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Amy Lusher
Project Manager/Lead Author

Bert van Bavel
Quality Assurer

Morten Jartun
Research Manager

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Amy L. Lusher, Ian Allan, Laura Rohler, Vilde K. Snekkevik, France Collard, Sverre Hjelset	Aquaculture	Open
Client(s)	**Client's contact person**	
FHF	Eirik Sigstadsto	

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Abstract
Plastic material has been collected from three recirculating aquaculture systems (RAS) and their infrastructure. All plastic sources were analysed to identify their polymer and additive contents and a reference library created. This was used for targeted analysis when investigating the presence of microplastics within RAS facilities, specifically looking at intake water, recirculating water, sludge, fish, and fish feed. Analysis of these samples showed low levels but identified several potential sources of microplastics, including plastic infrastructure and consumables. There were low numbers of microplastics >300 µm in RAS samples. Comparatively, particle analysis of the smaller size fraction (50-300µm) suggests some degree of microplastic generation within the RAS although data is limited. Potential sources originating from the RAS infrastructure were identified as the fix bed bioreactor (FBBR) and moving bed bioreactor (MBBR), and biomedia. Initial analysis of fish tissue and stomachs showed low levels of microplastic and based on these results no conclusion could be drawn on ingestion by the fish. Screening for additives identified that several plastic-related chemicals were ubiquitous in RAS recirculating waters, including selected antioxidants and plasticisers. The source and distribution of these chemicals was compound specific. Fewer chemicals were quantified in sludge and fish compared with water. But specific compounds including antioxidants and plasticisers were consistently found in fish.

Keywords: Microplastic, Plastic additives, aquaculture
Emneord: Mikroplast, tilsetningsstofffer i plast, akvakultur

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Preface

This report presents the outcome of WP1 of the REMIRA project until 31st January 2024. It provides an overview of the activities performed and plans for data synthesis across subsequent WPs.

Amy Lusher and Ian Allan led the microplastics and chemical additives elements of the project, respectively. Amy Lusher was the project manager. Sampling was performed by Laura Röhler, Sverre Hjelset, Jarle Hårvardstun and Ian Allan. Sample processing was performed by Sverre Hjelset, Laura Röhler, Moe Moe Thwe Win, Dorna Misaghian, France Collard, Amy Lusher, Vilde K. Snekkevik and Svetlana Pakhomova. Data analysis was performed by Amy Lusher, France Collard, Vilde K. Snekkevik and Ian Allan. Amy Lusher and Ian Allan lead the report with support from Laura Röhler, France Collard, Sverre Hjelset and Vilde K. Snekkevik. Bert van Bavel quality assured the scientific content of the project.

The project team received external support from Sebastian Primpke (AWI) in the generation of the FTIR library and Julien Gigault (CNRS) for support with the F-search.

Oslo, 14/03/2024

Summary

The following activities have been performed within WP1 of the REMIRA project:

- **Task 1.1 Sampling in RAS:** Sampling was performed at all three RAS involved in the project. Samples were collected across the different RAS lines and returned to NIVA for processing. Detailed information is presented in Section 2.1 Sampling.

- **Task 1.2 Occurrence and origin of microplastics in RAS:**
    - *Plastic infrastructure* – The majority of the plastics at RAS#1 consisted of polypropylene and polyethylene whereas RAS#2 and RAS#3 mostly contained polyethylene materials.
    - *Water samples* – No relation with any RAS sources could be established for the large size fraction (>300 µm) because of the low number of microplastic particles in the samples and the random distribution of particles. Particle analysis of the smaller size fraction suggests some microplastic are generated from within the RAS. Polyamide and polypropylene are used in the RAS infrastructure, for example the fixed bed bioreactor (FBBR), mixed bed bioreactor (MBBR), and biomedia.
    - *Sludge* – Fibres dominated the larger size fraction (>300 µm), no fragments from the RAS were observed. Particle analysis of the smaller size fraction suggests some microplastic generation from within the RAS, including polyamide.
    - *Fish feed* – Samples from all three RAS were found to have low numbers of microplastics, but cellulose acetate fibres (>300 µm) were found in the feed samples.

