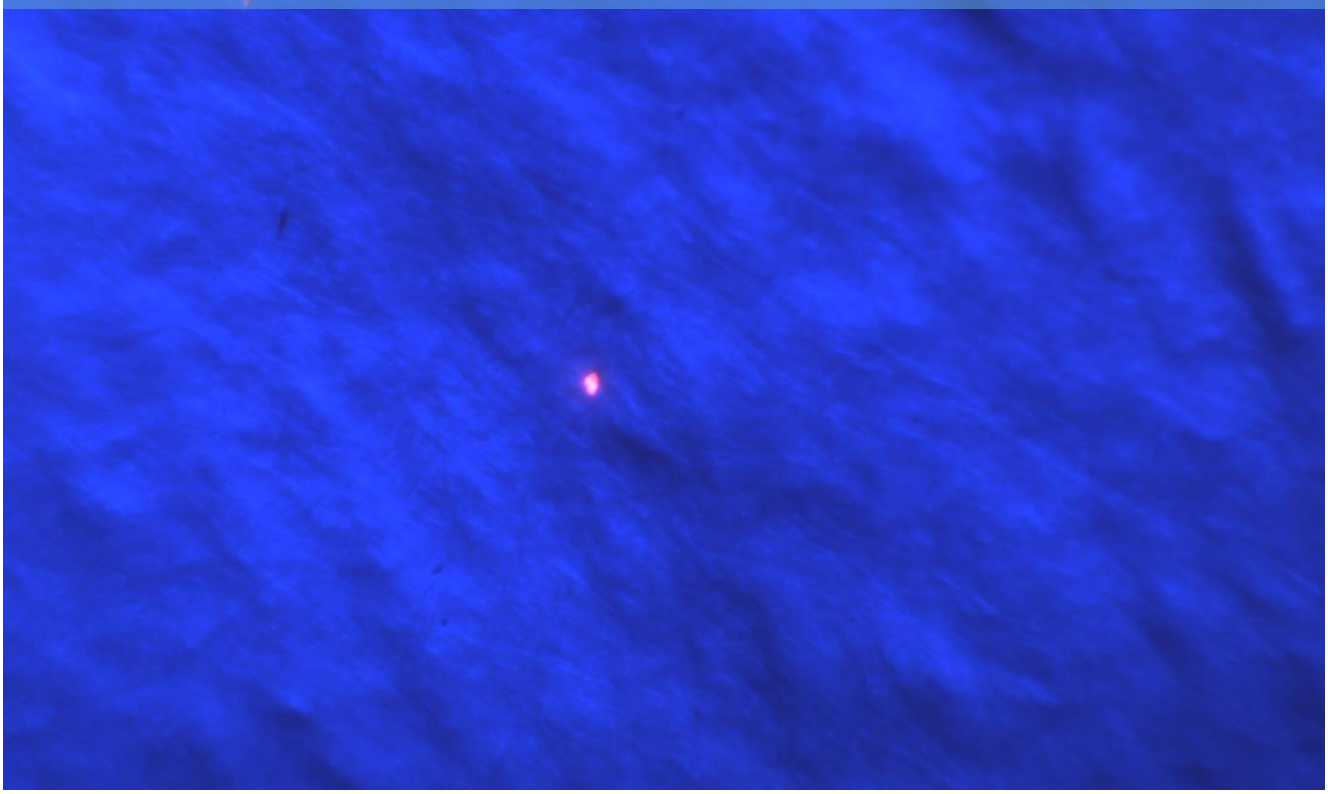
Microplastics in drinking water sources and distribution systems in Iceland



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Contents

Abstract

This study was commissioned by Veitur, HS Orka and Norðurorka in order to assess microplastics contamination in drinking water across the majority of consumer sources in Iceland. A modified fluorescence microscopy method was used to tag microplastics of between 27µm and 5000µm with Nile red indicator dye. A total of 44 200 L triplicate samples were collected from boreholes and distribution systems using forensic methods and filter blanks to prevent and quantify processing contamination. Based on blank results, a detection threshold of 0,09particles per litre (ppl) was set corresponding to a 1% false positive rate above the threshold. All samples and all blanks contained fluorescent particles; 22 samples exceeded the detection threshold; the mean particle count was 0,22ppl and the maximum 2,96ppl. In international context, this appears to be lower than other studies using similar methods but sufficient data for a thorough comparison is lacking. Lower values may be due to choices for lower limits of particle size, cleanliness of sampling and analysis procedures, or that Icelandic drinking water has less microplastic than in other studied sources.

Authorship

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1 Executive Summary

Context

In recent years, scientists and the general public have gained an awareness of the presence of microplastics (defined as synthetic polymers <5mm in all dimensions) in essentially all environments, in food, in the air, and in the water we drink. Measuring microplastics in drinking water is still fraught with difficulties and relatively uncommon. Contamination from airborne and other sources is extremely difficult to control for, and there are still significant methodological challenges in differentiation of plastic from its surroundings – the main methods are either expensive, slow and small-scale, or fast and less precise. International estimates of likely contamination range from 0 to 61 particles per liter (ppl), with the widest study finding a mean of around 5ppl (>100μm).

This study

This study attempted to balance scope and size of the study with precision; it represents one of the largest national studies of drinking water and microplastics to date. It used the latest Nile red fluorescent tagging methodology and significantly improved and developed this in the face of previously unreported methodological issues. 44 samples were taken in Akureyri, Reykjavík, Reykjanes, Akranes and Hvanneyri using forensic methodology, thereby sampling the vast majority of consumer drinking water supplies in Iceland. Particles of 27-5000μm were measured in samples of c.200l; particles under this size range are difficult to assess with the human eye. Each sample had a paired blank to assess contamination, which was subtracted from the final total and each point was sampled in triplicate.

Results

A detection limit (after subtraction of individual sample blanks) of 0,09 ppl was set based on the distribution of all blank results; values over this amount are unlikely to be due to chance contamination ($p<0,01$). As can be seen in Figure 13 (p. 18), 22 results exceeded this threshold; it should be noted that the highest of these were associated with high organic loading which may have affected results. Most positive samples were below the limit of quantification, meaning it is only possible to state that fluorescent particles (assumed to be microplastics) are present in the source, but at levels below <0,74ppl. Fluorescent particles were found in all samples and all blanks. The median particle count was 0,09ppl, the mean 0,22ppl and the maximum 2,96ppl; considering only boreholes (and thus excluding higher results from surface water), the mean was 0,12ppl.

Interpretation

The results indicate that there are certainly microplastics in some water sources in Iceland, including in boreholes themselves. The results are relatively low (a maximum of 0,71ppl for boreholes, under the limit of quantification) compared to other international studies using similar methods on bottled and tap water. This may be due to threshold effects (i.e. that most particles are < 27μm), it may be that this study used a better analysis protocol, or it may be that there is less pollution in Icelandic drinking water sources. The main priority of this study in method development was to avoid inaccurate overestimation due to contamination, so results must be taken as a lower boundary of the current state of Icelandic water. The method developed here is fast and replicable and uses equipment available in Iceland and is thus well-suited for regular monitoring.

2 Introduction

Research about microplastics in the environment and especially in drinks and food has been intensified internationally during recent years. The newest research estimates that around 50 000 particles are ingested annually via air, food and drinks by one person (Cox *et al.*, 2019); this amounts to around 137 particles per day.

The European Chemicals Agency refers to microplastics *as "any polymer, or polymer-containing, solid or semi-solid particle having a size of 5mm or less in at least one external dimension"* (ECHA, 2018). The definition has been expanded to exclude *"polymers that occur in nature that have not been chemically modified"* (ECHA, 2019).

