for single-laboratory validation of methods of analysis - (IUPAC technical report)." Pure and Applied Chemistry 74(5): 835-855.

Tyree, C. and Morrison, D. (2017) "Invisibles - the plastics inside us." https://orbmedia.org/stories/Invisibles_plastics (accessed 26/7/2019)

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M. and Reifferscheid, G. (2014). "Microplastics in freshwater ecosystems: what we know and what we need to know." Environmental Sciences Europe 26(12).

Wright, S.L. and Kelly, F.J. (2017). "Plastic and Human Health: A Micro Issue?" Environ Sci Technol 51(12): 6634-6647.

Ziajahromi, S., Neale, P.A. and Leusch, F.D. (2016). "Wastewater treatment plant effluent as a source of microplastics: review of the fate, chemical interactions and potential risks to aquatic organisms." Water Sci Technol 74(10): 2253-2269.

Ziajahromi, S., Neale, P.A., Rintoul, L. and Leusch, F.D. (2017). "Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics." Water Res 112: 93-99.

Zubris, K.A. and Richards, B.K. (2005). "Synthetic fibers as an indicator of land application of sludge." Environ Pollut 138(2): 201-211.

Zuccarello, P., Ferrante, M., Cristaldi, A., Copat, C., Grasso, A., Sangregorio, D., Fiore, M. and Oliveri Conti, G. (2019). "Exposure to microplastics (<10 µm) associated to plastic bottles mineral water consumption: The first quantitative study." Water Res 157: 365-371.

Appendix A   Detailed Materials and Methods

A.1 Materials and general procedures

All dilutions were performed using reverse osmosis water (EuRO10, SG Water or TR Reverse Osmosis Unit RO S 130, conductivity 7 µS/cm). All reagents used for sample processing were pre-filtered through a 1.2 µm glass-fibre filter (Whatman 1822-047 GF/C). All processing was undertaken in a Class II Microflow Biological Safety Cabinet fitted with a 99.999% HEPA filter (MDH Contamination Control, Hitchings Clinical Services, UK). Detergent (Jangro Professional, UK) was used to wash all glassware and equipment. SDS (sodium dodecyl sulfate) was used in the storage of samples (Fluka, BioChemica, 98% purity). The Fenton's reaction used an Fe(II) solution (0.05 M FeSO4°7H20, Fischer Scientific, USA, >98% purity) acidified with 0.2% sulfuric acid (H_2SO_4, AnaTaR, 98.07% purity) and 30% hydrogen peroxide (H_2O_2, Fisher Scientific, USA). Density separation was performed in concentrated zinc chloride solution at a density of 1.7 g/ml ($ZnCl_2$, BonnyMans, UK, >98% purity). The enzyme digestion steps utilized cellulase (MP Biomedicals, USA) and trypsin (Sigma-Aldrich, Germany, 25 g porcine trypsin per liter in 0.9% sodium chloride). All samples were dispersed in ethanol (≥99.8% purity, Sigma-Aldrich, Germany) diluted to 50% with RO water for storage prior to FTIR. The filters used to deposit samples on for analysis by FTIR were 25 mm 5 µm silver membrane filters (Sterlitech, USA).

The laboratory equipment used in the processing was selected to limit the number of plastic components used. These were as follows: stainless steel filter cartridge (Wolftechnik, Germany), 47 mm stainless steel 2 and 10 µm filters (Wolftechnik, Germany), natural bristle scouring pad (coconut coir fibre, LoofCo, UK), PTFE lined lids for glass bottles when necessary as a substitute for polypropylene lids, stainless steel tweezers, natural fibre brush (natural hair, H G Rant Ltd, UK), woven wire cloth stainless steel mesh (178 µm filter size, Bridgewater Filters Ltd., UK)

Several general procedures were common to the sampling workflows for each sample type and so have been compiled in Appendix A Table 1 for reference. Where the general procedure aims to clean the equipment prior to use, it can be assumed that when the "clean" equipment is referred to in subsequent methods, this cleaning step has been performed.

Appendix A Table 1 A reference table for general procedures common to all sample processing workflows

General procedure	Description
Washing equipment prior to use	All glassware was washed between use with RO, detergent and a natural bristle scouring pad to remove any particles. These were then rinsed three times with RO under the tap before storing for use, covered with foil. To prevent contamination, glassware and natural hair brushes used for sludge samples were marked and only used for this sample type. Immediately prior to use, all glassware was washed once more, three times with RO water under the tap, and three times with RO water within the HEPA filter safety cabinet. This same washing procedure was employed to wash the stainless steel tweezers and brushes used during the processing workflow.

General procedure	Description
Vacuum filtering	To remove unwanted reagent whilst retaining the microplastics, it is necessary to filter the dispersion between each stage of sample processing. A 10 µm pore size stainless steel filter is loaded into a standard vacuum pump set-up, with the filter handled with stainless steel tweezers. The filter unit comprises of a conical flask for collecting the filtrate, the filter holder that fits into the mouth of the flask and a, straight edged glass funnel that is placed on top of the filter and held in place with a metal clasp. The reagent is then drained through the filter, aided by the vacuum formed by the pump. The glass beaker containing the original reagent is rinsed with RO and this is also drained through the filter to ensure that all material in the beaker is transferred. The inside wall of the filter unit top is also rinsed thoroughly with RO to ensure all particulate material is deposited on the filter. The filtrate is disposed of, or for $ZnCl_2$ recycled for subsequent use by filtering to 1.2 µm over a Whatman glass fibre filter. The stainless steel filter is then removed with steel tweezers and is transferred into dispersion for the following processing step (see "transferring microplastics from the filter into dispersion").
Transferring microplastics from the filter into a dispersed solution	Stainless steel tweezers were used to lift the 10 µm pore size stainless steel filter from the vacuum filter unit. This filter is submerged, face-down in the relevant reagent. The sample is covered with clean aluminium foil and is sonicated in a bath sonicator to dislodge the material from the filter (the time for sonication varies depending on the processing stage and is stated in the detailed processing methods descriptions). After sonication, when specified, the filter is lifted from the reagent and the microplastics are rinsed and brushed off the filter using a natural hair brush back into dispersed sample. Both the tweezers and the natural hair brush are also rinsed with the relevant media into the sample after use. In other cases, the filter is retained submerged in the liquid sample. Both procedures will be explicitly referred to within the text.
Cleaning of the stainless steel filters in a muffle furnace	All stainless steel filters were heated in a muffle furnace prior to use to oxidise any microplastics and ensure no contamination. Filters were heated to 350°C for 180 minutes before cooling then rinsed with RO water and transferred to washed glassware for storage until use.

A.2 Cleaning and re-use of filters and other equipment

Seven large cartridge filter units and filter cartridges (for raw water and sewage effluent) and six disk filter units (for potable water and as pre-filters for blanks, see below) as well as >100 47 mm diameter filter disks were purchased. All filter holders and the filter cartridges were individually labelled. After use all equipment was thoroughly washed using detergent (Jangro Professional, UK) and a coconut coir fibre and metal cleaning brush (LoofCo, UK) and then

rinsed first with tap water, then three times with reverse osmosis (RO) water from the tap (TR Reverse Osmosis Unit RO S 130, conductivity 7 μS/cm) and a further three times with water from a EuRO 10 system (SG Water, conductivity 21 μS/cm) inside a Class II Microflow Biological Safety Cabinet, which filters air through a 99.999% HEPA filter (MDH Contamination Control, Hitchings Clinical Services, UK) using a wash bottle before the filters were reassembled ready for use for the next sample. Additionally, the filter disks were muffled at 350°C for 3 hrs, then rinsed three times outside of the safety cabinet and three times inside the safety cabinet.

It was not practicable to find a non-plastic alternative to laboratory LDPE wash bottles, but the same distinctive yellow coloured wash bottle was used throughout to be consistent in case some shed more or fewer polymer particles depending on age or quality.

A natural hair artist's brush (4 mm diameter, H G Rant Ltd, UK) was used at several stages during processing to remove particles from the filters. Five separate brushes were labelled and used for the different types of samples as follows: potable water, sewage effluent, raw water and sewage influent, sludge samples and the spike recoveries. During the processing the brush was rinsed into the sample with the appropriate solvent (RO water, 50% alcohol, enzyme solution) for that stage in the process and afterwards it was additionally thoroughly rinsed with RO water first from the tap and then from a LDPE wash bottle inside the safety cabinet.

All glassware was washed after use using the detergent above and rinsed with RO water three times from the tap and a further three times from the wash bottle inside the safety cabinet.

A.3   Sample collection: raw and potable water

Attached to the sampling tap is a WRAS approved hose (SILEX Platinum Cured Silicone Braided Hose - 70° Shore - Translucent, 12.5 mm internal diameter) secured with a jubilee clip. The hose is connected to a compression fitting brass non-return valve (IDC Plumbing and Heating Technology, double check valve, non-return – 15 mm DZR), by means of a short piece of copper pipe. A WRAS approved non-return valve is required by UK water companies in such a rig, however, the mechanism inside all valves of this type and size is made of plastic. Downstream of the non-return valve, there is a T-piece with a blue quarter-turn valve and another red quarter turn valve. These allow the flow to be directed either through the filter or past the filter to waste. The next item is a stainless steel filter holder (Spectrum Inox economic filter housing, EFH-SBR) with a woven stainless steel cylindrical filter cartridge (9 ¾" length, nominal pore size 10 μm, ca 500 cm^2 filter area, Wolftechnik Germany) inside. The cartridge is sealed against the filter holder with a Viton rubber seal at the bottom and a PTFE seal plus a Viton rubber seal at the top, while the filter holder itself also uses Viton rubber seals. The filter is arranged so that the flow enters on the outside of the cartridge and passes through it into the centre and to the outflow (waste) side, where another blue quarter-turn valve is located. The two blue quarter-turn valves at the inlet and outlet of the filter can be secured in the shut position with re-usable cable ties, to avoid leakage and loss of sample during transport. This is followed by clear braided PVC hose (Hermes, non-toxic, 12.5 mm ID, supplied by Onecall, UK) to connect it with a water meter (ISO 4064 ½'' cold water meter, Q$_n$=1.5 m^3/h, accuracy class B, Diamond BFG), and then the waste pipe of the same braided PVC hose. Brass Hozelock quick connectors are used from the filter unit up to the water meter,

whereas the waste pipe downstream of the water meter is connected with a plastic Hozelock connector. The different colours and materials make it easier to connect the items in the correct order.

The same general setup was used for potable water collection, except that a smaller filter unit was used (47 mm anodised aluminium filter holder, Pall Life Sciences, Advantec) with a woven stainless steel filter disc (same filter material as the cartridge filter above, 10 μm pore size, Wolftechnik, Germany) since the expected suspended solid concentration is much lower in potable water. This provided a filter area of about 10 cm^2 compared to the ca. 500 cm^2 of the larger units.

Appendix A Figure 1 Raw water sampling

Appendix A Figure 2 Potable water sampling

Briefly, the sampling procedure was the following: The sampling rig was connected to the sampling tap using the hose and secured with a jubilee clip. Wherever possible, the official sampling tap was used, but at some works this had insufficient pressure, so an alternative pressurized sampling tap was found, for example in a site laboratory. It will be noted in the results section on potable and raw water the differing volumes collected reflect this pressure issue. Then the red valve on the rig was opened and the blue valve shut to direct the flow past the filter to waste. The sampling tap was then turned on (unless it was already permanently on, which was the case at some potable water sampling points) and pipes were flushed with at least 5 L water to remove any debris that may have rubbed off the inside of the hose when it was pushed over the tap outlet. The reading on the water meter was taken and the red valve closed and blue valves opened to start filtering through the cartridge. The filtering was carried out overnight (about 16 hrs) and the following day the flow was stopped by closing the blue taps and the end reading was taken to calculate the total volume passed through the filter, before sending the rig to CEH for processing. For the earlier samples the flow rate was

not measured and no target flow rate was specified, but later the instructions were altered to include estimating the initial flow rate and adjusting it to within a recommended range that would provide appropriate filtered volume during an overnight sampling. At one site (LR3) the only raw water sampling tap available (i.e. the only one supplying the correct mixture from both water sources used there, rather than only one of them) had a very low flow rate, so it was decided that the filter should be left on the tap for a whole week rather than overnight to provide sufficient sample. At other sites, on some occasions, the filters got blocked before the sampling finished due to high suspended solids loads.

A.4   Potable water protocol

The basic approach was as follows:

1. Washing of the particles from the filter taken from the field rig and dispersion in < 1L water

2. Division of the original dispersed sample for storage or processing

3. Protein and complex carbohydrate removal by enzymatic digestion

4. Preservation of sample in 50% ethanol prior to analysis by FTIR.

Transfer of the particles from the filter rig into a dispersed solution

On receiving potable water samples from the field, the tubes of the filter unit were drained of surplus water and the 10 μm stainless steel filter disc removed with steel tweezers and placed face-down into a clean, pre-weighed 250 mL beaker. The inside of the filter unit was thoroughly rinsed with RO water into this beaker, submerging the filter. This was made up to 150 mL, with ~5 ml of SDS solution (150 g/L). If the sample was not processed on the same day as its arrival in the laboratory, the beakers were covered with foil and stored in the fridge at 4°C. When ready for processing, samples were sonicated for 2 minutes to release particles from the filter. Using stainless steel tweezers, the filter was lifted from the beaker and washed with RO water and a natural hair brush to rinse any remaining particles back into the beaker and, once clean; the filter was secured in the vacuum filtration unit. It should be noted that throughout the potable water processing, the same stainless steel filter was used for subsequent vacuum filtrations and particle recovery unless otherwise stated.