- **Task 1.3. Microplastics in fish:** Few plastic fragments were identified in the stomachs (13% frequency occurrence) although they could not be matched to any of the known plastic infrastructure. The particles observed in the fish fillet were likely a result of procedural contamination. The smaller fraction (<100 µm) of both stomach and fillet samples could not be used for data analysis due to error.

- **Task 1.4. Method development and optimization for plastic additives:** Samples of various plastics were obtained from RAS#1 and RAS#3 and analysed by pyrolysis-GCMS. Full scan spectra were interpreted with help of the NIST library and F-search software from Frontier. Our method for the quantification of plastic additives was subsequently optimised to include the 6 additives found during the thermal desorption/pyrolysis-GC-MS screening.

- **Task 1.5. Initial screening of plastic additives in RAS:** Freshwater and seawater intake waters as well as recirculating waters were sampled at the three RAS facilities. Sludge samples from two RAS facilities and fish samples from three RAS were also extracted and analysed. This sampling allowed the identification of the presence of selected chemicals in water of RAS systems. For some compounds including selected antioxidants and plasticisers, their presence in re-circulating waters was the result of their presence in intake waters, while for others the source was within the RAS system, whether this was plastic surfaces in contact with water, fish feed or the atmosphere.

- **Task 1.6. Complementary screening of plastic additives in RAS:** Samples of plastic materials used in RAS system and operation (e.g., biofilter media) were collected and analysed for plastic additives. Samples of fish feed were also analysed. Biofilter media samples appeared to present a relatively wide range of the additives measured here. Detectable concentrations of additives (including antioxidants and plasticisers) were found in the fish feed samples.

Sammendrag

Følgende aktiviteter er utført innenfor prosjektets AP1:

- **Oppgave 1.1 Prøvetaking i RAS:** Prøvetaking ble utført ved alle tre RAS stasjonene som var involvert i prosjektet. Prøvene ble samlet inn på tvers av de ulike RAS-linjene og returnert til NIVA for behandling. Detaljert informasjon om prøvetakingen er beskrevet under Section 2.1 Sampling.

- **Oppgave 1.2 Forekomst og opprinnelse av mikroplast i RAS:**
    - *Plastinfrastruktur* - Majoriteten av plastpartiklene i RAS#1 besto av polypropylen og polyetylen, mens RAS#2 og RAS#3 for det meste inneholdt polyetylenmaterialer.
    - *Vannprøver* – Det kunne ikke fastslås noen sammenheng mellom aktuelle RAS-kilder og den store størrelsesfraksjonen (>300 µm) på grunn av det lave antallet mikroplastpartikler i prøvene og den tilfeldige fordelingen av partikler. Resultatene fra mindre størrelsesfraksjoner indikerer noe mikroplast fra kilder i RAS. Funn av polyamid- og polypropylenpartikler kan stamme fra RAS-infrastrukturen, som FBBR og MBBR og biomedia.
    - *Slam* - Størrelsesfraksjonen >300 µm dominertes av fibre, og ingen mikroplastpartikler ble påvist. Partikkelanalyse av den mindre størrelsesfraksjonen antydde noe mikroplastgenerering fra RAS inkludert polyamid.
    - *Fiskefôr* – Det ble funnet lavt antall mikroplastpartikler (>300 µm) fra alle tre RAS. Partiklene ble dominert av celluloseacetatfibre.

- **Oppgave 1.3. Mikroplast i fisk:** Få plastfragmenter ble identifisert i magen (13% deteksjonsfrekvens), og de kunne ikke matches med noen av de kjente plastinfrastrukturene. Partiklene som ble observert i fiskefilet var sannsynligvis et resultat av prosedyreforurensning. Den mindre fraksjonen (<100 µm) i både mage- og filetprøver kunne ikke brukes til dataanalyse.

- **Oppgave 1.4. Metodeutvikling og optimalisering for plasttilsetninger:** Prøver av ulike plastmaterialer ble hentet fra RAS#1 og RAS#3, og analysert med pyrolyse-GCMS. Full skanningsspektra ble tolket ved hjelp av NIST-biblioteket og F-search-programvaren fra Frontier. GC-MSMS-metoden for kvantifisering av plasttilsetningsstoffer er optimalisert for å vurdere til 6 tilsetningsstoffer funnet under termisk desorpsjon/pyrolyse-GC-MS-screening.