Tap and bottled water has been tested in several studies with different sampling and analytical methods. Most use Raman or FTIR spectroscopy, precise but expensive techniques which limit sample size; others use Rose Bengal or Nile red staining, which allows for faster sample processing at the expense of precision and extent of the lower size range. Table 1 shows a selection of studies on this topic. The measured range of microplastic contamination lies between 0 to over 600ppl for tap water. This high range reflects the lack of a standard lower limit on particle size, variations in the thoroughness contamination prevention procedures, variations in the method used for analysis, and actual differences in microplastics content of water. Thus, it is currently not possible to adequately compare the results of most studies internationally.

The literature comparison reveals a high dependency on the investigated size ranges, with the newest studies suggesting that the majority of particles are well below 10µm. Smaller particles are, however, much more laborious to detect and background contamination in these sizes is also more difficult to prevent. In general, due to the presence of microplastics in air (particularly fibres) and most surfaces and substances that might be used in labs, contamination can make up the vast majority of detected particles, and therefore blanks must be used and strict procedures taken to reduce contamination.

In total 44 sample points, water from boreholes, groundwater, water in the distribution system, storage tanks and for the end user, were chosen for this project after discussion with water companies. The samples were taken from February to May 2019.

Table 1: Literature comparison of microplastic contamination in tap and bottled water. LOD = Limit of Detection; LOQ = Limit of Quantification.

Authors	Mean (ppl)	Range (ppl)	Sample size	Size Range	Blanks Mean (ppl)	Method	Comments
Tap Water							
Kosuth, Mason and Wattenberg (2018)/ORB Media	5,45	0 - 61	159 bottles (457 – 603 mL)	> 100µm	0,33	Rose Bengal Tagging, dissection microscope	Tap water
Pivokonsky *et al.* (2018)		338±76 - 628±28	1 L (54 L total)	> 1µm 95% of particles < 10µm	< 5% of the microplastics detected in the samples	Wet Peroxide Oxidation; SEM; FTIR; Raman; Elemental micro analysis	Treated water from treatment plants
Mintenig *et al.* (2019)		0 – 0,007	1200 – 2500 L	> 20µm	0,67 particles 0,3 fibres	FTIR spectroscopy	Treated drinking water
Uhl, Eftekhardadkhah and Svendsen (2018)/NIVA	< LOD (0,9) or <LOQ (4,1)		72 bottles (1 L)	> 60µm were analysed	0,5	FTIR spectroscopy	Water before and after treatment plants
Strand *et al.* (2018)/DCE	> 100µm: 0,312 (LOD=0,58)	10-100 µm: 0 – 0,8 *(LOD=0,3)*	3 samples; 17 sites (50 L)	10 – 100 µm > 100 µm	0,26	Dissection Microscope FTIR spectroscopy	Tap water from private households, workplaces and institutions
Bottled Water							
Orb Media /Mason, Welch and Neratko (2018)	6,5-100µm: 315 > 100µm: 10,4		259 bottles (500 mL – 2 L)	>6,5 µm	6,5-100µm: 23,5 particles > 100µm: 4,15 particles	Nile red Tagging (>6µm), FTIR spectroscopy (>100µm)	
Oßmann *et al.* (2018)	PET bottles: 2649 ± 2857 glass: 6292 ± 10521		32 bottles (250 mL)	> 0,4µm; 90% of particles < 5µm	384	Micro-Raman spectroscopy	

3 Method

Microplastic quantification in the laboratory is a fast-developing area of study, with several crucial method papers published in the last year alone. The method here was developed to balance comparability with international studies (particularly one funded by ORB Media, which led to the commissioning of this report (Kosuth *et al.*, 2017)), with precision, accuracy and generalisability (i.e. volume and breadth of sampling). Most studies attempt to prioritise only one of these three factors, giving results that are either very precise but small-scale and thus unrepresentative, or with such small samples or serious methodological flaws that they do not adequately represent reality. In addition, blanks were used to measure environmental contamination. The method developed here detects microplastics larger than 27µm in at least one dimension and the results are given in numbers of particles and fibres per volume drinking water.

3.1 Sampling

In all cases, water was sampled using filters on site and the filters themselves were transported and analysed afterwards in the lab.

Equipment

The sampling equipment and set-up can be seen in Figure 1 to Figure 4.

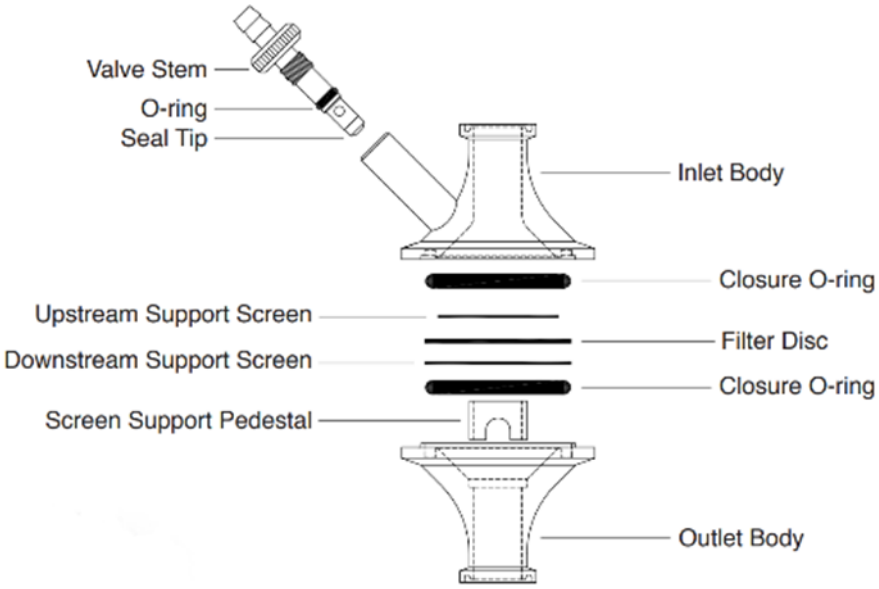

Figure 1: Exploded view of a filter holder unit. Upstream support screen was not required for the study. (Meissner Filtration Products Inc.)

The total set up (see Figure 3) consists of two filter holders. One was connected with PVC hoses[1] (Tricoflex Crystal, France) to the system and the other is set aside in a stand. The inlet was connected to the drinking water system and has a valve to turn the water on and off, as well as throttle the flow rate. Before the sample filter was a t-valve with air filter attached. The air valve was used to purge the hoses of water after sampling using a hand pump filtered by a PTFE

[1] The food-grade PVC hose could (in theory) shed particles that would not display in the blank. However, Mintenig et al. (2019) do not report finding particles of PVC hose material in FTIR analysis of their blanks despite its use in similar sample sizes, so this was not considered a high risk.

membrane with 0,45µm pore diameter. A flow meter was placed after the sample filter (Gardena, Germany) and the filtered water was discharged.

The samples were taken on laser-engraved stainless-steel filter meshes (Figure 2; custom-made by Inoxia Ltd., Great Britain). The wire mesh has a nominal aperture of 27µm and an open area of 28%. The filters are 47mm in diameter, ca. 17,35cm² and the grid is 3,1mm * 3,1mm, thus approximately 180 marked squares per filter.