Sub-sampling

The total volume of sample in the beaker was calculated from the difference in mass between the empty and full beaker. This was mixed by pouring the sample between the original beaker and a pre-weighed 250 mL beaker 6 times and then roughly half of the original sample was poured into the second beaker. The volume of both was calculated from the difference in mass between the empty beakers and that with the sample. These two samples were labelled as S1 and S2. The sample to be stored (S1) was vacuum filtered onto the original filter disc to remove the 5% SDS solution. The material on this filter disc was then transferred into dispersion in ~5 mL 50% ethanol following the general procedure for storage (Appendix A Table 1). This stored sample is available should a challenge to the results be made. The other

half (S2) was used for the full enzymatic digestion steps in preparation for analysis by FTIR (see below).

Enzyme digestion

The sample was vacuum filtered onto a clean muffled 10 µm stainless steel filter disc, following the general procedure (Appendix A Table 1). The particulates caught on the filter were transferred into dispersion in 20 ml cellulase to remove any plant residues (200 mg/L in pH 5 phosphate buffered saline solution, which equates to 12,000 enzyme units/L as suggested by Löder *et al.* (2017)). Samples were then covered with foil and incubated for 48 h at 50°C on a rotating platform at 60 rpm. The temperature was maintained at 50°C to balance the optimum temperature for the enzyme with the need to avoid the plastics degrading. After the 48 h incubation, samples were sonicated for 2 minutes to dislodge any particles from the filter, and the filter was lifted out and microplastics on the filter washed back into the sample using RO water and a natural fibre brush. Thus, at this stage the solution contains microplastics, carbohydrates and cellulase enzyme. The particles were retained and the waste solutes removed by once again vacuum filtering this dispersion through the 10 µm stainless steel filter disc. The filter disc was then placed face-down and submerged in 16 ml RO water to which 4 ml trypsin (2.5%) was added, giving an overall enzyme concentration of 0.5%, as recommended by Courtene-Jones *et al.* (2017) (who found 0.31% to be sufficient). This sample was incubated for 30 minutes at 37°C on a rotating platform at 60 rpm. Any microplastics on the filter were transferred into dispersion through sonication using the general procedure before microplastics on the filter were washed back into the sample using RO water and a natural fibre brush. As before, the microplastics were separated from any waste degraded proteinaceous material and enzyme by vacuum filtering the sample back onto the 10 µm filter.

Transfer into ethanol for storage

The filter disc was then placed face-down into a clean 250 mL beaker. To transfer the now clean microplastics from the filter disc, the disc was submerged in ~4-5 mL ethanol and sonicated for 15 minutes. The filter disc was lifted out and rinsed with ethanol using a glass pipette, to ensure all particles were removed from the filter and transferred into a clean 20 mL glass vial for storage prior to analysis by FTIR.

A.5   Raw water, WwTW effluent, WwTW influent processing steps

The basic approach was as follows:

1. Washing of the particles from the large filter cartridge taken from the field rig and dispersion in a liquid

2. Division of the original dispersed sample for storage or processing

3. Fenton's reaction to oxidise general organic materials

4. Protein and complex carbohydrate removal by enzymatic digestion

5. Preservation of sample in 50% ethanol prior to analysis by FTIR.

Transfer of the particles from the filter rig into dispersion

Transferal of the particles from both the raw water and WwTW effluent filter rigs into a suspension followed the same principles as for the potable water disc filters, however, the protocol was adapted to the much larger filter cartridges. The filter was removed from the rig and placed on a metal grid over a clean (pre-weighed) 1 L beaker. Any residual liquid in the filter rig casing was rinsed into the sample beaker (S1) as this was in contact with the outer surface of the filter and so must also be sampled to ensure maximum recovery of particles. The sample was washed off the filter using RO water and a natural fibre brush until visibly clean, and the filter rinsed a further three times after this with RO water into the S1 beaker.

Sub-sampling

The full sample was weighed (to calculate the volume of the total sample), and then divided following the same procedure as for potable water, pouring the sample back and forth six times from the original beaker into a clean 1 L beaker to homogenize the sample. Then 200 mL of the mixed sample was poured into a new 1 L beaker (S2). If S2 was not being processed that same day, the sample was placed into a freezer for storage. The original beaker (S1) was weighed once more to calculate the volume of both sub-samples. The S1 sample was transferred to a 1 L glass Duran bottle and frozen for long term storage at -18˚C. The S2 sample was used for processing.

The WwTW influent was sampled as a 24 hour composite volume directly into a 5 L Schott Duran glass bottle and so no transfer of the particles from a filter was required. Rather, these samples, once returned to the laboratory, were sub-sampled by vigorously shaking the Schott Duran glass bottle and pouring out approximately 0.5 L into two sub-sample Duran bottles, with S1 being stored at -18˚C. S2 was also frozen at -18˚C until further processing. Before processing, S2 was removed from the freezer and allowed to defrost at room temperature. This sample was then sub-sampled by pouring between 2 pre-weighed beakers. Approx. 200 mL was set aside for further processing (exact volume determined by weight of sample within the beaker) and the remaining S2 sample was placed back into the freezer.

Fenton's Reaction

The Fenton's reaction was carried out in order to break down the complex organic macromolecules by oxidation. The 200 mL sample was placed in a 600 mL beaker and then placed in a shallow iced water bath to ensure the Fenton's reaction remained <50°C. Then 70 mL of H_2O_2 and 30 mL 0.05 M Fe(II) solution were measured out, and the H_2O_2 was added to each sample, followed by 30 mL 0.05 M Fe(II). Over the incubation period of one hour, the temperature of the samples was monitored using a Mini RayTemp infrared thermometer (Electronic Temperature Instruments Ltd (ETI), Worthing, UK) to ensure that samples did not exceed 50°C. Overheating could be prevented by adding extra ice to the ice bath. The samples were covered loosely with foil and left in a fume hood overnight for the reaction to complete. If any iron precipitates had formed in the sample, these were removed by pipetting 1% H_2SO_4 drop-wise until they dissolved.

Enzyme Digestion and Storage in Ethanol

Particles were separated from the Fenton's reagent by vacuum filtering each sample onto a stainless steel 10 µm disc filter following the methods in the table of general procedures (Appendix A Table 1). This was handled in the same manner as potable water samples for enzyme digestion, detailed above, digested with both cellulase and trypsin. It should be noted that throughout the processing of each sample, the same stainless steel filter was used for each vacuum filtering step to limit potential losses of microplastics to the filter. The resulting sample is transferred from the filter after both steps of the enzyme digestion into dispersion in 50% ethanol and stored in a clean 20 mL glass vial until analysis by FTIR.

A.6 Potable and raw water blanks

Several blanks were run, the first samples taken in October 2018 and then once a week from January to February 2019. Two filter rigs were affixed in sequence to a mains tap. The first filter rig was fitted with a 2 µm stainless steel filter to act as a "pre-filter" to remove any microplastic material present in the mains tap water prior to entering the second filter rig, thus any particles deposited on the second filter should be too small to be observed as part of our analytical procedures. This second filter rig represented the filter 'in the field' and was fitted with a 10 µm stainless steel filter – a cartridge filter for the raw water blanks or a 47 mm disc filter for the potable water (Appendix A Figure 3) . Before commencing filtering through the second rig, the flow rate was measured and adjusted by diverting the flow through the by-pass tap (red tap, Appendix A Figure 1) to a flow meter and allowing ~5 L to flow. Once a desired flow rate was achieved of between 2-3 L/min the bypass tap was closed and the flow continued through the second filter rig, containing the 10 µm filter before passing out to drain into the sink (Appendix A Figure 3). This filtration was left running overnight for ≈16 hours to mimic the sampling procedure followed at WTWs (section A.3). The volume filtered was recorded by the attached flow meter. The second filter rig fitted with the 10 µm stainless steel filter was then processed using the workflow for potable water (section A.4).

Appendix A Figure 3 Schematic setup of raw and potable water blanks
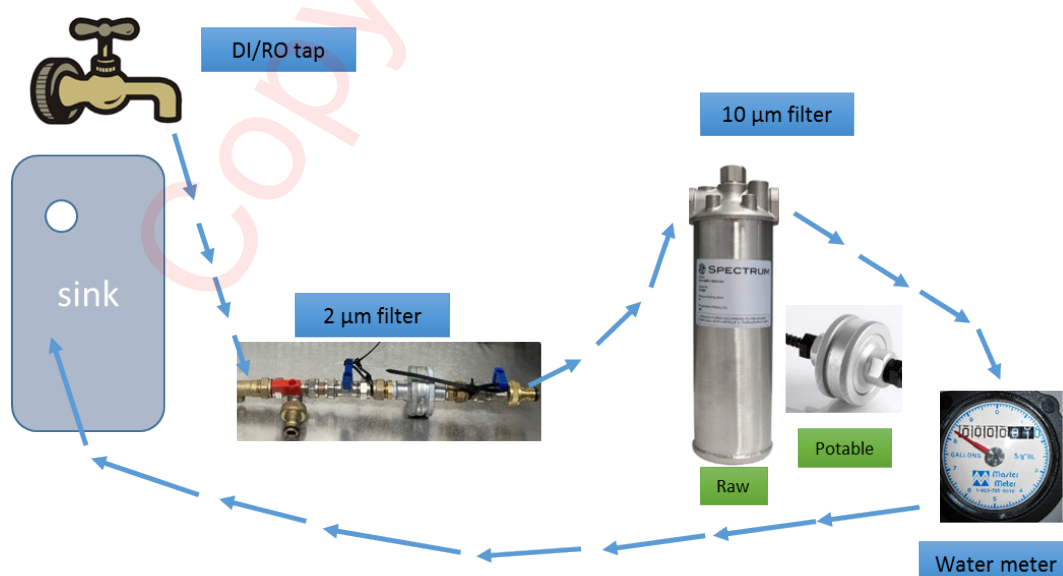
The workflow for raw water sample blanks was identical to that of the potable water, save for the second filter rig being the large 10 μm cylindrical stainless steel filter cartridge for raw water and a smaller 10 μm filter disc for potable water. After ≈16 hours of flow of "pre-filtered" RO water passing through this filter cartridge, this rig was processed using the workflow for raw water (Section A5). A total of ten potable sample blanks and eight raw water sample blanks were run at periodic intervals throughout the project, so as to be representative of any contamination arising during laboratory processing.

A.7   Sludge protocol (WTW and WwTW)

The general workflow for processing sludge samples was as follows:

1. Drying the sludge for storage and to get a dry sample for subsequent processing

2. Sub-sampling 1 g of material following breaking up aggregated material and sieving to <1 mm

3. Fenton's reaction to oxidize organic material

4. Density separation through flotation in concentrated $ZnCl_2$ solution to remove heavier residuals

5. Protein and complex carbohydrate removal by enzymatic digestion

6. Filtering to "coarse" (>178 μm) and "fine" (<178 μm) fractions and dispersion in 50% ethanol for storage prior to analysis by FTIR.

Sludge drying and sub-sampling

Samples were oven dried upon receipt at 50°C, for approximately one week. After the samples had dried, they were weighed. Several grams of sludge solids were decanted into a porcelain mortar and pestle and disaggregated, crushing larger clusters of material. This was done to generate sufficient material <1 mm in size from which the subsample of 1 g of material could be taken for processing, whilst limiting the potential for the grinding to wear microplastics within the samples. The disaggregated material was sieved through a stainless steel 1 mm sieve, 1 g dry mass sludge sampled from the sieved material was transferred to a clean 600 mL beaker. The remaining sludge solids were decanted back into the original sample jar.

Fenton's reaction

For the Fenton's reaction, 200 mL of RO water was added to the sludge samples. This solution was then digested with Fenton's reagent as described for the raw water, WwTW influent and effluent as described previously. However, because of the relatively high level of organic material and the need to manage a potentially vigorous reaction, 5 mL of the Fe(II) solution was added every 5 minutes for the first 15 minutes of the reaction. If the sample temperature remained <50°C, the remaining 15 mL of Fe(II) was added and the temperature was monitored over the following hour. The samples were covered loosely with foil and left in a fume hood overnight for the reaction to complete. If the reaction had not completed

overnight, samples were placed into the incubator at 50°C, and rotated at 60 rpm until bubbles ceased to form in the sample, an indication that the reaction had been completed.

Flotation

Once the Fenton's reaction was complete, samples were filtered onto 47 mm 10 μm stainless steel filter disc through a vacuum pump to remove the waste solutes as outlined in the general procedures (Appendix A Table 1). The particulate matter caught by the filter was transferred into dispersion in $ZnCl_2$ in a 250 mL beaker following the general procedure, with a sonication time of 5 minutes. On removal, the filter was brushed and rinsed with a natural fibre brush and $ZnCl_2$ to transfer all the sample into the 250 mL beaker. The stainless steel filter was retained and stored in a covered glass petri dish for use in subsequent processing steps. The sample dispersed in $ZnCl_2$ was poured into conical separation funnels using additional $ZnCl_2$ to rinse the beaker and agitate the sample. A total of 225 mL of $ZnCl_2$ was used per sample in each conical flotation funnel. This was left quiescent for 20 hours to separate. A number of previous studies have utilised density separations in various solutions as a method for separating microplastics from a range of solid matrices including soils and sediments (Coppock *et al.* 2017, Horton *et al.* 2017, Quinn *et al.* 2017). This 20 hour period was adapted from previous studies that successfully recovered polystyrene particles >4 μm and calculations indicate this is sufficient to completely recover particles ≥20 nm in size up to a density of 1.675 g/cm^3 (Wang *et al.* 2018). After a minimum of 20 h, the bottom quarter of the settled material in $ZnCl_2$ was released through the outflow tap into a waste beaker. The remaining sample was left to settle for an additional 5 minutes before releasing more of the sample. One quarter of the original volume was retained in the separation funnel, and this was poured carefully through the top of the funnel into a clean 250 mL beaker, and the funnel was rinsed thoroughly with RO water to ensure all particles were washed into the beaker. This sample then proceeded to the enzyme digestion step.