- **Oppgave 1.5. Innledende screening av plasttilsetninger i RAS:** Ferskvanns- og sjøvannsinntaksvann samt resirkulerende vann ble prøvetatt ved de tre RAS-anleggene. Det ble også tatt ut og analysert slamprøver fra to RAS-anlegg og fiskeprøver fra tre RAS. Denne prøvetakingen gjorde det mulig å identifisere tilstedeværelsen av utvalgte kjemikalier, i vann i RAS-systemer. For noen forbindelser inkluderer plastmykgjører og antioksidanter, er hovedkilden i resirkuleret vann selve inntaksvannet, mens for andre forbindelser var kilden innenfor RAS-systemet, enten plastutstyr i kontakt med vann, i fiskefôr eller gjennom atmosfærisk tilførsel.

- **Oppgave 1.6. Kompletterende sikting av plastmyknere i RAS:** Prøver av plastmaterialer brukt i RAS-system og drift (f.eks. biofiltermedier) ble samlet inn og analysert for plasttilsetningsstoffer. Prøver av fiskefôr ble også analysert. Prøver av biofiltermedier som ble testet, så ut til å presentere et relativt bredt spekter av tilsetningsstoffene som ble målt her. Påvisbare konsentrasjoner av tilsetningsstoffer (inkluderer plastmykgjører og antioksidanter) ble funnet i fiskefôrprøvene.

1 Introduction

Microplastic contamination is a global environmental threat, and these plastic materials may contain up to 30 % potential harmful additives/plastic softeners. Plastic materials are widely used in the aqua- and mariculture industries and the potential for them to release microplastics and additives over time has come into debate. Systems such as recirculating aquaculture systems (RAS) contain many plastic components although the main sources for microplastics and plastic additives are yet to be quantified and tracked within a RAS.

RAS systems have been queried to present accumulation of contaminants, such as heavy metals, drug residues and metabolites (e.g., Wei et al., 2024). Since RAS heavily reply on plastic components, the release of plastics and associated additives should be investigated. It is essential to inventory sources to determine the potential for RAS systems to up concentrate these pollutants. We hypothesise that RAS systems will have several potential sources of plastics and additives, such as water treatment (particle filtration), bioreactors, and other plastic installations. The efficiency of water treatment and filtration systems are likely to remove microplastics in the form of sludge. Thus, not compromising the production of commercial fisheries products. There may also be differences between the different bed types within a RAS which could influence the dynamics and sedimentation of microplastics. Therefore, this project set out to characterize and understand the complexity of microplastics and plastic additives in RAS with an aim to suggest mitigation measures, should there be a requirement.

Sub-goal 1: Quantifying the occurrence of MP and PS in feed, water, fish and sludge / sediment in RAS.
Sub-goal 2: Propose and document relevant measures to reduce the occurrence and release of MP and PS from RAS

The project is divided into two scientific and one management work packages displayed in Figure 1.

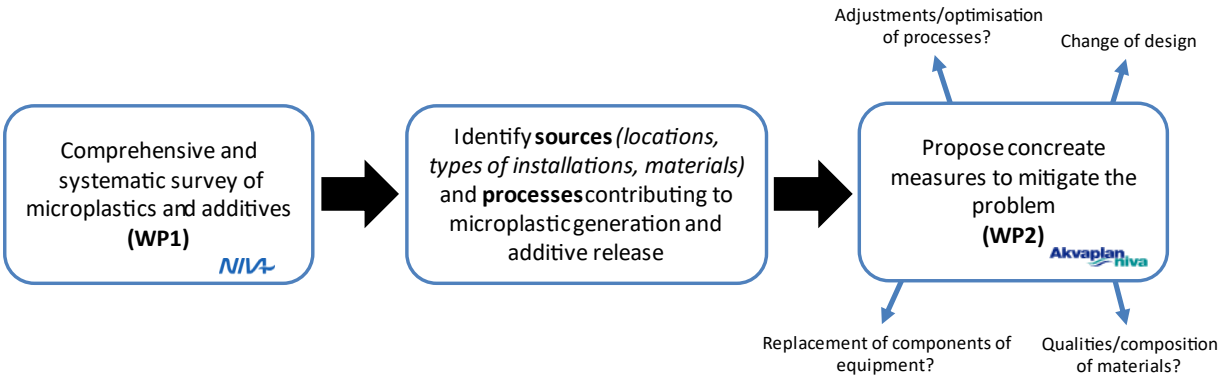


Figure 1 Conceptual diagram of the REMIRA project.