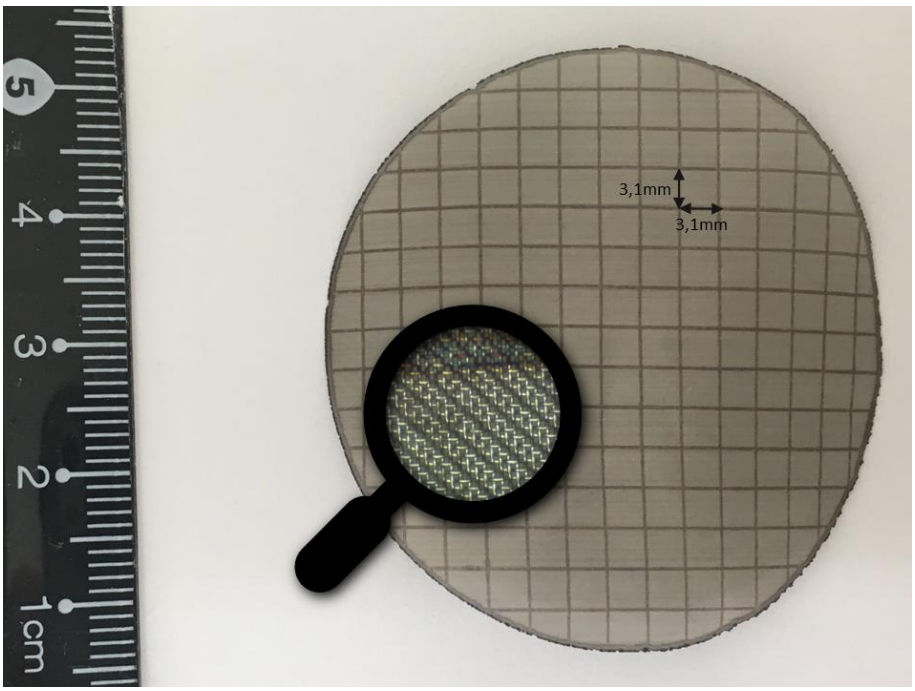

Figure 2: Picture of a 47mm stainless steel filter with a close-up of the wire mesh (RSI, 2018)

The pore size of 27µm was chosen to meet the practical limits of possible detection with optical microscopy (Erni-Cassola *et al.*, 2017). Uncertainties are known to rise considerably with the optical detection of particles below 100µm in size. However, with the aid of staining the polymeric particles (see analysis method) it was possible to lower the detection limit below 100µm.

Figure 3: Sampling set-up (RSI, 2019)

A custom-built wood/glass glove box (Figure 4) and glass petri dishes were used to receive the samples and store sample filters before usage, to prevent environmental contamination from plastic particles in the atmosphere. Filters and glass dishes were incinerated at 450°C for 4 hours in a muffle furnace in order to remove all contamination prior to sampling; filters were incinerated in petri dishes wrapped in aluminium foil and these were only opened during sampling.

Figure 4: Glove box (RSI, 2019)

Sampling procedure

The samples were taken as triplicates (with each replicate having its own blank) to allow calculation of random error. Sampling volume was set at 200 litres in order to maximise the size of the sample within a reasonable timeframe (10-40 minutes) to allow for replication. No filter clogged during this study, and sampling volume could therefore be increased with a corresponding increase in sampling time. For the most part, pumps and boreholes were activated during sampling periods.

The sampling equipment is shown in Figure 3. The equipment was plugged to a side stream valve of the drinking water system, except for surface water samples which were sampled using a stainless steel submersible pump with metal outlet piping. First the hoses were flushed with 200 L of the sampling water without filter holders, in order to remove impurities from the pipe, such as rust and sediment. Both filter holders were then inserted in the system and flushed with 200 L to clean them of any residual contamination. After flushing with a total of 400 L, both filter holders were taken into the glove box to insert the stainless-steel filter meshes. The open ends of the hoses and of the filter holders were covered with aluminium foil. The sample filter was then clamped into the system again and the blank filter is set aside in its holder. To take a sample, 200 L of water was passed through the filter. Both filters were then unclamped, open ends covered with aluminium foil and the filter meshes placed from the holders into petri dishes inside the glove box. The dishes were then sealed with Parafilm, labelled and stored at 4 °C prior to processing.

The volume and the approximate flow rate was noted for each sample. The flow rate was between 6l/min and 20l/min; this was set as a maximum to avoid as much as possible water pressure forcing material through the filters.

No water was run through the blank filter meshes, as initial testing revealed that they collected high numbers of particles (sediment and plastics). This was likely from material near 27µm being inadvertently pushed through the first filter rather than from environmental contamination. Including this would have meant blanks were no longer just an indication of actual contamination but could rather cause false negative results for the samples through overestimation. However, they were still moistened by the water remaining in the blank filter holder after its initial flushing; this is important as humidity greatly affects plastic adhesion to surfaces. Additionally, whenever the sample filter holder was unclamped and briefly exposed to the surrounding air before covered with tinfoil pieces, the process was mimicked for the blank filter holder where the tinfoil on the open ends was exchanged in a similar manner.

Forensic methods in sampling

To reduce or eliminate contamination of sampling the following actions in Table 2 were introduced. By following a strict forensic protocol, (Woodall *et al.*, 2015) showed that the fibre contamination can be reduced by 90%. This applies to the contamination risk during sampling as well as to the contamination risk while processing the samples (cf. Table 3).

Table 2: Actions to lower the contamination risk during sampling

Overall	Procedual blanks alongside the sampling and analysing
Before Sampling	Incinerating filters and petri dishes before use (450 °C, 4 h)
	Rinsing the equipment prior to sampling with source water
Equipment	Using non-plastic filter holders
	Sealed Transport of filters to the lab

	Use of a custom-built non-plastic glovebox for adding and removing filters to petri dishes
	Covering open hoses and filter holders with aluminium foil
Sampling Environment	Cotton clothing and non-shedding Tyvek suits
	Separate Tyvek suit for laboratory and sampling
	As few people as possible permitted in the sampling area
	All doors kept shut where possible

3.2 Analysis

A fluorescence microscopy method developed in 2017 by (Mason, Welch and Neratko, 2018) and (Maes *et al.*, 2017) using Nile red as a marker dye, was the basis of this method. Nile red only binds to non-polar compounds (e.g. lipids, plastics) and fluoresces red, pink or orange in the presence of blue light when viewed through a high-pass filter to exclude blue incident light. Most polymers are stained at room temperature and pressure, however the staining process is dependent on the surfaces, porosity, plasticisers, elastomers, pigments and other factors (Mayes, 2018). This process is fast and much cheaper than infrared or chromatographic spectrographic processing, allowing a large number of samples, which is critical given the natural variability in concentrations. It also returns the number of microplastic particles rather than the mass. This is the most common reporting standard currently.

Analysis was conducted in the RSI lab dedicated to the microplastics project. Lab personnel wore non-shedding Tyvek-suits when processing the samples, which were not removed from the RSI premises for e.g. sampling. The samples were processed in a closed, clean environment – a custom-built overpressure box with filtered (HEPA-filter) air inflow from a high-powered blower motor (Figure 8). The workspace was regularly wiped down with cellulose cloths and then swept with a lint roller.

The analysis scheme can be seen in Figure 5. The process in the laboratory consisted of the initial counting; staining and digestion; rinsing and transfer; and fluorescence counting. It was applied to all the sample filters and also to all blank filters to record and monitor the background contamination.