Enzyme Digestion and separation into coarse and fine fractions

To remove the unwanted $ZnCl_2$ solution and retain the floated microplastics, the sample in dispersion in $ZnCl_2$ was vacuum filtered following the general procedure. The solid material was then transferred off the stainless steel filter and into dispersion in 20 mL cellulase following the general procedure with a sonication time of 5 minutes. The enzyme digestion steps were performed in the same manner as for the water samples.

After enzymatic digestion, the solid material on the filter was sonicated for 5 minutes once more to dislodge any particles from the filter, brushed and rinsed with RO water into dispersion and then divided into coarse and fine fractions. The coarse fraction was collected first, representing particles >178 μm in size, vacuum filtering the spent enzyme solutions over a 178 μm stainless steel filter. The "coarse" fraction of material captured on this filter was transferred from the filter into dispersion in 50% ethanol for storage following the general procedure. The filtrate that passed through the 178 μm filter represents the "fine" fraction of particles that can be analysed with FTIR. These have to be vacuum filtered over a 10 μm stainless steel filter to remove the excess enzyme solution and are then re-dispersed in 50% ethanol for storage in clean glass vials.

A.8   Depositing different types of samples onto silver filters prior to FTIR analysis

For FTIR analysis, samples were vacuum filtered onto 25 mm 5 µm silver filters. The set up for this vacuum pump was the same as for the 47 mm diameter, 10 µm steel filters but using smaller glassware apparatus (13 mm filter funnel) to fit the smaller size of the 25 mm silver filter discs (thus producing a filtered area of 13 mm diameter). For the potable samples, the whole sample was vacuum filtered and rinsed thoroughly onto the silver filter with RO water. This silver filter was then stored in a clean glass petri dish covered with foil. As such, the FTIR image analysis of these samples represents the entirety of the processed potable water sub-sample (representing approximately half that which was filtered on site). For the raw/effluent/influent and sludge samples, the sheer number of particles (plastic and non-plastic) meant analysis of the whole sample on one filter would be too concentrated for analysis by FTIR. Therefore, for these sample types, a sub-sample was deposited on the filter.

First, the mass in grams of the 20 mL glass storage vial containing the sample was measured to 4 decimal places, then, using a vortex, the vial containing the sample was mixed for 10 seconds until homogenously dispersed. Using a glass pipette, ~100-200 µL was deposited onto the silver filter held in place in the vacuum pump unit. Around ~1 ml of RO water was added to this sample before switching on the vacuum pump to draw the liquid through the filter. This excess RO water was added to disperse the particles across the whole filter, with the aim to improve the distribution of microplastics on the filter and so the quality of the image for analysis by FTIR. The vacuum pump remained running whilst the upper half of the vacuum filtration unit was unclamped and removed to fix the silver filter in place whilst disassembling the filter unit. The pump was then switched off and the silver filter was removed and placed face up in a clean glass petri dish covered with foil. The glass storage vials containing the remaining sample were then weighed to calculate the volume of sample that was deposited for FTIR analysis. A calculation was applied to account for the fact that ethanol is slightly less dense than water (0.9327 g/mL).

A.9   FTIR Data collection

A Perkin Elmer Spotlight 400 FTIR micro spectrometer was used for the analysis of samples generated in this project. Silver filters containing the processed samples were mounted on a glass slide and held in place with a stainless steel O-ring and fixed with clamps to prevent movement during mapping. The spectrometer was set up to collect spectra in the range between 4000 and 700 cm^{-1} (wavenumbers). A background spectrum was collected for each sample from a blank area of silver filter, at a resolution of 8 cm^{-1}, with a total of 90 scans per pixel with an interferometer speed of 2.2 cm/s. To generate an FTIR map, an optical image was first collected by tiling single field of view images together to cover an area of approximately 13 mm x 13 mm. The mapping area covering the filter was selected to be 11.6 mm x 11.6 mm due to the limitations of the file size generated by the Perkin Elmer SpectrumIMAGE software, which resulted in 92 % of each filter being mapped. Preliminary analysis showed that there was no significant accumulation of particles at the boundary of the filter unit and therefore this was considered representative of the whole filter. Mapping was carried out using 4 accumulations (i.e. four scans per spectra) at a resolution of 25 µm per pixel, which reflects a trade-off between mapping time and spectral quality. Mapping the 11.6 x 11.6 mm area took approximately 2 h 30 minutes per sample.

A.10 FTIR Data analysis with MPHunter

Files containing the spectra map information were first processed to remove signals from atmospheric water vapour and carbon dioxide (atmospheric correction). The processed files were then loaded into the FTIR particle analysis software MPhunter, available on request from the originator, Professor Jes Vollertsen (jv@civil.aau.dk) at Aalborg University, Denmark. Due to the fact that processing and analysing large spectral maps containing upwards of 160,000 spectra per file is computationally intensive, all subsequent analyses were performed on a dedicated 20 core PC with 64 GB of RAM and a 1 TB SSD hard drive. MPhunter first converts Perkin Elmer .fsm files into .spe files that can be read by MPhunter. Converted files are then loaded into the software along with a custom polymer database comprising reference spectra of target plastics. Due to the short time-scale of this project it was decided to focus on a sub-set of nine common plastic polymers. There are many rare polymer types, but quantifying such additional targets in the reference database would have added considerably to the analysis time. The most common plastics in the reference database, supplied with MPhunter, were acrylonitrile butadiene styrene (ABS), polyamide (PA), polyethylene (PE), polyethylene terephthalate (PET), poly(methyl methacrylate) (PMMA), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC-U), and polyurethane (PU). Spectra were matched against this database using a threshold of 0.65 (where 1.0 is a perfect match and 0 is a complete mismatch). There is the potential to optimise the matching process by defining different thresholds for different polymers for different sample types. However, the conservative approach of applying the same threshold across all polymers was taken. There is currently no established practice for thresholds in the literature, so a threshold of 0.65 was chosen as a compromise between allowing for spectral modifications that occur when microplastic particles weather in the environment and having a reasonable confidence in the polymer assignment. After matching against the database (a process that takes anywhere from 3-6 h per sample) clusters of spectra that match against the reference database are identified as particles and exported from the software in the form of an image file and a .csv file containing particles, their identity, location, size and estimated volume.

For final reporting on the number of particles the results from the 92% of a filter scanned were extrapolated to 100% (i.e. particles on 100% of filter = (particles counted on 92% of filter / 92) * 100). A worked example of the detailed calculations is given in Appendix D.

A.11 References

Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M. and Galloway, T.S. (2017). "A small-scale, portable method for extracting microplastics from marine sediments." Environmental Pollution 230: 829-837.

Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S.F. and Narayanaswamy, B.E. (2017). "Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics." Analytical Methods 9(9): 1437-1445.

Horton, A.A., Svendsen, C., Williams, R.J., Spurgeon, D.J. and Lahive, E. (2017). "Large microplastic particles in sediments of tributaries of the River Thames, UK - Abundance, sources and methods for effective quantification." Marine Pollution Bulletin 114(1): 218-226.

Löder, M.G., Imhof, H.K., Ladehoff, M., Löschel, L.A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I. and Laforsch, C. (2017). "Enzymatic purification of microplastics in environmental samples." Environmental science & technology 51(24): 14283-14292.

Quinn, B., Murphy, F. and Ewins, C. (2017). "Validation of density separation for the rapid recovery of microplastics from sediment." Analytical Methods 9(9): 1491-1498.

Wang, Z., Taylor, S.E., Sharma, P. and Flury, M. (2018). "Poor extraction efficiencies of polystyrene nano- and microplastics from biosolids and soil." PLoS One 13(11): e0208009.

Appendix B Sample and site details

Appendix B Table 2 Volumes filtered: WTW. The solids collected on the filter were processed and subsampled, so only a proportion was used in the final analysis by FTIR.

Code	WTW	Sample	Date	Raw water (code R)		Potable water (code P)	
				Total volume filtered [L]	Volume [L] represented in final FTIR sample	Total volume filtered [L]	Volume [L] represented in final FTIR sample
01	**LR1**	1	29/08/2018	309	63	5,494	2,713
		2	24/09/2018	831	287	2,642	1,334
		3	08/10/2018	1,264	241	790	370
	Lowland river, direct abstraction	4	25/10/2018	1,657	724	2,946	1,542
		5	14/12/2018	236	1.6	2,800	1,504
			Total	4,297	1,316	14,672	7,463
02	**LR2**	1	03/10/2018	334	6.1	3,178	1,574
		2	22/10/2018	5,853	61	4,166	2,245
		3	05/11/2018	247	1.1	3286	1,628
	Lowland river, direct abstraction	4	26/11/2018	63	18	3,605	1,695
		5	11/12/2018	1.4(?)	0.4	2,969	1,433
			Total	6,497	87	17,204	8,575
03	**LR3**	1	27/09/2018	5,661	3,354	4,448	2,206
		2	12/11/2018	5,469	57	8,713	4,132
		3	21/11/2018	51,839	429	-	-
			11/01/2019	-	-	3,925	1,856
		4	18/02/2019	41,143	448	-	-
	Lowland river, direct abstraction		06/03/2019	-	-	12,710	5,893
		5	01/05/2019	39,491	5,032	-	-
			Total	143,603	9,320	29,796	14,087
04	**LRS1**	1	03/10/2018	1,506	16.7	4,165	2,089
		2	24/10/2018	121	1.4	876	461
		3	07/11/2018	179	2.2	2715	1,469
	Lowland river, pumped storage	4	29/11/2018	14	0.08	3510	1,699
		5	10/12/2018	2,087	35	2,200	1,023

Code	WTW	Sample	Date	Raw water (code R)		Potable water (code P)	
				Total volume filtered [L]	Volume [L] represented in final FTIR sample	Total volume filtered [L]	Volume [L] represented in final FTIR sample
			Total	*3,907*	*55*	*13,466*	*6,741*
05	**LRS2**	1	18/09/2018	2,429	19	2,204	1,062
		2	02/10/2018	5,484	1,557	5,902	2,798
		3	26/10/2018	655	202	20495	9,938
	Lowland river, pumped storage	4	14/11/2018	9,502	4,332	12,190	6,279
		5	04/12/2018	85	26	14338	7,057
			Total	*18,155*	*6,135*	*55,129*	*27,134*
06	**GWC**	1	08/10/2018			7917	4,309
		2	22/10/2018			6662	3,642
		3	05/11/2018	n.a.	n.a.	3701	2,013
		4	20/11/2018			5890	2,750
	Groundwater, chalk	5	05/12/2018			6212	3,381
			Total			*30,382*	*16,095*
07	**GWS**	1	08/10/2018			5,990	2,787
		2	22/10/2018			4,499	2,360
		3	05/11/2018	n.a.	n.a.	3,094	1,560
		4	20/11/2018			6,222	2,848
	Groundwater, greensand	5	05/12/2018			7,308	3,605
			Total			*27,113*	*13,160*
08	**UR**	1	20/09/2018	2,460	25	1,418	766
		2	09/10/2018	2,816	829	786	355
		3	25/10/2018	2,852	1,392	271	141
	Pristine upland reservoir	4	13/11/2018	2,224	805.5	1,478	725
		5	04/12/2018	412	228	173	89
			Total	*10,764*	*3,280*	*4,126*	*2,076*

Appendix B Table 3 Volumes analysed WwTW. The nominal collected volume was 4.8 L for influent and 360 L for effluent, each collected as a composite of 48 samples every 0.5 hrs. Normally a subsample of 0.2 L of influent and all the effluent were filtered and a subsample of the processed solids was analysed by FTIR.

Code	WwTW	Secondary treatment	Tertiary treatment	Sample	Date	Influent (code I) Volume [mL] represented in final FTIR sample	Effluent (code E) Volume [mL] represented in final FTIR sample
01	ASTC1	activated sludge	cloth filter	1	26/11/2018	1.2	721
				2	08/01/2019	1.4	692
02	ASTS1	activated sludge	sand filter	1	29/11/2018	1.2	1,348
				2	10/01/2019	1.1	2,829
03	ASTS2	activated sludge	sand filter	1	03/12/2018	1.1	2,049
				2	14/01/2019	1.3	1,106
04	ASTC2	activated sludge	cloth filter	1	05/12/2018	1.1	1,089
				2	21/01/2019	1.1	2,195
05	TFP	plastic trickling filter	pond	1	12/12/2018	1.4	2,396
				2	23/01/2019	1.2	3,138
06	AS1a	activated sludge		1	16/01/2019	4.2	1,052
				2	31/01/2019	1.1	1,653
07 AS	AS2b	activated sludge	n.a. (sampled before tertiary)	1	11/01/2019	1.3	1,848
				2	17/02/2019	1.1	2,383
07 TF	TFSb	stone trickling filter	n.a. (sampled before tertiary)	1	11/01/2019	3.1	1,637
				2	17/02/2019	1.3	149
08	BAFF	biological aerated flooded filter		1	29/01/2019	1.2	6,348
				2	05/02/2019	1.4	2,551

Appendix B Table 4 Weights analysed for WTW sludge. About 0.8 L wet sludge by volume was collected. This was dried at 50°C and then, 1 g sieved sludge (<1 mm) was processed and a subsample of the fine fraction (<178 µm) was analysed by FTIR.

Code	WTW sludge (code PS)	Sample	Date	sludge weight [mg] represented in final FTIR sample
02	LR2	1	03/10/2018	13.9
		2	11/12/2018	12.0
03	LR3	1	27/09/2018	2.7
		2	12/11/2018	2.1
04	LRS1	1	04/10/2018	9.3
		2	11/12/2018	8.3
08	UR	1	21/09/2018	22.3
		2	04/12/2018	11.6

Appendix B Table 5 Weights analysed for WwTW sludge. About 0.8 L wet sludge by volume was collected. This was dried at 50°C and then, 1 g sieved sludge (<1 mm) was processed and a subsample of the fine fraction (<178 µm) was analysed by FTIR.