2 Materials and methods

2.1 Sampling

An initial site visit was carried out on the 26th of May 2023. Project members from NIVA and Akvaplan-niva RAS#1 to tour the facilities, discuss sampling plans, identify the RAS system to focus on, and take provisional samples. Provisional samples were identified to allow NIVA to begin to build the database of plastics and additives used in the RAS system, confirm the methods for processing, and to allow the team to identify which element of the RAS to target.

Sampling was carried out at all three RAS systems between September and December 2023. The RAS infrastructure utilise different bioreactors: fixed bed bioreactor (FBBR) and mixed bed bioreactor (MBBR) Further information on the site visits is presented in Table 1.

2.1.1. Overview of sampling locations at RAS facilities

RAS#1: Two separate lines were tested in this RAS (Figure 2). Sampling of the intake of freshwater and saltwater into the facility before water treatment (UV and particle filtration) and after the filter. In addition, two fish tanks were sampled. ***RAS#1A*** – "post-smolt", the sampling was at the outside of the fish tank where the clean water after-water treatment was pumped back into the fish tank. ***RAS#1B*** – "Påvekst" - the sampling was at the outlet of the heat exchanger system after the combined bed filter (FBBR + MBBR). The two RAS systems are denoted as RAS#1A and RAS#1B moving forward.

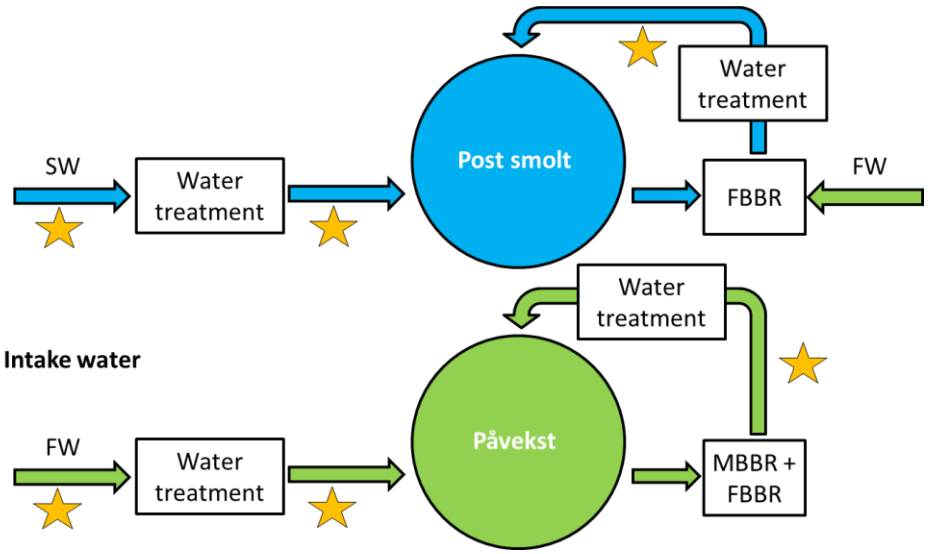


Figure 2 Water sampling locations in RAS#1. Stars denote the sampling locations. Sampling was performed in replicate or triplicate. SW – seawater, FW – freshwater, FBBR – fixed bed bioreactor, MBBR – moving bed bioreactor.

RAS#2: Sampling of the intake of freshwater and saltwater into the facility was performed after water treatment (particle filtration, 80-100 µm). Sampling was carried out in one fishtank inside the sewage-water treatment part. The water samples were taken from the same spot where the facility performs their own sensor monitoring of water quality (Figure 3). The RAS system is denoted as RAS#2 moving forward.