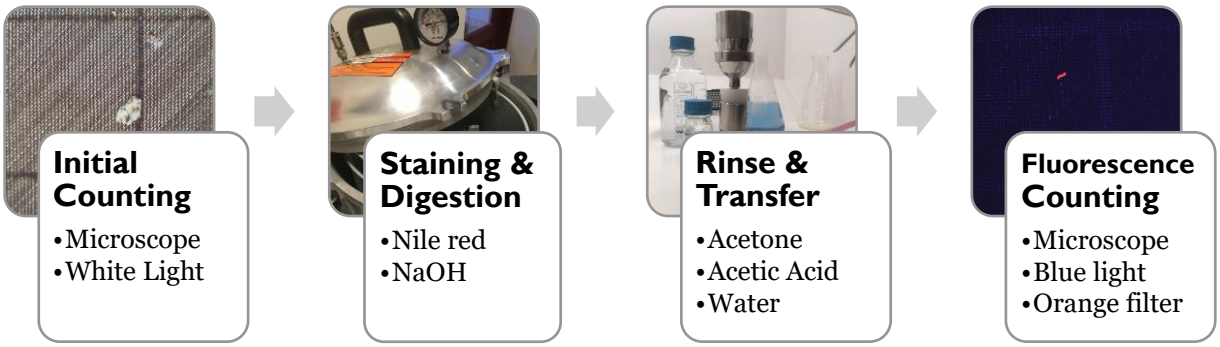

Figure 5: Analysis process scheme (RSI, 2019)

Pre-Counting

All particles on the filters were counted and sub-divided into fibres, light-coloured particles and dark-coloured particles (Figure 6) prior to staining. This indicated whether there was a correlation between particle number and plastic frequency. Particles were viewed under a light microscope (AmScope 20X & 40X Trinocular Stereo Microscope SW-3T24, AmScope) with 20 x magnification.

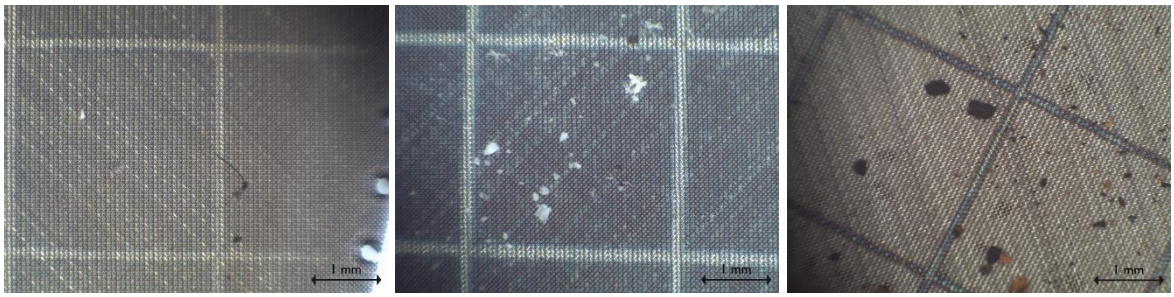

Figure 6: Example microscope pictures; left: fibre; middle: light particles; right: dark particles (RSI, 2019)

Due to the uneven background of the stainless-steel filters, it cannot be guaranteed that all transparent particles and fibres were detected during white-light counting. The different colours of the stainless-steel filters themselves result from incinerating the filters and the steel's tempering colours. The water from some sample points, for example the point shown in Figure 7, had many mineral particles in it, making it impossible to count.

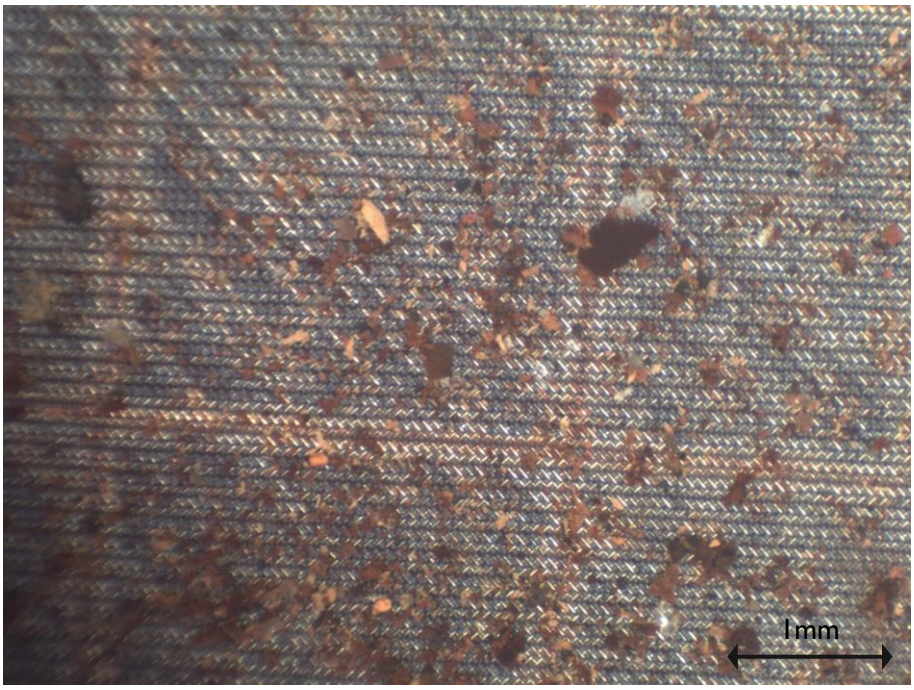

Figure 7: Microscope picture of a filter with too many particles to count (RSI, 2019)

Staining & Digestion

100 µl of Nile red solution (1 mg/ml in acetone) was used to stain potential polymers as per previous studies. However, it was found that this did not satisfactorily stain polyester, a major source of fibres in the environment. To stain polyester, samples were processed at pressure (1 bar) and temperature (120°C) for 20 minutes in an autoclave after staining. This

unfortunately had the effect of also permanently staining wool fibres (a natural polymer); in order to digest these, sodium hydroxide (NaOH) was added (10ml; 1mol/L) to petri dishes containing sample filters and Nile red prior to autoclaving. It was observed in pilot work that this had no effect on all the most common plastic polymers. Nile red also has no effect on black or extremely dark pigmented particles, as the dye content of these absorbs the fluorescent light wavelengths. However, prior laboratory work determined that the method should be sufficient to exclude wool, cotton and hemp. Nile red may also stain mineralised chitin found in insect shells (Maes *et al.*, 2017) but this is known in the literature to be digested in NaOH (Einbu *et al.*, 2004) of a similar molarity.

All laboratory glass ware was incinerated, and all chemicals were filtered before use (Table 3).

Rinse & Transfer

Samples were poured out of the petri dishes after heat treatment and rinsed onto new filters. The original filter was rinsed with filtered acetone onto the new filter. The new filter was then set to be soaked in filtered 14% acetic acid for one minute. The acetone and acetic acid de-stained organic particles (e.g. cotton and hemp fibres) and also neutralised and dissolved residual NaOH crystals. New filters were stored in petri dishes until analysis and the equipment was rinsed and cleaned with filtered water.