Code	WwTW sludge (code S)	Sample	Date	Sludge weight [mg] represented in final FTIR sample
01	AAD1a	1	26/10/2018	9.1
		2	16/01/2019	23.9
	Advanced anaerobic digestion	3	31/01/2019	28.3
		4	07/02/2019	10.2
		5	21/02/2019	13.6
02	AAD2	1	10/09/2018	12.2
		2	17/09/2018	28.1
	Advanced anaerobic digestion	3	24/09/2018	19.6
		4	08/11/2018	23.9
		5	13/12/2018	16.9
03	LS	1	06/09/2018	25.3
		2	17/07/2018	7.2
		3	09/10/2018	7.2
	Limed sludge	4	19/11/2018	4.2
		5	14/12/2018	3.2

Code	WwTW sludge (code S)	Sample	Date	Sludge weight [mg] represented in final FTIR sample
04	**ADb**	1	19/10/2018	22.1
		2	24/10/2018	24.0
		3	03/01/2019	24.1
	Conventional anaerobic digestion	4	06/11/2018	13.9
		5	27/11/2018	21.0
05	**AAD3**	1	26/07/2018	27.7
		2	20/09/2018	24.1
		3	28/09/2018	26.3
	Advanced anaerobic digestion	4	04/10/2018	30.6
		5	12/10/2018	27.7

Appendix C   Results tables and figures

Appendix C Table 6 Individual results for each of the 8 blank samples for raw water (also used for WwTW influent and final effluent) with calculated LOD and LOQs. Particles per processed blank (corrected to 100% filter area).

Sample Polymer	1	2	3	4	5	6	7	8	Mean	SD	LOD	LOQ
ABS	1	-	2	2	2	-	9	-	2.0	2.9	9.5	29
PA	-	-	1	2	1	-	5	1	1.4	1.8	6.0	18
PE	39	27	13	9	5	-	14	5	14	13	43	130
PET	46	26	13	3	9	1	36	11	18	16	53	161
PMMA	-	-	-	-	-	-	-	-	-	-	1.1	3.3
PP	22	20	17	9	3	1	10	5	11	8	26	78
PS	1	-	-	-	1	-	2	2	0.8	1.0	3.2	9.6
PVC-U	-	-	-	-	-	-	-	-	-	-	1.1	3.3
PU	-	-	-	-	-	-	-	-	-	-	1.1	3.3

Appendix C Figure 4 Plotted values for the microplastics found in the eight raw water blanks corrected to 100 % filter area (also used for influent and effluent).
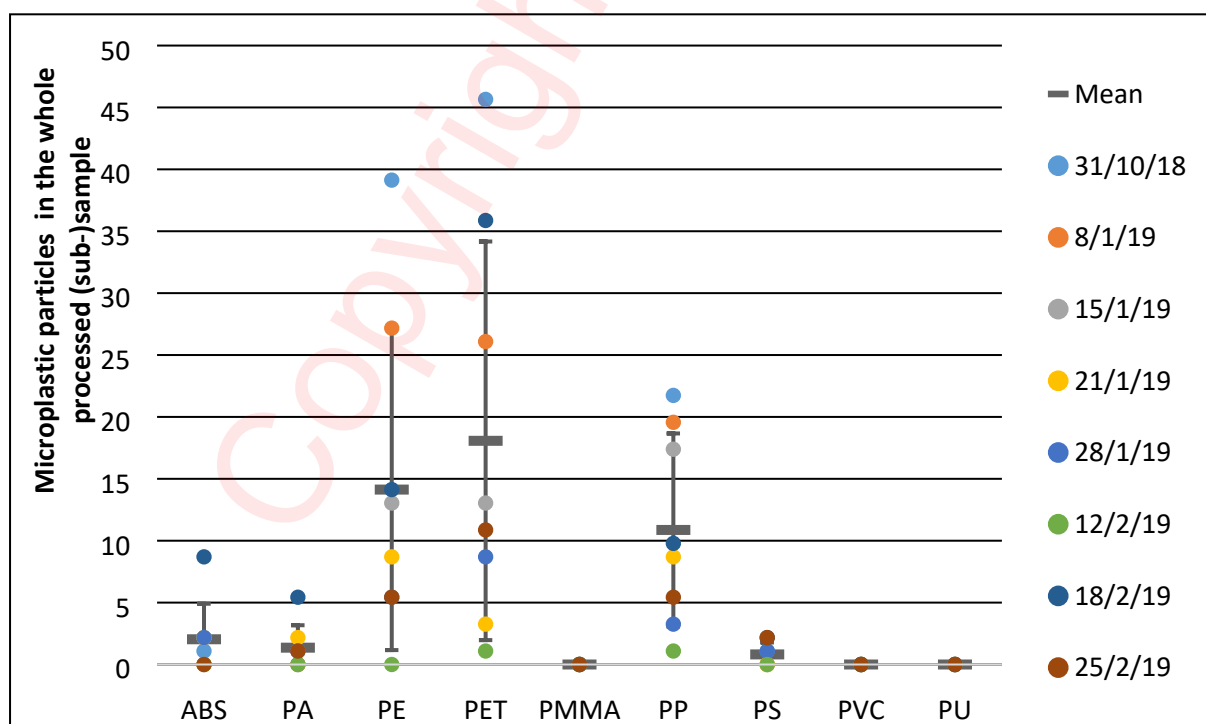
Appendix C Table 7 Microplastic particles in raw water samples, particles counted on each filter (not corrected for blanks or by volume or visible filter area).

Site name	Date	ABS	PA	PE	PET	PMMA	PP	PS	PVC	PU
LR1	29/08/2018	-	-	1	-	-	1	-	-	-
LR1	24/09/2018	3	4	85	15	-	3	-	-	-
LR1	08/10/2018	-	-	-	-	-	-	-	-	-
LR1	25/10/2018	3	-	11	16	-	9	-	-	-
LR1	14/12/2018	-	-	7	2	-	3	-	-	-
LR2	02/10/2018	-	-	55	1	-	20	-	1	-
LR2	22/10/2018	-	-	12	3	-	3	-	-	-
LR2	05/11/2018	-	-	16	1	-	2	-	-	-
LR2	26/11/2018	1	-	56	3	1	17	-	-	-
LR2	11/12/2018	-	-	75	3	-	7	-	-	-
LR3	27/09/2018	2	1	16	29	-	30	-	-	-
LR3	12/11/2018	-	-	90	2	1	8	-	1	-
LR3	11/01/2019	-	-	-	-	-	-	-	-	-
LR3	18/02/2019	-	-	-	-	-	-	-	-	-
LR3	01/05/2019	1	1	39	11	-	54	-	-	-
LRS1	03/10/2018	-	-	-	-	-	-	-	-	-
LRS1	24/10/2018	-	-	1	-	-	-	-	-	-
LRS1	07/11/2018	-	-	5	-	-	3	-	-	-
LRS1	29/11/2018	-	-	9	2	-	-	-	-	-
LRS1	10/12/2018	-	-	4	2	-	-	-	-	-
LRS2	18/09/2018	-	-	4	2	-	-	-	-	-
LRS2	02/10/2018	-	2	40	18	-	20	1	-	1
LRS2	26/10/2018	1	-	35	6	-	22	8	-	-
LRS2	14/11/2018	-	-	32	3	-	4	-	-	-
LRS2	04/12/2018	-	-	66	7	-	17	1	-	-
UR	20/09/2018	-	-	1	-	-	-	-	-	-
UR	09/10/2018	-	-	3	-	-	2	-	-	-
UR	25/10/2018	4	1	17	30	-	39	2	-	1
UR	13/11/2018	1	-	15	3	-	2	-	-	-
UR	04/12/2018	-	6	17	17	-	17	-	-	-

Appendix C Table 8 Concentrations as microplastic particles per L in raw WTW water, values <LOD are qualified by "<" and those above the LOD but below the LOQ by "≈". Values above the LOD are coloured yellow and those above the LOQ green, LOD and LOQ values depend on the processed volumes.

Name	Date	% S2 on final filter	Resolution*	ABS MP/L	PA MP/L	PE MP/L	PET MP/L	PMMA MP/L	PP MP/L	PS MP/L	PVC MP/L	PU MP/L
LR1	29/08/18	100%	0.02	<0.2	<0.10	<0.7	<0.8	<0.02	<0.4	<0.05	<0.02	<0.02
LR1	24/09/18	100%	0.004	<0.03	<0.02	≈0.3	<0.2	<0.004	<0.09	<0.011	<0.004	<0.004
LR1	08/10/18	56%	0.005	<0.02	<0.014	<0.10	<0.12	<0.005	<0.06	<0.007	<0.005	<0.005
LR1	25/10/18	100%	0.002	<0.01	<0.008	<0.06	<0.07	<0.002	<0.04	<0.004	<0.002	<0.002
LR1	14/12/18	2.4%	0.7	<0.7	<0.7	4.4	≈1.1	<0.7	≈1.8	<0.7	<0.7	<0.7
LR2	02/10/18	10%	0.2	<0.2	<0.2	9.6	<0.9	<0.2	3.4	<0.2	≈0.2	<0.2
LR2	22/10/18	4.6%	0.02	<0.02	<0.02	0.2	<0.04	<0.02	≈0.04	<0.02	<0.02	<0.02
LR2	05/11/18	2.3%	0.98	<0.98	<0.98	15	<1.08	<0.98	≈1.7	<0.98	<0.98	<0.98
LR2	26/11/18	100%	0.06	<0.5	<0.3	≈2.6	<3.0	≈0.061	<1.4	<0.2	<0.06	<0.06
LR2	11/12/18	100%	2.5	<21.8	<13.8	≈155	<122	<2.5	<59	<7.3	<2.5	<2.5
LR3	27/09/18	100%	0.0003	<0.003	<0.002	<0.01	<0.02	<0.0003	<0.008	<0.0009	<0.0003	<0.0003
LR3	12/11/18	2.0%	0.02	<0.02	<0.02	1.70	≈0.03	≈0.02	0.15	<0.02	≈0.02	<0.02
LR3	11/01/19	3.2%	0.003	<0.003	<0.003	<0.003	<0.004	<0.003	<0.003	<0.003	<0.003	<0.003
LR3	18/02/19	3.1%	0.002	<0.002	<0.002	<0.003	<0.004	<0.002	<0.002	<0.002	<0.002	<0.002
LR3	01/05/19	100%	0.0002	<0.002	<0.001	<0.009	<0.011	<0.0002	≈0.01	<0.001	<0.000	<0.0002

Name	Date	% S2 on final filter	Resolution*	ABS MP/L	PA MP/L	PE MP/L	PET MP/L	PMMA MP/L	PP MP/L	PS MP/L	PVC MP/L	PU MP/L
LRS1	03/10/18	4.5%	0.07	<0.07	<0.07	<0.12	<0.14	<0.07	<0.07	<0.07	<0.07	<0.07
LRS1	24/10/18	5.0%	0.8	<0.8	<0.8	<1.5	<1.9	<0.8	<0.9	<0.8	<0.8	<0.8
LRS1	07/11/18	4.0%	0.5	<0.5	<0.5	≈2.2	<1.0	<0.5	≈1.3	<0.5	<0.5	<0.5
LRS1	29/11/18	2.8%	13	<13	<13	113	≈20	<13	<13	<13	<13	<13
LRS1	10/12/18	4.8%	0.03	<0.03	<0.03	≈0.11	<0.07	<0.03	<0.04	<0.03	<0.03	<0.03
LRS2	18/09/18	2.9%	0.06	<0.06	<0.06	0.20	≈0.09	<0.06	<0.06	<0.06	<0.06	<0.06
LRS2	02/10/18	100%	0.0007	<0.006	<0.004	<0.03	<0.03	<0.0007	<0.02	<0.002	<0.0007	≈0.0007
LRS2	26/10/18	100%	0.005	<0.05	<0.03	<0.21	<0.26	<0.005	<0.13	≈0.04	<0.005	<0.005
LRS2	14/11/18	100%	0.0003	<0.002	<0.001	<0.010	<0.01	<0.0003	<0.006	<0.0007	<0.0003	<0.0003
LRS2	04/12/18	100%	0.042	<0.4	<0.23	≈2.21	<2.04	<0.04	<1.0	<0.12	<0.04	<0.04
UR	20/09/18	2.0%	0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
UR	09/10/18	100%	0.0013	<0.011	<0.007	<0.05	<0.06	<0.001	<0.03	<0.004	<0.001	<0.001
UR	25/10/18	100%	0.0008	<0.007	<0.004	<0.03	<0.04	<0.0008	≈0.023	<0.002	<0.0008	≈0.0008
UR	13/11/18	100%	0.0013	<0.012	<0.007	<0.05	<0.07	<0.0013	<0.03	<0.004	<0.0013	<0.0013
UR	04/12/18	100%	0.005	<0.04	<0.03	<0.2	<0.2	<0.005	<0.11	<0.014	<0.005	<0.005

* Resolution refers to the amount that the calculated microplastic particles/L changes from a single detected particle on the silver filter in the FTIR

Appendix C Table 9 Individual results for each of the 10 potable blank samples with calculated LOD and LOQs. Particles per processed blank (corrected to 100% filter area).