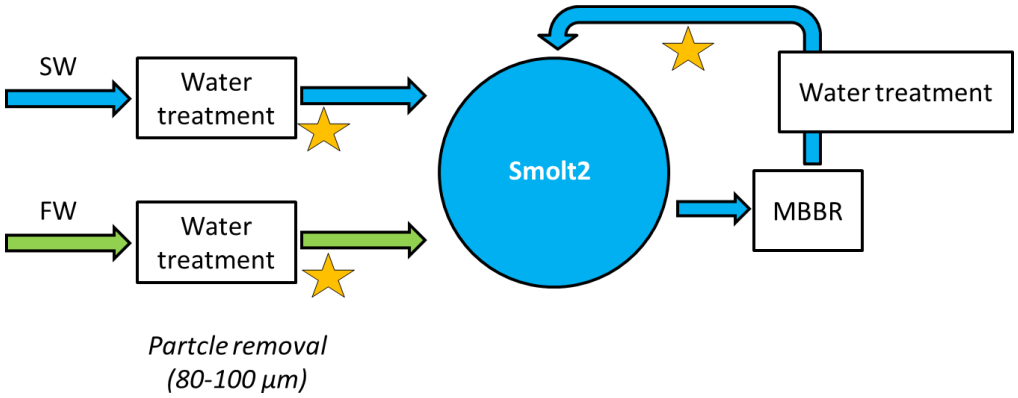


Figure 3 Water sampling locations in RAS#2. Stars denote the sampling locations. Sampling was performed in triplicate. SW – seawater, FW – freshwater, FBBR –MBBR – moving bed bioreactor.

RAS#3: Sampling of the intake of freshwater into the facility was performed before the water treatment. Sampling of the intake of saltwater was performed after the filter. Sampling was carried out in one fishtank inside the sewage-water treatment part, sampling at the heat exchanger after the biofilter (Figure 4). The RAS systems is denoted as RAS#3 moving forward.

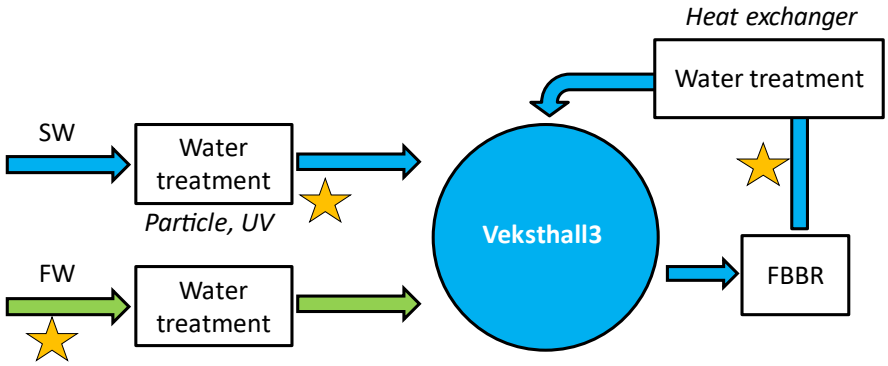


Figure 4 Water sampling locations in RAS#3. Stars denote the sampling locations. Sampling was performed in triplicate. SW – seawater, FW – freshwater, FBBR – fixed bed bioreactor.

Table 1 Overview of REMIRA sampling at RAS facilities.

Site ID	Date of sampling	Inlet	Water treatment	Tank ID	Bed	Effluent	Sludge	Fish
RAS#1A	20.09.2023 – 03.11.2023	FW and SW	SW: pressure filters with ultrafiltration (0.1 µm) and two UV in parallel.	Post smolt	FBBR	Recirc. H_2O	n = 3 (+ PC, AB)	n = 15
RAS#1B			FW: 4 x sand filters and 3 UV in parallel.	Påvekst	MBBR + FBBR	Recirc. H_2O	n = 3 (+ PC, AB)	n = 10
RAS#2	10.10.2023 – 24.10.2023	FW and SW	Particle removal with pressure filter (80-100 µm), UV filter.	Smolt2	MBBR	Recirc. H_2O	n = 3 (+ PC, AB)	2 x tank, n = 10 / tank
RAS#3	30.11.2023 – 15.12.2023	FW and SW	SW: 100 µm filtration with 3 UV in parallel.	Veksthall3	FBBR	Recirc. H_2O	n.a.	n = 10