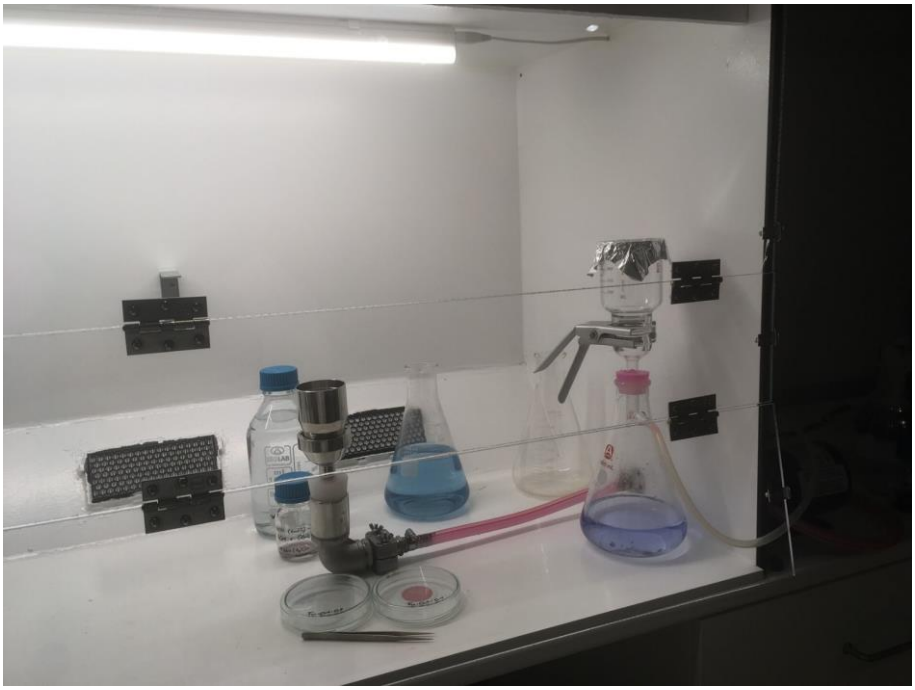

Figure 8: Filtering equipment inside the blower box, RSI lab (RSI, 2019)

Fluorescence Counting

The new filters and the original filters were examined under a blue light source (Epistar, beam angle 15°, 460 – 470 nm) through a microscope masked with a high-pass filter (Filter G350 Dark Amber by Rosco Laboratories Inc). Observations were repeated by an independent lab tech and a mean was counted unless the deviation on any observation or the total was greater than 0,1ppl. In this case, a third independent lab tech recounted the sample and a mean of the two most similar values was taken. To gain consistent results by independent personnel, extensive training was conducted.

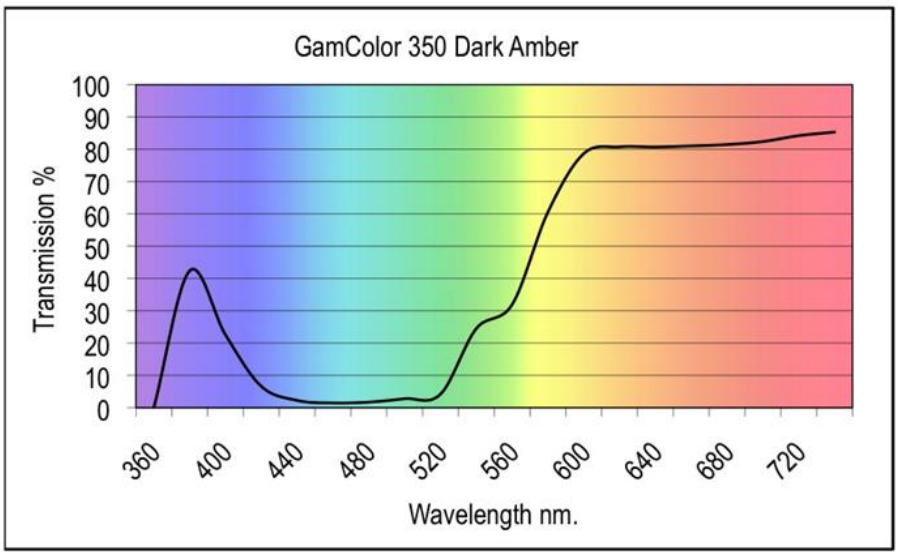

Figure 9: Extract of technical data sheet - G350 Dark Amber filter (Rosco Laboratories Inc, USA)

Red, pink or orange glowing particles are counted and divided into fibres, small (< 51µm) and large (> 51µm) fragments. Wire width plus the gap size is 51µm and could be clearly seen with the microscope, see Figure 10. Only particles with at least one dimension larger than 27µm are held back by the filters and thus analysed. It is possible that some particles larger than 27µm were forced through the filters by mains water pressure but there was no satisfactory way to measure this within the constraints of this study.

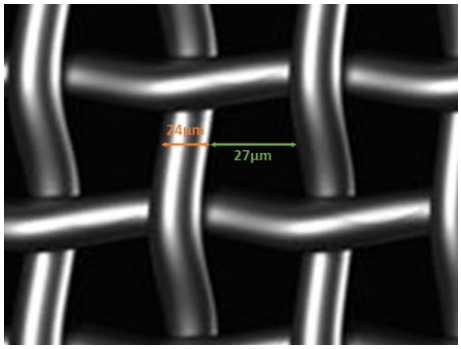

Figure 10: Close-up of wire mesh and its sizes (Inoxia Ltd.)

The same procedure was applied to all the sample filters as well as blank filters. The number of particles per filter was is then divided by the number of sampled litres. Background contamination as measured by the paired blank filter, was subtracted from the sample counts to calculate the results. Throughout the processing microscope pictures are taken, especially during the step of initial counting.

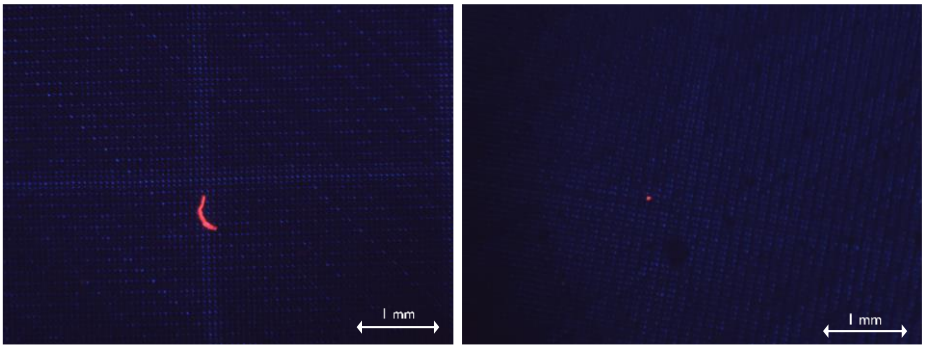

Figure 11: Examples of fluorescent particles on the filter meshes (RSI, 2018)

Forensic processing in laboratory

Table 3 below shows actions that were done to reduce the contamination risk during the analysis. Some of the actions apply to both the sampling and processing, as for example to wear cotton clothing and non-shedding Tyvek suits.

Table 3: Actions to lower the contamination risk during processing

Laboratory and Personnel	Cotton clothing and non-shedding Tyvek suit, and do not wear this out of the lab area; Keep in a separate place from normal lab clothes
	Clean lab and analysis area: wiping (all surfaces) well before use with wet paper towels or cotton cloths
	Storing unused equipment in boxes
	All doors kept shut where possible
	As few people as possible permitted in the laboratory
	Avoid air currents (from open windows etc.) when doing extended work without a lid
Lab Equipment	Rinsing the non-glass equipment: funnel and filtration equipment
	Incinerating the metal filters and glass ware (450 °C, 4 h)
Chemicals	Filtering the water, acetone, sodium hydroxide, Nile red solution through glass fibre filter membranes
	Reagents stored in glass bottles
During the Analyses	The tops of the petri dishes were not lifted more than necessary. When tops were lifted, it was done slowly to avoid any air stream and particles to be blown away
	Work was done inside the blower box in a slightly over pressured environment

4 Results

4.1 Pre-Counting Results

The pre-counting results are presented in Figure 12. Counts range from 0,1 to 14,7ppl with a mean of 2,68ppl. Nine samples were not counted due to overloading, so the true sample mean is higher.

The particles counted in the pre-counting are seemingly mostly mineral inorganic particles. Fragments in the colours white, black or rusty brown were commonly found and the mean value of the counted samples is 2,63 fragments per litre. The mean share of fibres is low with 0,04 fibres per litre.