Sample Polymer	1	2	3	4	5	6	7	8	9	10	Mean	SD	LOD	LOQ
ABS	1	-	-	1	-	-	-	-	-	-	0.2	0.5	1.5	4.6
PA	-	1	3	1	1	1	1	-	-	-	0.9	1.0	3.3	10
PE	10	15	9	15	12	4	9	-	-	-	7.4	6.0	20	60
PET	61	21	1	18	15	16	39	-	2	-	17	20	65	197
PMMA	-	-	-	-	-	-	-	-	-	-	-	-	1.1	3.3
PP	8	7	-	10	18	1	4	-	-	-	4.8	6.0	20	60
PS	-	-	-	-	-	-	-	-	-	-	-	-	1.1	3.3
PVC-U	-	-	-	-	-	-	-	-	-	-	-	-	1.1	3.3
PU	-	-	-	-	-	-	-	-	-	-	-	-	1.1	3.3

Appendix C Figure 5 Plotted values for the microplastics found in the potable blanks (corrected to 100% filter area).
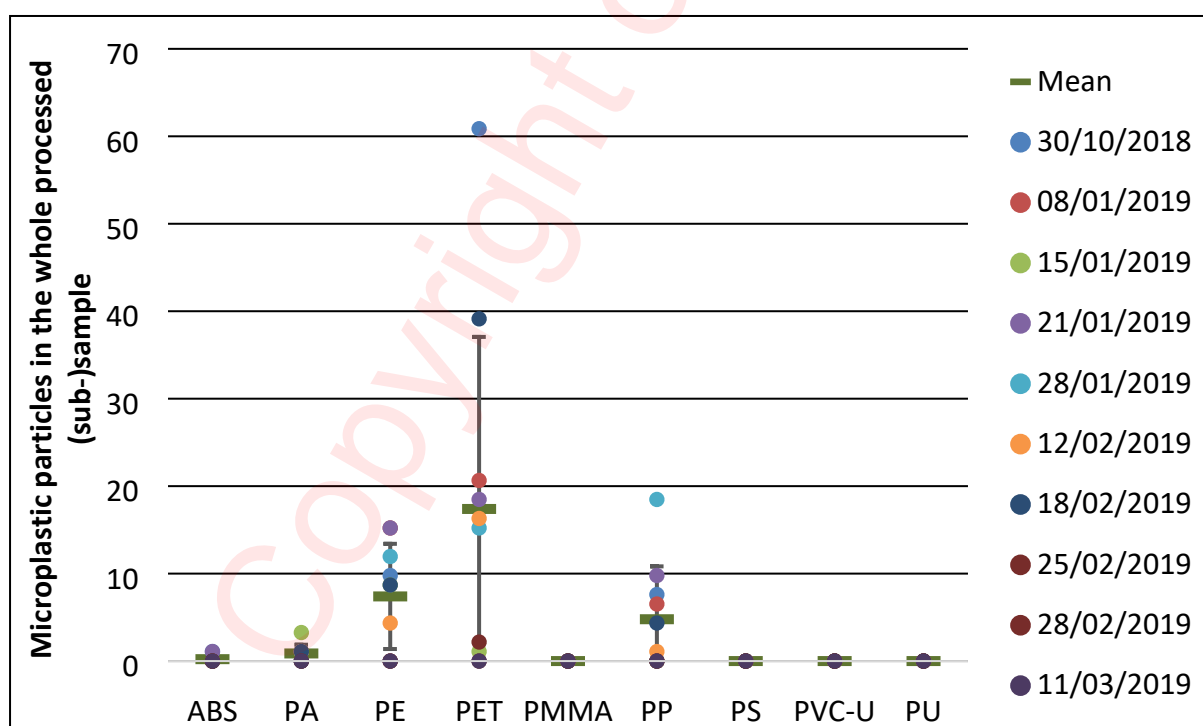
Appendix C Table 10 Microplastic particles in potable water samples, particles counted on each filter (not corrected for blanks or by volume or visible filter area).

Site name	Date	ABS	PA	PE	PET	PMMA	PP	PS	PVC	PU
LR1	29/08/2018	-	-	8	21	-	24	-	1	-
LR1	24/09/2018	-	-	-	-	-	-	-	-	-
LR1	08/10/2018	1	1	23	43	-	8	-	-	-
LR1	25/10/2018	-	-	10	17	-	14	-	-	-
LR1	14/12/2018	3	4	11	25	2	14	-	-	-
LR2	02/10/2018	-	-	11	24	-	5	-	-	-
LR2	22/10/2018	-	1	6	8	-	5	1	-	-
LR2	05/11/2018	1	7	1	18	-	10	1	-	-
LR2	26/11/2018	-	-	13	26	-	9	-	-	-
LR2	11/12/2018	-	1	2	9	-	11	-	-	-
LR3	27/09/2018	-	-	5	8	2	2	-	-	-
LR3	12/11/2018	-	-	1	2	-	-	-	-	-
LR3	21/11/2018	-	-	4	3	-	4	-	-	-
LR3	06/03/2019	-	-	7	1	-	4	-	-	-
LR3	Discounted due to contamination									
LRS1	03/10/2018	-	1	21	33	-	11	-	-	-
LRS1	24/10/2018	1	1	5	29	-	18	-	-	-
LRS1	07/11/2018	-	-	22	14	-	6	2	-	-
LRS1	29/11/2018	-	3	3	3	-	6	-	-	-
LRS1	10/12/2018	-	2	4	3	-	20	-	2	-
LRS2	18/09/2018	-	-	5	2	-	6	1	-	-
LRS2	02/10/2018	-	-	6	9	-	7	-	-	-
LRS2	26/10/2018	-	2	2	19	-	16	-	-	-
LRS2	14/11/2018	-	-	6	4	-	-	9	-	-
LRS2	04/12/2018	-	-	8	1	-	14	-	-	-
GWC	08/10/2018	8	1	38	52	-	19	3	-	-
GWC	22/10/2018	-	-	8	16	-	4	-	-	-
GWC	05/11/2018	3	-	19	18	-	6	-	-	-
GWC	20/11/2018	-	1	10	25	-	16	1	-	-
GWC	05/12/2018	2	-	5	11	-	8	-	-	-

Site name	Date	ABS	PA	PE	PET	PMMA	PP	PS	PVC	PU
GWS	08/10/2018	4	-	8	21	-	1	1	-	-
GWS	22/10/2018	1	1	13	54	-	14	-	-	-
GWS	05/11/2018	-	-	12	37	-	-	-	-	-
GWS	20/11/2018	2	-	8	14	-	9	-	-	-
GWS	05/12/2018	-	1	5	26	-	13	-	-	-
UR	20/09/2018									
UR	09/10/2018	-	-	20	37	-	14	-	-	-
UR	25/10/2018	-	1	4	24	-	6	-	-	-
UR	13/11/2018	-	1	5	43	-	19	1	-	-
UR	04/12/2018	-	-	2	4	-	8	-	2	-

Appendix C Table 11 Concentrations as microplastic particles per L in <u>potable water</u>, values <LOD are qualified by "<" and those above the LOD but below the LOQ by "≈". Values above the LOD are coloured yellow and those above the LOQ green, LOD and LOQ values depend on the processed volumes.

Name	Date	vol repr. (L)*	resolu-tion**	ABS MP/L	PA MP/L	PE MP/L	PET MP/L	PMMA MP/L	PP MP/L	PS MP/L	PVC MP/L	PU MP/L
LR1	29/08/18	2,713	0.0004	< 0.0006	< 0.0012	< 0.0073	< 0.0239	< 0.0004	≈ 0.0079	< 0.0004	≈ 0.0004	< 0.0004
LR1	24/09/18	1,334	0.0007	< 0.0011	< 0.0025	< 0.0149	< 0.0487	< 0.0008	< 0.0150	< 0.0008	< 0.0008	< 0.0008
LR1	08/10/18	370	0.0027	< 0.0041	< 0.0089	< 0.0537	< 0.1756	< 0.0029	< 0.0539	< 0.0029	< 0.0029	< 0.0029
LR1	25/10/18	1,542	0.0006	< 0.0010	< 0.0021	< 0.0129	< 0.0421	< 0.0007	< 0.0129	< 0.0007	< 0.0007	< 0.0007
LR1	14/12/18	1,504	0.0007	≈ 0.0020	≈ 0.0023	< 0.0132	< 0.0432	≈ 0.0014	< 0.0133	< 0.0007	< 0.0007	< 0.0007
LR2	02/10/18	1,574	0.0006	< 0.0010	< 0.0021	< 0.0126	< 0.0412	< 0.0007	< 0.0127	< 0.0007	< 0.0007	< 0.0007
LR2	22/10/18	2,245	0.0004	< 0.0007	< 0.0015	< 0.0088	< 0.0289	< 0.0005	< 0.0089	≈ 0.0005	< 0.0005	< 0.0005
LR2	05/11/18	1,628	0.0006	< 0.0009	≈ 0.0041	< 0.0122	< 0.0399	< 0.0007	< 0.0123	≈ 0.0007	< 0.0007	< 0.0007
LR2	26/11/18	1,695	0.0006	< 0.0009	< 0.0019	< 0.0117	< 0.0383	< 0.0006	< 0.0118	< 0.0006	< 0.0006	< 0.0006
LR2	11/12/18	1,433	0.0007	< 0.0011	< 0.0023	< 0.0138	< 0.0453	< 0.0008	< 0.0139	< 0.0008	< 0.0008	< 0.0008
LR3	27/09/18	2,206	0.0005	< 0.0007	< 0.0015	< 0.0090	< 0.0294	≈ 0.0010	< 0.0090	< 0.0005	< 0.0005	< 0.0005
LR3	12/11/18	4,132	0.0002	< 0.0004	< 0.0008	< 0.0048	< 0.0157	< 0.0003	< 0.0048	< 0.0003	< 0.0003	< 0.0003
LR3	21/11/18	1,856	0.0005	< 0.0008	< 0.0018	< 0.0107	< 0.0350	< 0.0006	< 0.0107	< 0.0006	< 0.0006	< 0.0006
LR3	06/03/19	5,893	0.0002	< 0.0003	< 0.0006	< 0.0034	< 0.0110	< 0.0002	< 0.0034	< 0.0002	< 0.0002	< 0.0002
LR3							discounted due to contamination					

Name	Date	vol repr. (L)*	resolu-tion**	ABS MP/L	PA MP/L	PE MP/L	PET MP/L	PMMA MP/L	PP MP/L	PS MP/L	PVC MP/L	PU MP/L
LRS1	03/10/18	2,089	0.0005	< 0.0007	< 0.0016	< 0.0095	< 0.0311	< 0.0005	< 0.0095	< 0.0005	< 0.0005	< 0.0005
LRS1	24/10/18	461	0.0022	< 0.0033	< 0.0072	< 0.0431	< 0.1409	< 0.0024	< 0.0433	< 0.0024	< 0.0024	< 0.0024
LRS1	07/11/18	1,469	0.0007	< 0.0010	< 0.0022	< 0.0135	< 0.0442	< 0.0007	< 0.0136	≈ 0.0015	< 0.0007	< 0.0007
LRS1	29/11/18	1,699	0.0006	< 0.0009	< 0.0019	< 0.0117	< 0.0382	< 0.0006	< 0.0117	< 0.0006	< 0.0006	< 0.0006
LRS1	10/12/18	1,023	0.0010	< 0.0015	< 0.0032	< 0.0194	< 0.0635	< 0.0011	< 0.0195	< 0.0011	≈ 0.0021	< 0.0011
LRS2	18/09/18	1,062	0.0009	< 0.0014	< 0.0031	< 0.0187	< 0.0611	< 0.0010	< 0.0188	≈ 0.0010	< 0.0010	< 0.0010
LRS2	02/10/18	2,798	0.0004	< 0.0005	< 0.0012	< 0.0071	< 0.0232	< 0.0004	< 0.0071	< 0.0004	< 0.0004	< 0.0004
LRS2	26/10/18	9,938	0.0001	< 0.0002	< 0.0003	< 0.0020	< 0.0065	< 0.0001	< 0.0020	< 0.0001	< 0.0001	< 0.0001
LRS2	14/11/18	6,279	0.0002	< 0.0002	< 0.0005	< 0.0032	< 0.0103	< 0.0002	< 0.0032	0.0016	< 0.0002	< 0.0002
LRS2	04/12/18	7,057	0.0001	< 0.0002	< 0.0005	< 0.0028	< 0.0092	< 0.0002	< 0.0028	< 0.0002	< 0.0002	< 0.0002
GWC	08/10/18	4,309	0.0002	0.0020	< 0.0008	≈ 0.0079	< 0.0151	< 0.0003	< 0.0046	0.0008	< 0.0003	< 0.0003
GWC	22/10/18	3,642	0.0003	< 0.0004	< 0.0009	< 0.0055	< 0.0178	< 0.0003	< 0.0055	< 0.0003	< 0.0003	< 0.0003
GWC	05/11/18	2,013	0.0005	≈ 0.0015	< 0.0016	< 0.0099	< 0.0322	< 0.0005	< 0.0099	< 0.0005	< 0.0005	< 0.0005
GWC	20/11/18	2,750	0.0004	< 0.0005	< 0.0012	< 0.0072	< 0.0236	< 0.0004	< 0.0073	≈ 0.0004	< 0.0004	< 0.0004
GWC	05/12/18	3,381	0.0003	≈ 0.0006	< 0.0010	< 0.0059	< 0.0192	< 0.0003	< 0.0059	< 0.0003	< 0.0003	< 0.0003
GWS	08/10/18	2,787	0.0004	≈ 0.0015	< 0.0012	< 0.0071	< 0.0233	< 0.0004	< 0.0072	≈ 0.0004	< 0.0004	< 0.0004
GWS	22/10/18	2,360	0.0004	< 0.0006	< 0.0014	< 0.0084	< 0.0275	< 0.0005	< 0.0085	< 0.0005	< 0.0005	< 0.0005