2.1.2. Water samples

Microplastics – Volumes of water were collected from water systems at all three RAS in similar ways. A hose was attached to each water body, and the flow rate adjusted (when possible) to approximately 10 L/min. The flow rate calculated using a stopwatch and a 10L container. A PEX hose (polyethylene) was connected to a simple filtering stage housing two metal sieves (300 µm, 20 µm) under a wooden cover. The 50 µm sieve held a 20 µm metal filter. The start and end time of the sampling was recorded. Samples collection times varied between 30 mins and 1 h. The volume of water filtered was calculated based on the predetermined flow rate. Approximately 200 L of water was sampled for each replicate. After the allotted sampling time, the hose was removed with the cover. The particles collected on the 300 µm sieve were flushed into glass jar using pre-filtered water. The 20 µm metal filter was remove from the stand, folded to protect the particles, and placed directly into a glass jar. Subsequent samples were taken without adjusting the flow rate. One atmospheric blank was collected for each sampling point (glass jar with open aluminium foil during the entire sampling time for the replicates) to act as an atmospheric contamination control. Total water volumes calculated for each site are presented in Table 2.

Table 2 Volumes of water (litres/L) collected for each RAS.

	RAS#1 [L]	RAS#2 [L]	RAS#3 [L]
Freshwater influent before treatment	203	n.a.	200
	203	n.a.	200
	n.a.	n.a.	200
Seawater influent before treatment	194	n.a.	n.a.
	194	n.a.	n.a.
	n.a.	n.a.	n.a.
Freshwater influent after treatment	206	200	n.a.
	206	200	n.a.
	n.a.	200	n.a.
Seawater influent after treatment	217	200	200
	217	200	200
Påvekst / veksthall	n.a.	200	200
	160	n.a.	200
	160	n.a.	200
	160	n.a.	200
Post-smolt / smolt	203	200	n.a.
	203	200	n.a.
	203	200	n.a.

2.1.3. Sludge

Sludge was collected differently depending on the sludge composition. The method varied slightly per RAS depending on access to the collecting tanks. Although the sample collection was similar for both microplastic and additive analysis at each RAS. For RAS#1, samples were collected from the settling tank. Whereas with RAS#2 sludge was sampled from the belts before the drum filters. No samples were collected for RAS#3. Triplicate samples of sludge were collected in glass bottles or jars (depending on the volume) for microplastic analysis. One atmospheric blank was collected in parallel. A single sample (2.5 L) was collected for plastic additives.

2.1.4. Fish

Fish were collected by RAS personal according to their standard protocols. Fish were shipped frozen to NIVA for further analysis.

2.1.5. Plastic materials used within RAS

Plastic samples were collected from within each RAS facility by onsite personal. A description of the items and where they came from were sent to NIVA for polymer and chemical mapping.

2.2. Sample processing

All processing followed NIVAs internal methods unless otherwise stated.

2.2.1. Water Samples

The volume-reduced samples collected at the RAS were divided into two samples in the field: one with a small fraction ranging from 20 µm to 300 µm, and another with a large fraction exceeding 300 µm. Upon arrival at the lab, the samples were refrigerated and processed as soon as possible to prevent any growth.

Processing of water samples was performed inside a fume hood, and all glass wear were thoroughly rinsed with RO water. The large fraction (>300 µm) was sieved on a 300 µm steel mesh to remove excess water. Then, the sample was transferred solution into a glass beaker using 10% KOH. Subsequently, samples were topped up with additional 10% KOH solution to a volume of 100 mL. The samples were then left in an incubator for 24 hours at 100 RPM and 40°C. The small fraction (20-300 µm) on metal mesh filters and was rinsed onto a small mesh sieve (20 µm) before being further rinsed with a 10% KOH solution into a clean glass beaker. Additional KOH was added during the transfer to increase the sample volume to 100 mL. The sample was then incubated for 24 hours at 40°C with 100 RPM.