Pre-Counting Results

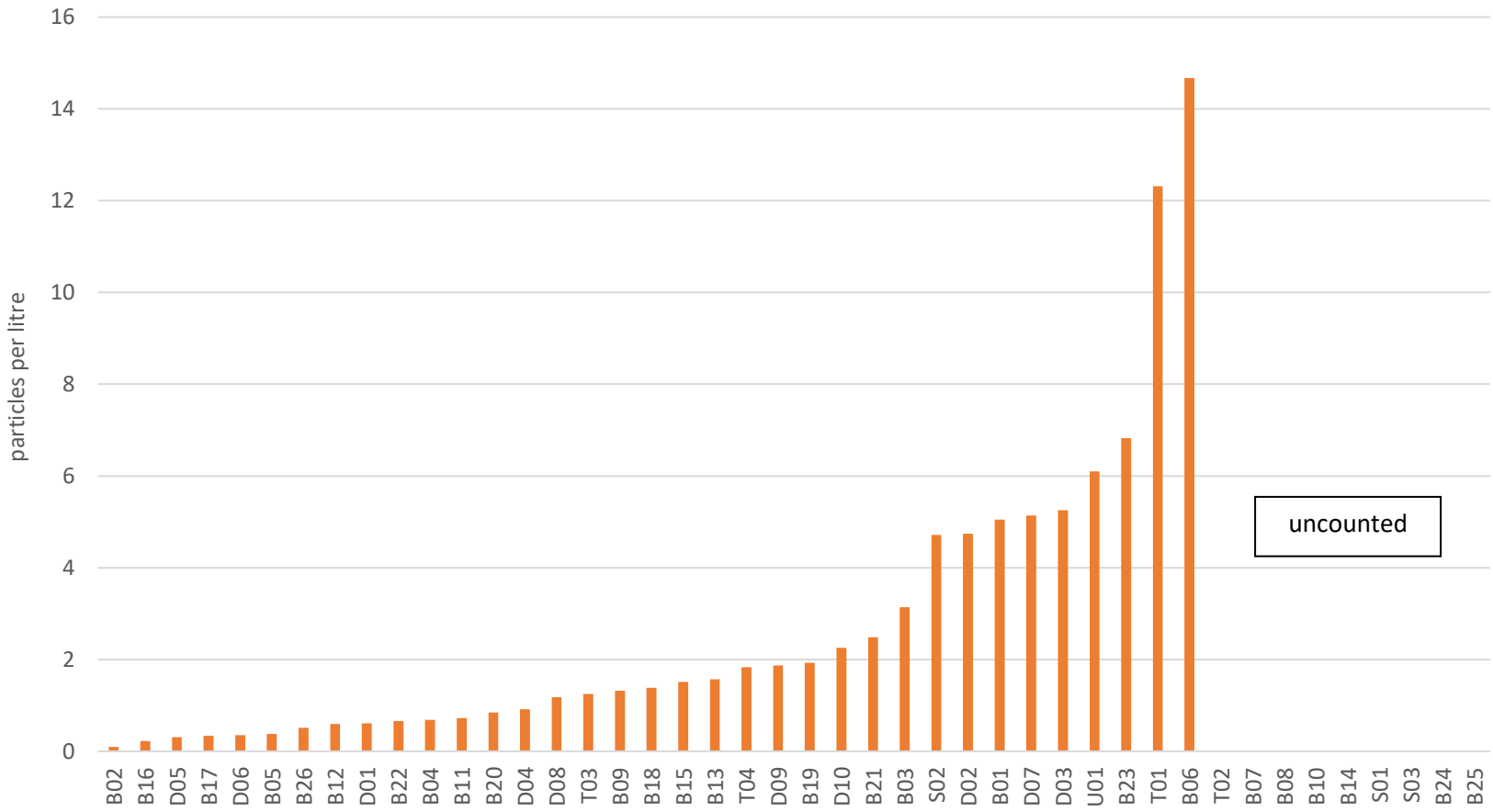

Figure 12: Pre-Counting Results

4.2 Results for particles fluorescing

Table 4: Summary of the presented study (cf. Table 1)

Mean (ppl)	Range (ppl)	Sample size	Size Range	Blanks Mean (ppl)	Method
0,22	-0.03 – 2,96	132 (200 L)	> 27 µm	0,07	Nile red Tagging Fluorescence Microscopy

The results as well as the comparison between the categories water supply, storage and distribution system are shown below in Table 5. The results show a mean of 0,22ppl for all 44 samples within a range of -0,03 to 2,96ppl. Negative results can occur when blanks contained more fluorescing particles than the samples.

Table 5: Sample results and statistics for each type of location.

	Overall	Boreholes / Groundwater	Distribution System	Storage Tanks	Other
Number of Samples	44	26	10	4	4
Mean	0,22	0,12	0,14	0,06	1,25
Median	0,09	0,08	0,10	0,03	0,81
Minimum	-0,03	-0,03	0,02	0,02	0,43
Maximum	2,96	0,71	0,42	0,16	2,96

An overview of counts for all sampling points (mean of triplicates) is given in Figure 13, with corresponding blank values subtracted. The red line at 0,09ppl (once paired blanks are subtracted) shows the limit of detection (LOD). This level is determined by the mean background contamination rate observed in all blanks and the distribution of outliers; here it is set at 2,33 times the standard deviation of these, after the distribution was normalised by using a square root transformation. The probability of false positive sample results above this line is small (<1%), but there may be a considerable number of false negatives below it.

The so-called limit of quantification (LOQ) is set to 10 times the standard deviation of blanks, thus 0,74ppl (also after blank subtraction). All values above the LOQ are said to be quantifiable, and therefore the number of plastic particles, that are fluorescent tagged can be given rather than just an indication of their presence or absence. Between the LOD and LOQ it can only be said that microplastics are present ($p<0,01$) but not to what extent. The two samples with results higher than the LOQ were surface water samples. However, these must be interpreted with caution (see Discussion).

Particle Shape

Figure 13 shows the mean results for the different categories. There is no significant difference ($p=0,62$) between the results for the boreholes and the distribution system. With the limited number of samples, no correlation along the waterway from source to sink is evident. As discussed later, surface water samples must be considered separately.