Name	Date	vol repr. (L)*	resolution**	ABS MP/L	PA MP/L	PE MP/L	PET MP/L	PMMA MP/L	PP MP/L	PS MP/L	PVC MP/L	PU MP/L
GWS	05/11/18	1,560	0.0006	< 0.0010	< 0.0021	< 0.0127	< 0.0416	< 0.0007	< 0.0128	< 0.0007	< 0.0007	< 0.0007
GWS	20/11/18	2,848	0.0004	≈ 0.0007	< 0.0012	< 0.0070	< 0.0228	< 0.0004	< 0.0070	< 0.0004	< 0.0004	< 0.0004
GWS	05/12/18	3,605	0.0003	< 0.0004	< 0.0009	< 0.0055	< 0.0180	< 0.0003	< 0.0055	< 0.0003	< 0.0003	< 0.0003
UR	20/09/18	766	0.0013	< 0.0020	< 0.0043	< 0.0259	< 0.0847	< 0.0014	< 0.0260	< 0.0014	< 0.0014	< 0.0014
UR	09/10/18	355	0.0028	< 0.0043	< 0.0093	< 0.0560	< 0.1831	< 0.0031	< 0.0563	< 0.0031	< 0.0031	< 0.0031
UR	25/10/18	141	0.0071	< 0.0107	< 0.0234	< 0.1407	< 0.4603	< 0.0077	< 0.1414	< 0.0077	< 0.0077	< 0.0077
UR	13/11/18	725	0.0014	< 0.0021	< 0.0045	< 0.0274	< 0.0895	< 0.0015	< 0.0275	≈ 0.0015	< 0.0015	< 0.0015
UR	04/12/18	89	0.0112	< 0.0170	< 0.0370	< 0.2229	< 0.7291	< 0.0122	< 0.2241	< 0.0122	≈ 0.0244	< 0.0122

* Volume represented on the silver filter. ** Resolution: The amount by which the calculated microplastic particles/L changes from a single detected particle on the silver filter in the FTIR

Appendix C Table 12 Individual results for each of the 5 blank samples for WTW sludge with calculated LOD and LOQs. Particles per processed blank (corrected to 100% filter area and proportion of the sample added to the silver filter in the FTIR).

Sample	1	2	3	4	5	Mean	SD	LOD	LOQ
amount in FTIR	100%	100%	3.0%	58%	70%				
ABS	-	-	-	-	2	0	0.7	2.3	7.0
PA	-	-	36	-	8	9	15.8	52	158
PE	2	7	-	21	19	10	9.5	31	95
PET	27	9	764	226	16	208	324	1,068	3,236
PMMA	-	-	-	-	-	-	-	1.1	3.3
PP	22	91	109	75	34	66	37.3	123.0	373
PS	5	3	-	-	2	2	2.3	7.7	23
PVC-U	-	-	-	2	-	0	0.8	2.8	8.4
PU	-	-	-	-	-	-	-	1.1	3.3

Appendix C Table 13 Microplastic particles in WTW sludge, particles counted on each filter (not corrected for blanks or by volume or visible filter area).

Site name	Date	ABS	PA	PE	PET	PMMA	PP	PS	PVC	PU
LR2	03/10/18	-	1	-	2	-	1	-	-	-
LR2	11/12/18	-	-	9	1	-	-	-	-	-
LR3	27/09/18	-	-	2	3	-	3	-	-	-
LR3	12/11/18	-	-	11	119	-	39	-	1	-
LRS1	04/10/18	-	-	2	-	-	3	-	-	-
LRS1	11/12/18	-	1	-	-	-	-	-	-	-
UR	21/09/18	-	1	2	3	-	5	-	-	-
UR	04/12/18	-	-	3	2	-	5	-	-	-

Appendix C Table 14 WTW sludge. Plastic particles per g dry weight. One g dry weight was processed.

Source	Date	used in FTIR	resolution* MP/g	ABS MP/g	PA MP/g	PE MP/g	PET MP/g	PMMA MP/g	PP MP/g	PS MP/g	PVC MP/g	PU MP/g
LR2	03/10/18	1.4%	78	< 78	< 78	< 78	< 1,068	< 78	< 123	< 78	< 78	< 78
LR2	11/12/18	1.2%	90	< 90	< 90	808	< 1,057	< 90	< 122	< 90	< 90	< 90
LR3	27/09/18	0.27%	399	< 399	< 399	≈ 789	< 1,068	< 399	≈ 1,132	< 399	< 399	< 399
LR3	12/11/18	0.21%	509	< 509	< 509	5,589	60,357	< 509	19,783	< 509	< 509	< 509
LRS1	04/10/18	0.93%	117	< 117	< 117	≈ 225	< 1,068	< 117	≈ 286	< 117	< 117	< 117
LRS1	11/12/18	0.83%	131	< 131	< 131	< 131	< 1,068	< 131	< 131	< 131	< 131	< 131
UR	21/09/18	2.2%	49	< 49	< 52	≈ 88	< 1,068	< 49	≈ 177	< 49	< 49	< 49
UR	04/12/18	1.2%	94	< 94	< 94	≈ 272	< 1,068	< 94	404	< 94	< 94	< 94

* Resolution: The amount by which the calculated microplastic particles/g changes from a single detected particle on the silver filter in the FTIR

Appendix C Table 15 Microplastic particles in WwTW influent samples, particles counted on each filter (not corrected for blanks or by volume or visible filter area).

Site name	Date	ABS	PA	PE	PET	PMMA	PP	PS	PVC-U	PU
ASTC1	26/11/2018	-	-	2	1	-	6	-	-	-
ASTC1	29/11/2018	-	-	4	6	-	7	-	-	-
ASTS1	03/12/2018	-	-	1	2	-	8	1	-	-
ASTS1	05/12/2018	-	-	1	1	-	10	1	-	-
ASTS2	12/12/2018	-	-	2	3	-	17	-	-	-
ASTS2	16/01/2019	-	-	5	-	-	4	-	-	-

Site name	Date	ABS	PA	PE	PET	PMMA	PP	PS	PVC-U	PU
ASTC2	11/01/2019	-	-	3	-	-	4	-	-	-
ASTC2	29/01/2019	-	-	1	-	-	4	-	-	-
TFP	08/01/2019	-	-	4	-	1	2	1	-	-
TFP	10/01/2019	-	-	3	1	-	2	-	-	-
AS1a	14/01/2019	-	-	4	2	-	2	-	-	-
AS1a	21/01/2019	-	-	9	3	-	3	-	-	-
AS2b	23/01/2019	-	-	1	2	-	2	-	-	-
AS2b	31/01/2019	-	-	1	-	-	1	-	-	-
BAFF	19/02/2019	-	-	4	2	-	3	-	-	-
BAFF	05/02/2019	-	-	2	5	-	7	-	-	-

Appendix C Table 16 Concentrations as microplastic particles per L in WwTW influent, values <LOD are qualified by "<" and those above the LOD but below the LOQ by "≈". Values above the LOD are coloured yellow and those above the LOQ green, LOD and LOQ values depend on the processed volumes.

Name	Date	% S2 on final filter	reso-lution* MP/L	ABS MP/L		PA MP/L		PE MP/L		PET MP/L		PMMA MP/L	PP MP/L	PS MP/L		PVC MP/L		PU MP/L
ASTC1	26/11/18	0.7%	882	<	882	<	882	≈	1,688	<	882	<	882	5,232	<	882	<	882	<	882
ASTC1	08/01/19	0.7%	787	<	787	<	787		3,078		4,633	<	787	5,457	<	787	<	787	<	787
ASTS1	29/11/18	0.7%	875	<	875	<	875	<	875	≈	1,651	<	875	6,940	<	875	<	875	<	875

Name	Date	% S2 on final filter	reso-lution* MP/L	ABS MP/L	PA MP/L	PE MP/L	PET MP/L	PMMA MP/L	PP MP/L	PS MP/L	PVC MP/L	PU MP/L
ASTS1	10/01/19	0.6%	988	< 988	< 988	< 988	< 988	< 988	9,817	< 988	< 988	< 988
ASTS2	03/12/18	0.6%	1,016	< 1,016	< 1,016	≈ 1,958	≈ 2,954	< 1,016	17,214	< 1,016	< 1,016	< 1,016
ASTS2	14/01/19	0.7%	845	< 845	< 845	4,151	< 845	< 845	3,323	< 845	< 845	< 845
ASTC2	05/12/18	0.6%	963	< 963	< 963	≈ 2,816	< 963	< 963	3,796	< 963	< 963	< 963
ASTC2	21/01/19	0.6%	1,007	< 1,007	< 1,007	< 1,007	< 1,007	< 1,007	3,969	< 1,007	< 1,007	< 1,007
TFP	12/12/18	0.7%	762	< 762	< 762	2,979	< 762	≈ 762	≈ 1,471	< 762	< 762	< 762
TFP	23/01/19	0.6%	917	< 917	< 917	≈ 2,677	< 917	< 917	≈ 1,777	< 917	< 917	< 917
AS1a	17/01/19	2.3%	258	< 258	< 258	955	≈ 419	< 258	≈ 457	< 258	< 258	< 258
AS1a	01/02/19	0.6%	962	< 962	< 962	8,583	≈ 2,793	< 962	≈ 2,830	< 962	< 962	< 962
AS2b	11/02/19	0.7%	841	< 841	< 841	< 841	≈ 1,585	< 841	≈ 1,624	< 841	< 841	< 841
AS2b	17/02/19	0.6%	991	< 991	< 991	< 991	< 991	< 991	< 991	< 991	< 991	< 991
BAFF	29/01/19	0.7%	351	< 351	< 351	1,371	≈ 661	< 351	≈ 1,028	< 351	< 351	< 351
BAFF	06/02/19	0.7%	816	< 816	< 816	≈ 1,559	3,987	< 816	5,656	< 816	< 816	< 816

* Resolution: The amount by which the calculated microplastic particles/L changes from a single detected particle on the silver filter in the FTIR

Appendix C Table 17 Microplastic particles in WwTW final effluent samples, particles counted on each filter (not corrected for blanks or by volume or visible filter area).

Site name	Date	ABS	PA	PE	PET	PMMA	PP	PS	PVC	PU
ASTC1	26/11/2018	-	-	-	4	-	1	1	-	-
ASTC1	08/01/2019	-	-	1	3	-	2	-	-	1
ASTS1	29/11/2018	-	-	-	2	-	1	-	-	-
ASTS1	10/01/2019	-	-	4	1	-	1	-	-	-
ASTS2	03/12/2018	-	-	-	-	-	-	-	-	-
ASTS2	14/01/2019	-	-	1	1	-	1	-	-	-
ASTC2	05/12/2018	-	-	2	1	-	3	-	-	1
ASTC2	21/01/2019	-	1	-	5	1	3	-	-	1
TFP	12/12/2018	-	-	12	-	-	1	-	-	-
TFP	23/01/2019	-	-	7	2	-	2	1	-	-
AS1a	17/01/2019	-	-	1	5	-	4	2	-	-
AS1a	01/02/2019	-	-	3	4	-	-	-	1	-
AS2b	11/02/2019	-	-	2	1	-	3	-	-	-
TFSb	11/02/2019	-	-	6	1	-	-	-	-	-
AS2b	17/02/2019	-	-	2	1	-	1	-	-	-
TFSb	17/02/2019	-	1	4	4	-	1	-	-	-
BAFF	29/01/2019	-	-	-	-	-	2	-	-	-
BAFF	06/02/2019	-	-	5	5	-	105	-	-	-

The blanks for the wastewater effluent were the same as for WTW raw water (section 3.1.1).

Appendix C Table 18 - Concentrations as microplastic particles per L in WwTW effluent, values <LOD are qualified by "<" and those above the LOD but below the LOQ by. Values above the LOD are coloured yellow and those above the LOQ green, LOD and LOQ values depend on the processed volumes.

Name	date	% of S2 on final filter	resolution* MP/L	ABS MP/L	PA MP/L	PE MP/L	PET MP/L	PMMA MP/L	PP MP/L	PS MP/L	PVC MP/L	PU MP/L
ASTC1	26/11/18	1.9%	1.5	< 1.5	< 1.5	< 1.5	5.6	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
ASTC1	08/01/19	0.8%	1.6	< 1.6	< 1.6	< 1.6	≈ 4.5	< 1.6	≈ 3.0	< 1.6	< 1.6	≈ 1.6
ASTS1	29/11/18	1.9%	0.8	< 0.8	< 0.8	< 0.8	≈ 1.4	< 0.8	< 0.8	< 0.8	< 0.8	< 0.8
ASTS1	10/01/19	3.8%	0.4	< 0.4	< 0.4	≈ 1.3	< 0.7	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4
ASTS2	03/12/18	1.5%	0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
ASTS2	14/01/19	2.1%	1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
ASTC2	05/12/18	1.9%	1.0	< 1.0	< 1.0	≈ 1.8	< 1.0	< 1.0	≈ 2.8	< 1.0	< 1.0	≈ 1.0
ASTC2	21/01/19	3.7%	0.5	< 0.5	< 0.5	< 0.7	≈ 2.2	≈ 0.5	≈ 1.3	< 0.5	< 0.5	≈ 0.5
TFP	12/12/18	2.4%	0.5	< 0.5	< 0.5	5.3	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
TFP	23/01/19	4.0%	0.3	< 0.3	< 0.3	2.2	< 0.7	< 0.3	≈ 0.6	< 0.3	< 0.3	< 0.3
AS1a	17/01/19	1.4%	1.0	< 1.0	< 1.0	< 1.0	4.9	< 1.0	4.0	≈ 2.1	< 1.0	< 1.0
AS1a	01/02/19	2.2%	0.7	< 0.7	< 0.7	≈ 1.8	2.4	< 0.7	< 0.7	< 0.7	≈ 0.7	< 0.7
AS2b	11/02/19	2.2%	0.6	< 0.6	< 0.6	≈ 1.0	< 0.6	< 0.6	≈ 1.6	< 0.6	< 0.6	< 0.6
TFSb	11/02/19	2.3%	0.5	< 0.5	< 0.5	2.6	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

Name	date	% of S2 on final filter	resolu-tion* MP/L	ABS MP/L		PA MP/L		PE MP/L		PET MP/L		PMMA MP/L		PP MP/L		PS MP/L		PVC MP/L		PU MP/L
AS2b	17/02/19	1.1%	0.7	<	0.7	<	0.7	≈	1.2	<	0.7	<	0.7	<	0.7	<	0.7	<	0.7	<	0.7
TFSb	17/02/19	1.9%	7.3	<	7.3	<	7.3		27.4		26.8	<	7.3	<	7.3	<	7.3	<	7.3	<	7.3
BAFF	29/01/19	4.4%	0.2	<	0.2	<	0.2	<	0.3	<	0.4	<	0.2	≈	0.3	<	0.2	<	0.2	<	0.2
BAFF	06/02/19	4.7%	0.4	<	0.4	<	0.4	≈	1.9	≈	1.8	<	0.4		44.5	<	0.4	<	0.4	<	0.4

* Resolution: The amount by which the calculated microplastic particles/L changes from a single detected particle on the silver filter in the FTIR

Appendix C Table 19 - Microplastic particles in WwTW sludge, particles counted on each filter (not corrected for blanks or by volume or visible filter area).