Sample results: particles fluorescing

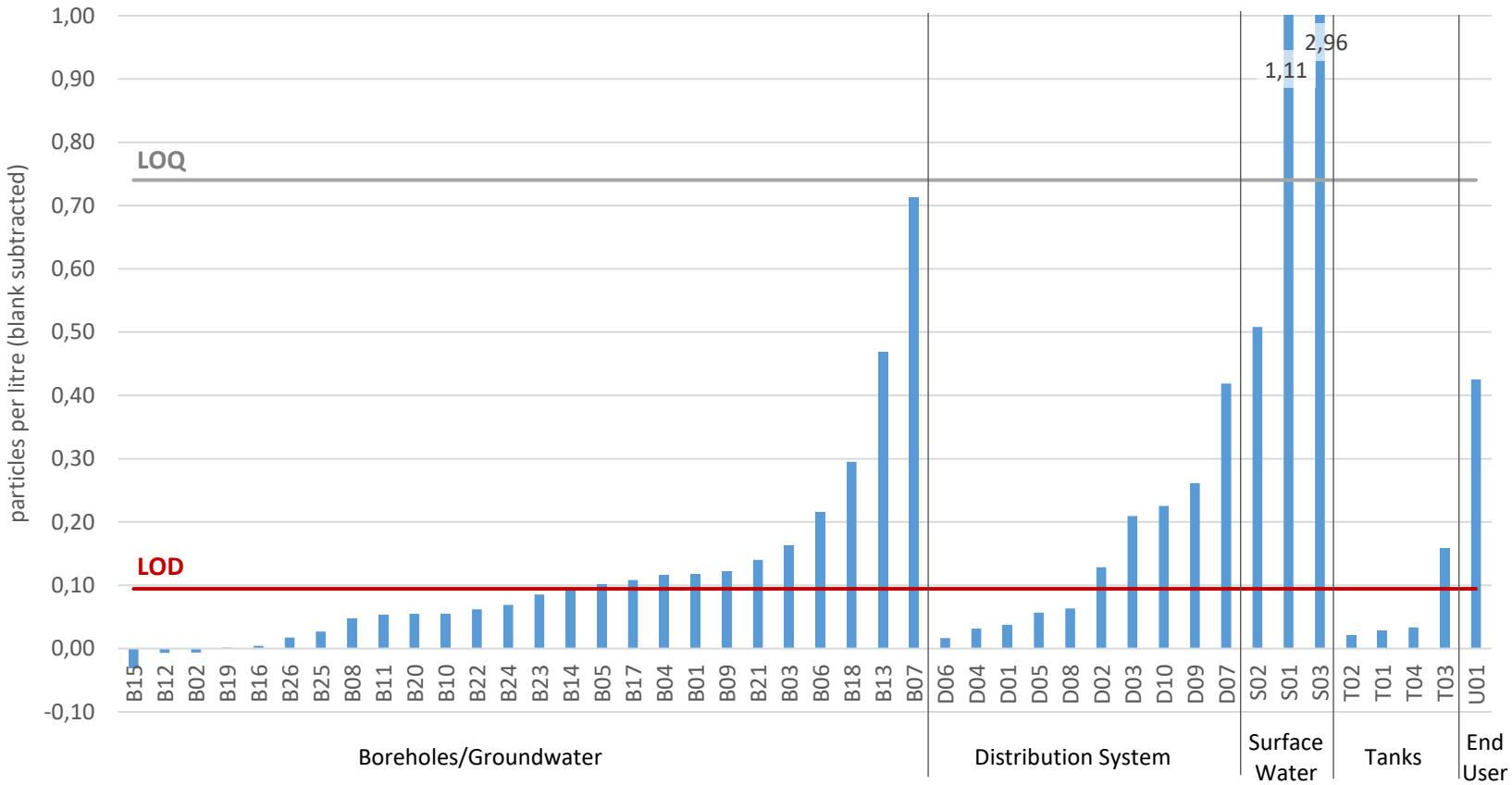

Figure 13: Overview of the combined results for particles fluorescing under Nile red and blue light and therefore assumed to be plastics. The red line shows the limit of detection and the grey line shows the limit of quantification. All values shown are difference between triplicate sample and triplicate blanks, hence some negative values are present. (RSI, 2019)

Blank results: particles fluorescing

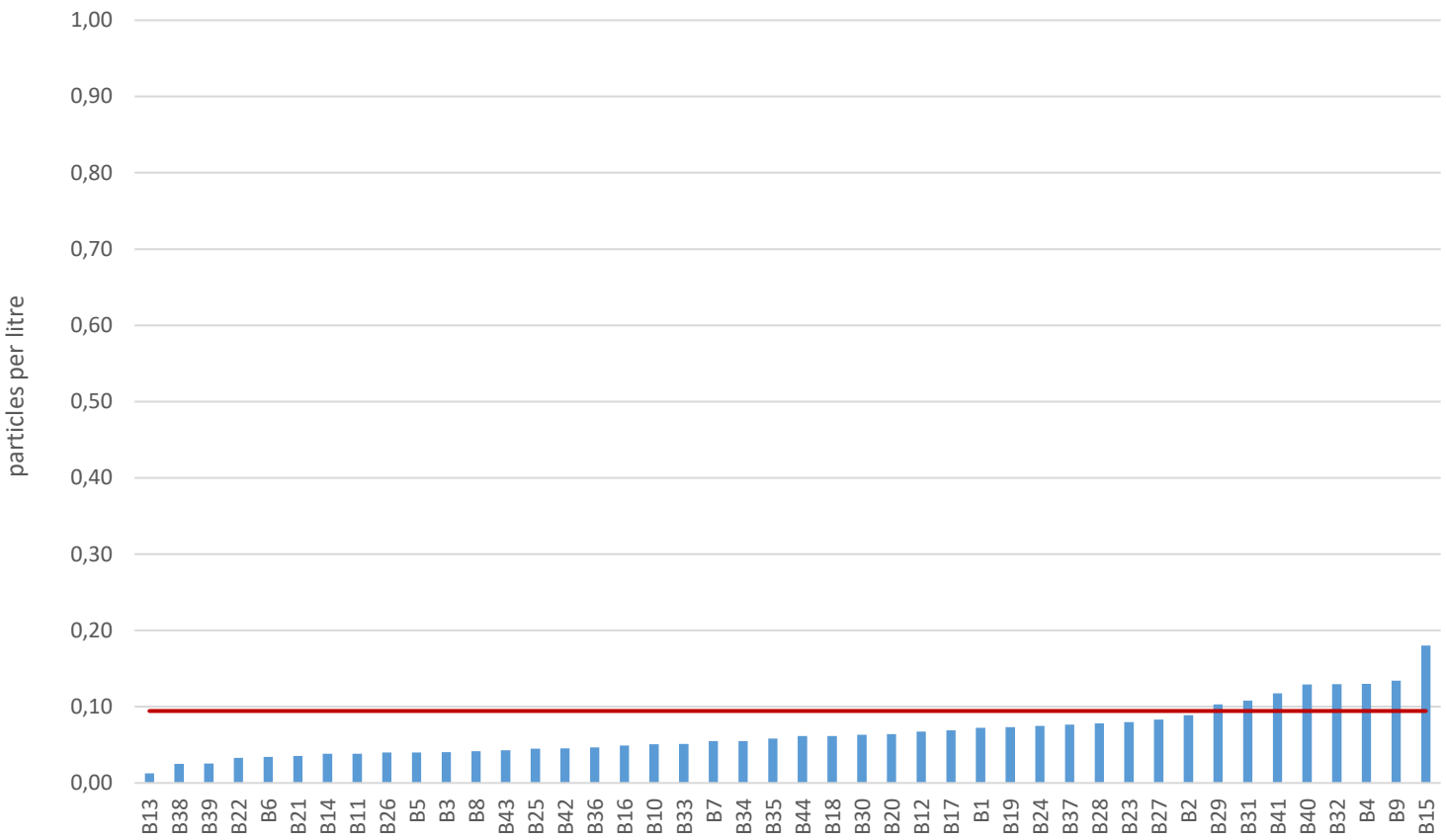

Figure 14: Results for the contamination control: blank filter meshes; the red line shows the limit of detection (RSI, 2019)

Fibres and fragments

The samples show low numbers of fluorescing fibres (mean=0,05/l) compared to fragments (mean=0,15/l). There is no linear correlation (R^2=0,20) between the fibres and fragments.

Blanks

Figure 14 shows the results of all 44 blank triplicate sets which have been processed parallel to the samples. The mean is 0,07ppl, the range lies between 0,01 and 0,18ppl. There is no evidence for a systematic contamination of the blanks. The fluorescent particles on the samples and their corresponding blanks do not correlate (R^2=0,12).

Pre- vs. Fluorescence-Counting

There is no linear correlation (R^2=0,09) between the pre-counting and fluorescent particle counts, indicating that filter loading (due to minerals, organics, rust etc.) is not an indicator for presence of microplastics.

5 Discussion

International comparison of the results

The methods and commissioning of this study were significantly informed by work funded by ORB Media which gained international attention in 2018 (Kosuth, Mason and Wattenberg, 2018; Mason, Welch and Neratko, 2018). The findings in this study are one to two orders of magnitude lower than those found in the one of these focusing on tap water, where the mean particle count was 5,45ppl (with a range of 0 – 61ppl; see Table 1). This must be considered in the context that the study used a higher size range (100µm vs 27µm) so the true difference is likely to be much higher. In addition, 98,3% of particles found in that study were fibres, compared to 12% here; fibres are disproportionately likely to be found in contamination (Woodall *et al.*, 2015).

This study has a limit of detection of 0,09ppl compared to 0,90ppl in a recent study of Norwegian drinking water (Uhl, Eftekhardadkhah and Svendsen, 2018) and 0,58ppl in a Danish study (Strand *et al.*, 2018). This is due to cleaner sampling and analysis and greater attention paid to blank samples, meaning that "noise" (contamination) was reduced and measurement of "signal" (i.e. what is in the water) can be more precise. The Norwegian study was unable to distinguish microplastics from background noise even with a false positive rate of 33% (vs. 1% in this study); it should also be noted that only particles >60µm were analysed.

The Danish study reported 0,312ppl as the average particle count for 17 tap water samples (vs. 0,22ppl here), below their LOD of 0,58ppl (at a 5% false positive rate, for particles >100µm). This meant again that for the average values, it was impossible to distinguish signal from noise. Both studies applied a combined method of stereomicroscopy and FTIR spectroscopy to count and identify the microplastic particles.

Lower limits

Optical counting of particles smaller than 100µm is fraught with difficulties, but Nile red can be usefully used to stain particles down to c.20µm (Erni-Cassola *et al.*, 2017). In their study on bottled water, Mason, Welch and Neratko (2018) used automated counting with a 6µm pixel size to count particles down to 6,5µm but the uncertainties at this level are high. However, one recent study indicates that 95% of microplastics particles in tap water may be below 10µm (Pivokonsky *et al.*, 2018); although current methods are insufficient to survey this size range at environmentally relevant scales, is important context. Close attention should be paid to method advances in the following years.

Sampling and analysis method

The sampling method developed here allows for large sample volumes and triplicates in an easy and replicable way. The sample volume of 200 litres was large enough for precise and accurate results but could be increased in future as desired.

It is important to choose the side streams and connection points for the sampling equipment carefully. In this study there were two samples (D07 and D09) with a high load of hemp fibres on the filter meshes. The fibres were de-stained (see Figure 15) and thus not counted as plastic. However, both of these filters have the highest amount of fluorescing particles present in the distribution systems, and it may be that the high organic loading in these cases inhibited the de-staining process in some way, giving false positives.

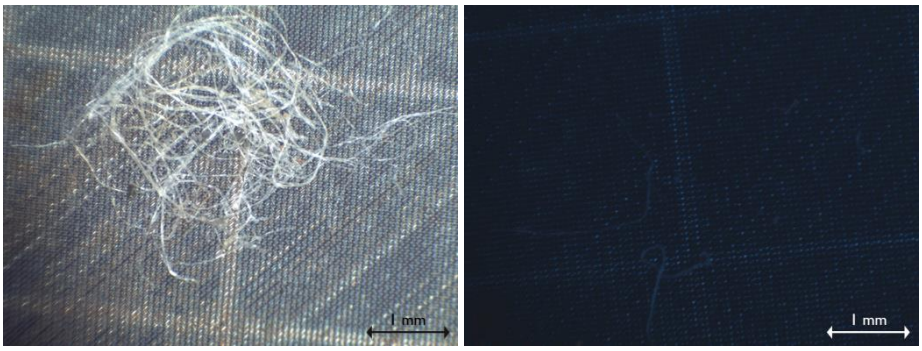

Figure 15: Hemp fibres on the filter meshes: left: pre-counting; right: fluorescence counting (RSI, 2019)

The same phenomenon as for hemp fibres was observed for the samples of surface water and their high organic loads (Figure 16); while it may be expected that plastic numbers are highest in surface waters, inhibition of de-staining may also have played a role in the high count of fluorescent particles in these samples. Future work should avoid cases of high organic loading if possible and test the effects of high organic loading on the amount of reagents required for de-staining processes. In addition, the literature is still developing regarding the exclusivity of the Nile red dye – it may be that future work must also exclude the possibility of mineralised chitin (Maes *et al.*, 2017), although it is likely that this is digested using the protocol in this study (Einbu *et al.*, 2004). Last, it should again be noted that black-dyed particles were not included in this study as the dye absorbs all fluorescent light.

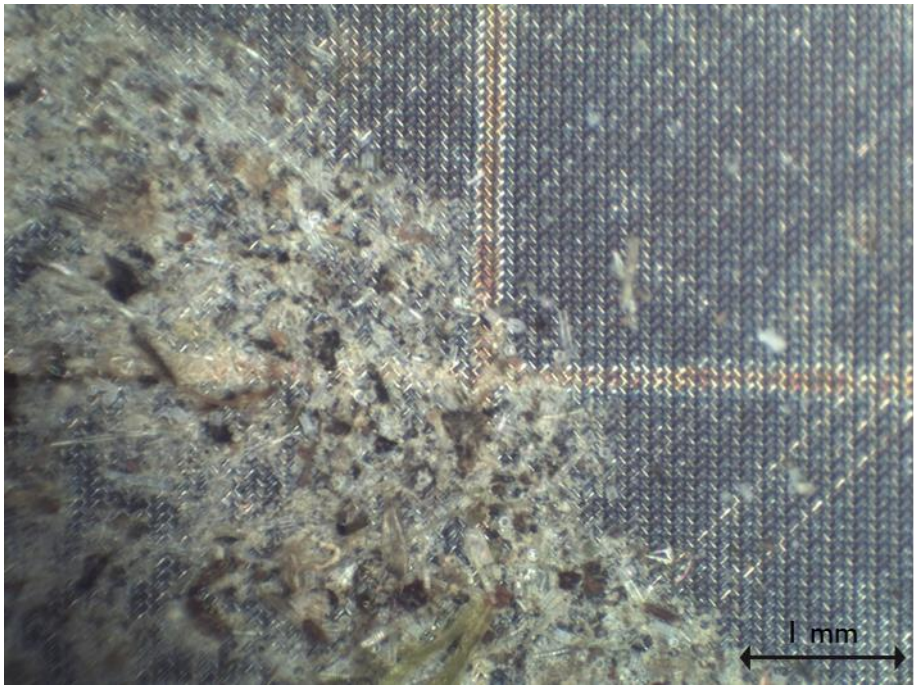

Figure 16: Sample of surface water with high organic load (RSI, 2019)

Type of Polymer

While this method does not allow an identification of different types of synthetic polymers, it is non-destructive, thus further analyses could be done by other labs. Internationally this is done with Fourier-transform infrared spectroscopy (FT-IR) as well as Raman spectroscopy; however these are laborious and costly and deal best with small samples, making them unsuitable for wide-scale monitoring. In the case that a sampling location returns high results, they may be productively used to confirm Nile red staining and to determine to origin of the polymers.

6 Conclusion

Drinking water throughout Iceland was sampled and analysed in Spring 2019. The results show a mean contamination of 0,22ppl particles fluorescing under Nile red and blue light and therefore assumed to be plastics for all 44 samples. These were detected in processing of all samples. However, the limit of detection based on background contamination was set at 0,09ppl and only 22 individual samples exceeded this limit.

The here-developed method for sampling and analysing is an improvement of the state-of-the-art of peer-reviewed literature. It is suitable for a high number of samples and large sample volumes and effectively removes background noise from contamination during sampling and analysis. It is also relatively low-cost and thus efficient for large-scale monitoring programs. Future work should focus on investigating the cause of detected microplastics and determining variability over the course of months and years, such as before and after maintenance of systems.

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