Site name	Date	ABS	PA	PE	PET	PMMA	PP	PS	PVC	PU
AAD1a	26/10/18	-	3	19	59	-	11	1	3	1
AAD1a	16/01/19	-	2	15	86	-	14	3	-	1
AAD1a	31/01/19	-	1	29	162	1	36	1	-	2
AAD1a	07/02/19	-	1	5	34	-	10	1	-	-
AAD1a	21/02/19	1	2	19	87	2	17	2	-	-
AAD2	10/09/18	-	-	6	-	-	-	-	-	-
AAD2	17/09/18	-	-	-	-	1	6	-	-	-
AAD2	24/09/18	-	-	3	4	1	7	-	1	-
AAD2	08/11/18	-	-	-	-	-	13	1	1	-
AAD2	13/12/18	-	-	-	6	-	8	-	-	1

Site name	Date	ABS	PA	PE	PET	PMMA	PP	PS	PVC	PU
LS	17/07/18									
LS	06/09/18				Sample too contaminated to be analysed					
LS	09/10/18									
LS	19/11/18	-	-	4	-	-	1	-	-	1
LS	14/12/18	-	-	12	1	-	1	-	-	-
ADb	19/10/18	1	2	21	11	-	25	3	2	-
ADb	24/10/18	2	2	40	9	2	13	-	1	-
ADb	06/11/18	-	1	17	9	-	15	1	-	-
ADb	27/11/18	-	-	38	12	1	16	2	-	2
ADb	03/01/19	-	-	6	1	-	6	-	-	-
AAD3 (rep A)	26/07/18	1	2	32	8	1	17	3	1	1
AAD3	20/09/18	2	-	42	11	-	42	9	1	-
AAD3	28/09/18	1	-	33	12	2	29	1	-	2
AAD3	04/10/18	1	3	31	19	1	11	1	1	1
AAD3	12/10/18	-	6	48	22	4	31	5	-	3
AAD3 (rep B)	26/07/18	-	5	24	12	1	18	2	-	-
AAD3 (rep C)	26/07/18	-	3	30	8	-	17	3	-	1
AAD3 (rep D)	26/07/18	1	-	30	12	3	15	1	-	-

Appendix C Table 20 - WwTW sludge cake. Plastic particles per g dry weight. One g dry weight was processed.

Source	Date	used in resolution* FTIR	MP/g		ABS MP/g		PA MP/g	PE MP/g	PET MP/g		PMMA MP/g	PP MP/g		PS MP/g		PVC MP/g		PU MP/g
AAD1a	26/10/18	0.91%	120	<	120	≈	351	2,267	6861	<	120	1,252	<	120	≈	359	≈	120
AAD1a	16/01/19	2.4%	46	<	46	≈	82	673	3705	<	46	571	≈	134	<	46	≈	46
AAD1a	31/01/19	2.8%	38	<	38	<	52	1,105	6018	≈	38	1,317	<	38	<	38	≈	77
AAD1a	07/02/19	1.0%	107	<	107	<	107	526	3431	<	107	1,004	<	107	<	107	<	107
AAD1a	21/02/19	1.4%	80	<	80	≈	151	1,506	6732	≈	160	1,290	≈	158	<	80	<	80
AAD2	10/09/18	1.2%	89	<	89	<	89	525	< 1068	<	89	< 123	<	89	<	89	<	89
AAD2	17/09/18	2.8%	39	<	39	<	52	< 39	< 1068	≈	39	≈ 166	<	39	<	39	<	39
AAD2	24/09/18	2.0%	55	<	55	<	55	≈ 157	< 1068	≈	55	≈ 322	<	55	<	55	<	55
AAD2	08/11/18	2.4%	45	<	45	<	52	< 45	< 1068	<	45	525	<	45	<	45	<	45
AAD2	13/12/18	1.7%	64	<	64	<	64	< 64	< 1068	<	64	449	<	64	<	64	≈	64
LS	17/07/18																	
LS	06/09/18																	
LS	09/10/18					Samples could not be analysed, because excess solids in the subsample obscured the microplastic particles													
LS	19/11/18	0.42%	261	<	261	<	261	1,035	< 1068	<	261	< 261	<	261	<	261	≈	261
LS	14/12/18	0.32%	338	<	338	<	338	4,047	< 1068	<	338	< 338	<	338	<	338	<	338
ADb	19/10/18	2.2%	49	<	49	≈	89	1,021	< 1068	<	49	1,161	≈	145	≈	98	<	49
ADb	24/10/18	2.4%	44	≈	90	≈	82	1,803	< 1047	≈	91	524	<	44	≈	45	<	44
ADb	06/11/18	2.4%	45	<	45	<	52	757	< 1068	<	45	610	<	45	<	45	<	45

Source	Date	used in FTIR	resolution* MP/g	ABS MP/g	PA MP/g	PE MP/g	PET MP/g	PMMA MP/g	PP MP/g	PS MP/g	PVC MP/g	PU MP/g
ADb	27/11/18	1.4%	78	< 78	< 78	2,956	< 1068	≈ 78	1,182	≈ 154	< 78	≈ 156
ADb	03/01/19	2.1%	52	< 52	< 52	301	< 1068	< 52	≈ 244	< 52	< 52	< 52
AAD3(repA)	26/07/18	3.2%	34	< 34	≈ 59	1074	< 1068	≈ 34	509	≈ 100	< 34	≈ 34
AAD3	20/09/18	2.3%	43	≈ 90	< 50	1,882	< 1027	< 43	1,828	403	≈ 45	< 43
AAD3	28/09/18	2.6%	41	< 41	< 52	1,353	< 1068	≈ 83	1,131	< 41	< 41	≈ 83
AAD3	04/10/18	3.1%	35	< 35	≈ 98	1,091	< 1068	≈ 35	≈ 324	< 35	< 35	≈ 35
AAD3	12/10/18	2.8%	39	< 39	227	1,877	< 1068	157	1,152	194	< 39	118

* Resolution: The amount by which the calculated microplastic particles/g changes from a single detected particle on the silver filter in the FTIR

Appendix C Table 21 Full repeat processing and analysis of four sub-samples from the same WwTW sludge.

Source	Date	used in FTIR	reso-lution* MP/g	ABS MP/g	PA MP/g	PE MP/g	PET MP/g	PMMA MP/g	PP MP/g	PS MP/g	PVC MP/g	PU MP/g
AAD3 (rep A	26.7.18	3.2%	34	< 34	≈ 59	1,074	< 1068	≈ 34	509	≈ 100	< 34	≈ 34
AAD3 (rep B)	26.7.18	3.7%	29	< 29	≈ 138	697	< 1068	≈ 29	463	≈ 57	< 29	< 29
AAD3 (rep C)	26.7.18	2.7%	40	< 40	≈ 112	1,196	< 1068	< 40	617	≈ 118	< 40	≈ 40
AAD3 (rep D)	26.7.18	2.2%	48	< 48	< 52	1,445	< 1068	145	661	< 48	< 48	< 48

* Resolution: The amount by which the calculated microplastic particles/g changes from a single detected particle on the silver filter in the FTIR

Appendix D Worked example for blank correction and reporting of concentrations in liquid or solid samples

Appendix D box 1 General principle for determining the limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection (LOD)   =   3.3 $*$ S_{blanks} **OR** 1 microplastic particle per 92% visible area of the filter

Limit of quantification (LOQ) =   10 $*$ S_{blanks} **OR** 3 microplastic particles per 92% visible area of the filter

where S_{blank} is the standard deviation of the blank and the measured values to which this is applied are the counts on the filter minus the average of the blanks.

In each case the higher of the two values is chosen, which means that where a polymer was **never** detected in the blanks or the fraction of the processed subsample that was added to the silver filter was very small (< $1.09/(3.3*SD_{blanks})$), the LOD was assumed to be one particle on the 92% analysed area of the filter (this makes the nominal LOD 1.09 particle for the whole filter) and the LOQ three detected particles. The LOQ and LOD values for the actual samples in particles per L or per g depend on the individual volumes or weights processed.

In the following paragraphs, the calculations are shown in detail for a real measured example. As is common practice with mathematical equations, various equations are given numbers in square brackets to be used as a shortcut for using the results from one equation in a later calculation (For readers not familiar with the concept, consider the following example: equation [1]: 3+5 = 8, equation [2]: 5 x 2 = 10, equation [3]: [2] - [1]= 10 - 8 = 2).

First part - Managing the blank values

D.1   **Blanks – raw counts on the visible 92% of the silver filter.**

Three sets of blanks have been produced depending on the processing steps used, for a) potable water, b) "dirty" water samples: WTW raw water and WwTW influent and effluent and c) WTW and WwTW sludge. The example shown is for the "dirty" water samples.

Microplastic particles seen on the visible 92% filtration area of the blank filters								
	Blank 1	Blank 2	Blank 3	Blank 4	Blank 5	Blank 6	Blank 7	Blank 8
ABS	1	-	2	2	2	-	8	-
PA	-	-	1	2	1	-	5	1
PE	36	25	12	8	5	-	13	5
PET	42	24	12	3	8	1	33	10
PMMA	-	-	-	-	-	-	-	-

PP	20	18	16	8	3	1	9	5
PS	1	-	-	-	1	-	2	2
PVC	-	-	-	-	-	-	-	-
PU	-	-	-	-	-	-	-	-

D.2 Correct the blank values for filter area by dividing all numbers in the previous table by 0.92.

Since 8% of the filtration area was invisible to the FTIR, the actual counts are increased by multiplying them by 100%/92%. This assumes that particles are evenly distributed and then gives a best estimate for the number of particles on the whole 100% of the filter. For Blank 1, the calculations are given as an example, for the other blanks only he resulting number is shown.

	Number of particles estimated to be on the whole filter, including the 8% of the filtration area not visible to the FTIR analysis							
	Blank 1	**Blank 2**	**Blank 3**	**Blank 4**	**Blank 5**	**Blank 6**	**Blank 7**	**Blank 8**
ABS	1/0.92 = 1.1	-	2.2	2.2	2.2	-	8.7	-
PA	-	-	1.1	2.2	1.1	-	5.4	1.1
PE	36/0.92 = 39.1	27.2	13.0	8.7	5.4	-	14.1	5.4
PET	42/0.92 = 45.7	26.1	13.0	3.3	8.7	1.1	35.9	10.9
PMMA	-	-	-	-	-	-	-	-
PP	20/0.92 = 21.7	19.6	17.4	8.7	3.3	1.1	9.8	5.4
PS	1/0.92 = 1.1	-	-	-	1.1	-	2.2	2.2
PVC	-	-	-	-	-	-	-	-
PU	-	-	-	-	-	-	-	-

D.3 Calculating the mean and standard deviation for the corrected blanks for each polymer to allow for blank-corrections and calculate the limits of detection (LOD) and limits of quantification (LOQ)

The **mean** is needed later for blank correction of the samples.

The mean of blanks was calculated without taking into account the volume of water filtered in each case as it was less likely that the contamination with detectable microplastic particles arose from the 2 µm pre-filtered water itself than from the other volume-independent sampling- and processing steps. For example, for ABS the mean was therefore (1.1+0+2.2+2.2+2.2+0+8.7+0)/8=2.0 and the standard deviation of the same numbers was 2.9.

For all samples except the potable water and the blanks, only a subsample of the prepared particle suspension in 50% ethanol could be applied to the silver filter for analysis by FTIR. The blank correction was applied to the whole (sub)sample that was processed rather than only to the -sometimes very small- proportion that was used on the silver filter in the FTIR. The argument for that decision is that, while it is unknown at which stage contamination occurred, it is very likely that it happened BEFORE the very last steps of dropping some of the 50% ethanol suspension onto the silver filter and analysing it. Indeed, when filters without any addition were analysed, no plastic particles were found at all despite the filters being open to the laboratory environment for at least 2.5 hours during FTIR analysis.

LOD for the blank corrected whole (sub)samples is EITHER 3.3 times the standard deviation of the blanks OR 1 particle on the visible area of the filter (1/0.92=1.1 particles for the whole filter), whichever number is higher.

Equally, LOQ is the higher number of 10 times standard deviation or 3 particles detected on the visible 92% of the filter, i.e. 3/0.92=3.3 for the whole filter.

	[1] Mean of filter-area corrected blanks	[2] Standard deviation of filter-area corrected blanks	[3] LOD - the higher number of 3.3 x [2] or 1.1	[4] LOQ - the higher number of 10 x [2] or 3.3
ABS	2.0	2.9	3.3 x 2.9 = 9.5	10 x 2.9 = 28.7
PA	1.4	1.8	3.3 x 1.8 = 6.0	10 x 1.8 = 18.1
PE	14.1	13.0	3.3 x 13.0 = 42.8	10 x 13.0 = 129.7
PET	18.1	16.1	3.3 x 16.1 = 53.1	10 x 16.1 = 161.0
PMMA	-	-	1.1	3.3
PP	10.9	7.8	3.3 x 7.8 = 25.7	10 x 7.8 = 77.9
PS	0.8	1.0	3.3 x 1.0 = 3.2	10 x 1.0 = 9.6
PVC	-	-	1.1	3.3
PU	-	-	1.1	3.3

Second part – the blank correction and reporting for a sample from a WwTW. This is the worked example for the TFP effluent taken on 23/01/2019

D.4 **Correct the counts in the sample for filter area, by dividing by 0.92, then adjust to the total volume processed**

[5] Proportion of the processed sub-sample used in FTIR *

In this example, the proportion of the processed sub-sample which was added to the silver filter was only **3.97% or 0.0397**

	[6] Raw counts on silver filter (92% visible)	[7] Particles on whole 100% silver filter: [6] / 0.92	[8] Particles in whole (sub)sample: [7] / [5] Here: [7]/0.0397
ABS	-	-	-

[5] Proportion of the processed sub-sample used in FTIR *

In this example, the proportion of the processed sub-sample which was added to the silver filter was only **3.97% or 0.0397**

	[6] Raw counts on silver filter (92% visible)	**[7] Particles on whole 100% silver filter: [6] / 0.92**	**[8] Particles in whole (sub)sample: [7] / [5] Here: [7]/0.0397**
PA	-	-	-
PE	7	7/0.92 = 7.6	7.6/0.0397 = 191.4
PET	2	2/0.92 = 2.2	2.2/0.0397 = 54.7
PMMA	-	-	-
PP	2	2/0.92 = 2.2	2.2/0.0397 = 54.7
PS	1	1/0.92 = 1.1	1.1/0.0397 = 27.3
PVC	-	-	-
PU	-	-	-

* for the potable and some of the raw water samples this number was 100%, simplifying the following calculations

D.5 **Blank correction of values for whole processed (sub) sample**

	[8] Particles in whole (sub)sample	**[1] Mean of filter-corrected blanks**	**[9] Blank-corrected particles in whole (sub)sample: [8] – [1]**
ABS	-	2.0	-
PA	-	1.4	-
PE	191.4	14.1	191.4 – 14.1 = 177.3
PET	54.7	18.1	54.7– 18.1 = 36.6
PMMA	-	-	-
PP	54.7	10.9	54.7– 10.9 = 43.8
PS	27.3	0.8	27.3 – 0.8 = 26.5
PVC	-	-	-
PU	-	-	-

D.6 **Calculate individual LOD and LOQ for the whole (sub) sample**

[5] Proportion of the processed (sub)sample used in FTIR *

[10] LOD (particles in whole sample) based on detecting at least one particle in 92% visible part of filter → 1/0.92/[5].

[11] equally LOQ based on three particles in the visible part of the filter= 3/0.92/[5]

In this example: [5] 3.97% or 0.0397.

[10] LOD=1/0.92/0.0397=27.3

[11] LOQ= 3/0.92/0.0397=82.0

	[3] LOD based on standard deviation of blanks	[12] Final LOD per processed (sub)sample The higher number of [3] or [10]	[4] LOQ based on standard deviation of blanks	[13] Final LOQ per processed (sub)sample The higher number of [4] or [11]
ABS	9.5	9.5 < 27.3 → 27.3	28.7	28.7 < 82.0 → 82.0
PA	6.0	6.0 < 27.3 → 27.3	18.1	28.7 < 82.0 → 82.0
PE	42.8	42.8 > 27.3 → 42.8	129.7	129.7 > 82.0 → 129.7
PET	53.1	53.1 > 27.3 → 53.1	161.0	161.0 > 82.0 → 161.0
PMMA	1.1	1.1 < 27.3 → 27.3	3.3	28.7 < 82.0 → 82.0
PP	25.7	25.7 < 27.3 → 27.3	77.9	28.7 < 82.0 → 82.0
PS	3.2	3.2 < 27.3 → 27.3	9.6	28.7 < 82.0 → 82.0
PVC	1.1	1.1 < 27.3 → 27.3	3.3	28.7 < 82.0 → 82.0
PU	1.1	1.1 < 27.3 → 27.3	3.3	28.7 < 82.0 → 82.0

* Note for the potable and some raw water samples, this was 100%, which makes this particular step unnecessary.

D.7 **Compare the blank corrected values to the individual LOD & LOQ. The measurement is valid, if ≥ LOQ & is regarded as an estimate & marked "≈" if between LOD and LOQ**

Microplastic numbers per whole processed (sub)sample

	[9] Blank-corrected particles	[12] Final LOD	[13] Final LOQ	[9] compared to [12] and [13]		[14] particles/ (sub)sample
ABS	-	27.3	82.0	0 < 27.3 →	< LOD	< 27.3
PA	-	27.3	82.0	0 < 27.3 →	< LOD	< 27.3
PE	177.3	42.8	129.7	177.3 > 129.7 →	> LOQ	177.3
PET	36.6	53.1	161.0	36.6 < 53.1 →	< LOD	< 53.1
PMMA	-	27.3	82.0	0 < 27.3 →	< LOD	< 27.3
PP	43.8	27.3	82.0	43.8>27.3,<82.0→> LOD, < LOQ		≈ 43.8
PS	26.5	27.3	82.0	26.5 < 27.3 →	< LOD	< 27.3
PVC	-	27.3	82.0	0 < 27.3 →	< LOD	< 27.3
PU	-	27.3	82.0	0 < 27.3 →	< LOD	< 27.3

D.8 **Relate the counts per sample to the original (sub) sample volume and report this final value**

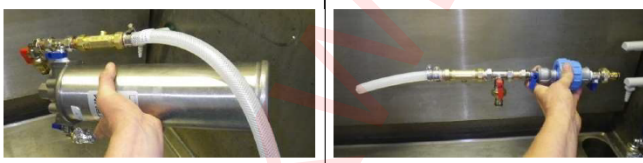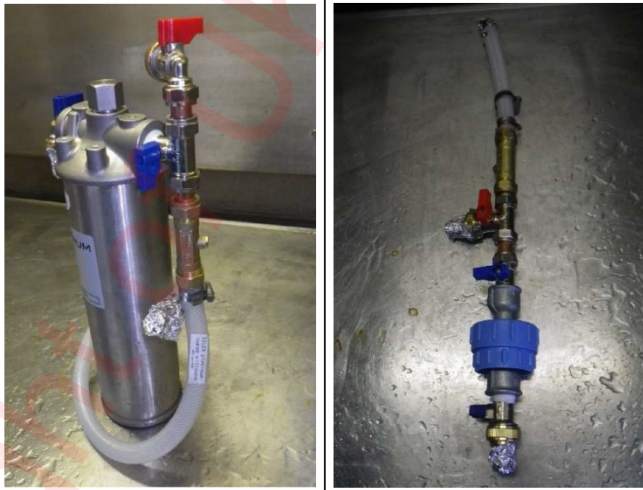**[15] Volume or weight represented in processed (sub)sample**

in this example the final effluent volume processed was 79 L

	[14] particles/ (sub)sample	[16] Final: [14]/[15] microplastic particles/L	
ABS	< 27.3	<27.3/79 =	< 0.35
PA	< 27.3	<27.3/79 =	< 0.35
PE	177.3	177.3/79 =	2.2
PET	< 53.1	<53.1/79 =	< 0.67
PMMA	< 27.3	<27.3/79 =	< 0.35
PP	≈ 43.8	≈43.8/79 =	≈ 0.55
PS	< 27.3	<27.3/79 =	< 0.35
PVC	< 27.3	<27.3/79 =	< 0.35
PU	< 27.3	<27.3/79 =	< 0.35

Appendix E   Instructions given by CEH to the operating staff at WTW plants

Raw water and drinking water sampling for microplastics

Steps	Raw water (large tall filter)	Treated water (small filter, now grey aluminium, not blue plastic)
0. Optional, if disinfection is required by the water company: With both **blue taps closed** the inlet hose may be disinfected using a water company approved method		
1. Ensure that both **blue taps are closed** and the **red tap is open** 2. Attach inlet pipe to sampling tap and secure with the supplied jubilee clip 3. Attach meter and wastepipe to red tap 4. Run at least 5 L water to waste to rinse the pipe and NRV. The flow rate should be 2.5-6 L/min if possible.		
5. **Close red tap** 6. Move the meter with waste pipe to the blue outlet tap 7. **Make note of the time and meter reading** 8. Loosen the cable ties and **open both blue taps** 9. Re-check flow rate: if >2 L/min go to next step, else see to the right 10. Run water through the filter overnight* 11. Check that the amount that was filtered is at least 2 m^3, otherwise continue (unless the flow stopped due to blockage).		 If the flow is too low (and the sampling tap isn't fully open yet): **Open the red tap and close the blue ones before increasing the flow.** Thoroughly flush out any pipe deposits through the red tap before returning to filtering through the blue taps. **Never increase the flow rate while filtering through the blue taps**
12. **Close** sampling tap and **both blue taps** 13. **Make note of the time and meter reading** 14. Disconnect the meter and the rig from the tap		

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15.	**For the large rig only:** using the spanner supplied remove the 7 mm plug at the bottom and open the **blue <u>outlet</u> tap** to drain excess water. 16. Replace the 7 mm plug	
17.	With **both blue taps closed** and **red tap open** drain any remaining water from the pipe and the red tap	
18.	Ensure **the blue taps are closed and secured with cable ties.** Cover all open ends with aluminium foil and post to CEH.	

* What to do if access to the sampling tap is required before the scheduled filtering period is finished:
A water sample can be taken any time through the red tap without disconnecting the filter unit.

If the filter unit needs to be disconnected:
if 2 m³ or more have run through, the sampling may be finished early following the instructions above, otherwise the sampling can be interrupted– **please record this on the sampling sheet**

Instructions for interrupting the sampling
1. Close both blue taps
2. Disconnect the sampler
3. When you are ready to resume, reconnect the sampler.
4. Flush the pipe thoroughly through the red tap. Either transfer only the waste pipe without the meter onto the red tap and flush thoroughly without measuring the amount of flushing water or (better) make a clear note of how many litres were flushed through, so this can later be subtracted from the total
5. Then continue sampling from step 5 as described above

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Appendix F Instructions given to the operating staff for collecting WwTW sludge

F.1 Collecting sludge samples for UKWIR microplastics project in 2018

F.1.1 Plan

We are requested to sample 5 separate WwTWs to quantify microplastics in treated/digested sludge. From each works we wish to collect 5 repeat samples. Of these 5 samples, we wish to collect 3 samples during the 'summer season' of July to end September 2018 (this will need to be slightly extended now) and 2 from the 'winter season' of October to December 2018. The date of collection is at the discretion of the Utility, however, as a minimum, there should be a 1 week difference between sampling dates.

Water Company	Site	Type
A	WwTW 6	Advanced AD
B	WwTW 7+8	Conventional AD
C	WwTW 9	Advanced AD
D	WwTW 10	Limed
E	WwTW 11	Advanced AD

F.1.2 Vessel

Use a new 1 L Kilner jar provided to you by the project and aim to half-fill it. Cover with aluminium foil then the two-part lid on top of that. Please label with the name of the WwTW, the date and name of operator who collected it.
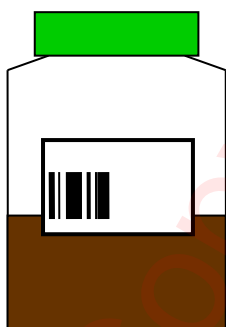
Health & Safety Warning:

Sludge samples will continue to digest even after being taken, hence they continue to produce small amounts of biogas. Therefore if the pot is overfilled or left un-refrigerated the pressure can build up and potentially explode!

F.1.3 Sample location

Sludge cake samples will be fresh on the day of production at the works. Ideally, this will be at the sampling point as the cake comes off the final belt press/centrifuge but before it is dropped into the cake pad.

F.1.4 Sampling technique

As we are trying to measure microplastic contamination, we want to avoid exposure to plastic materials during sampling. If you wish to use a trowel to scoop cake into the sampling vessel ensure it is not plastic or painted - un-painted stainless steel is best, aluminium is also suitable. It may be possible to simply hold the vessel under the press and allow cake to fall directly into your sampling vessel.

Sludge cake: you must ensure that all safety procedures are followed, and that the operators/contractors are aware & not operating any machinery.

If the only option is to take direct from the cake pad, then collect from at least 5 separate locations and add no more than 500 ml to the 1 L sample vessel. The samples should be representative of the bulk as much as possible.

F.1.5 Sample handling and initial storage:

Once samples have been taken they must be kept in the dark and between 1-8°C (fridge) where practically possible.

All samples must arrive at the water company's central lab facility within 48 h of being taken.

F.1.6 Useful additional information

If there are routine records kept on the sludge product, such as % solids content on the sample day this would be useful to report. Also, if a schematic diagram is available illustrating the sludge treatment process at the works that would be valuable to the project team. All such information could be e-mailed to Monika Jürgens (address below)

F.1.7 Storage at the lab and onward delivery to CEH

It would be preferable that the samples are frozen once received at the water company's labs, so they can be sent as a batch to CEH once the first 3 ("summer") samples are collected.