The next day, material from the large and small fractions was vacuum filtered onto a GF/A filter (43 mm) for the large fraction and a silver filter (13 mm) for the small fraction using a vacuum glass pump. Three laboratory procedural blanks were included per processed batch, using only RO water (1 L). The procedural blanks underwent the same processing as the samples.

2.2.2. Sludge

Sludge composition required the use of two slightly different methods because one sample was mostly liquid (RAS#1A) whereas the others were more solid in consistency (RAS#1B and RAS#2).

RAS#1A - each of the samples were sieved through a 20 µm metal sieve and transferred to a clean Erlenmeyer flask using a squirt bottle filled with 10% SDS. The sample was then topped up with SDS to achieve 100 mL of SDS per sample. SDS is used to disaggregate complex samples before processing.

RAS#1B and RAS#2 - before processing, all sludge samples were freeze dried. 20 mL of 10% SDS was added to 5g of freeze-dried sludge for each sample.

All samples were placed in the incubator over the weekend (Friday to Monday) at 50°C and 100 RPM. The samples were sieved again using a 20 µm sieve, and then transferred to a new clean Erlenmeyer flask using distilled water. Next, the Fenton's reaction was performed following Hurley et al., (2018). After the reactions were completed, samples were toped of with water, covered with aluminium foil and left inside the fume hood overnight. The next day the samples were split into two fractions of 50-300 µm and >300 µm. The small fractions were filtered onto 13 mm silver membrane filters, while the larger fraction was filtered onto 47 mm GF/A filters. Three procedural blanks were included for the sludge samples which were treated in the same way.

2.2.3. Fish

Salmon (*Salmo salar*) were provided by from all three RAS. Fish came from two tanks in both RAS#1 (RAS#1A and RAS#1B) and RAS#2 (further named RAS#2A and RAS#2B), and from one tank in RAS#3. Ten fish per tank were used for this study, except in RAS#1 where 15 were used. All fish were stored frozen at -20°C for further dissection and tissue collection.

The fish were thawed at room temperature and both the stomach content, a fillet, liver, and gills were collected during dissection. All samples were freeze dried (with both wet and dry weight, g, recorded. The liver and gills were preserved for further analysis (Table 3).

Table 3 Summary of fish length and weights obtained within the REMIRA project.

Lab code	n	Fish length (cm)	Fish weight (g)	Fillet (g, d.w)	GIT (g, d.w)
RAS#1A	15	23.1 (20-25.3)	148.9 (94.3-202.2)	4.5 (2.4-7.6)	2.1 (0.6-4.3)
RAS#1B	10	10.6 (9.9-11.4)	18.3 (15.1-22.1)	0.4 (0.3-0.6)	0.4 (0.2-0.5)
RAS#2A	10	19.8 (18.9-21.0)	109.4 (93.3-130.6)	4.6 (3.1-6.1)	2.6 (1.5-3.2)
RAS#2B	10	24.1 (22.8-26.0)	182.2 (148.0-218.5)	7.4 (5.3-11.6)	4.1 (3.2-5.1)
RAS#3	10	16.3 (14.8-17.3)	55.9 (39.0-69.0)	1.4 (1.0-2.1)	0.9 (0.7-1.3)

The stomach content (0.4-5.1 g) and fillet (0.3-11.6 g) were put into a glass jar, subsequently filled up with a 10% KOH solution (Rochman et al. 2015). All jars were left for organic matter digestion at 40℃ in an incubator gently shaking the jars at 100 RPM for 24 to 72 hours. After incubation, the solutions were filtered through a 100 µm mesh stainless steel sieve. To ensure the full digestion of organic matter and to ease further visual observations and spectroscopic analyses, the filter - with the extracted particles - was placed in a glass jar and a biological detergent (Alcojet) was added to remove any remaining organic matter (Hampton et al., 2023). After 24 hours in Alcojet, the filter was removed and rinsed, and the remaining solution was filtered through a 47 mm GF/A filters. That filter paper was then stored in a clean petri dish for further visual analyses.

Originally the lower size fraction (<100 µm) was intended for analysis. Unfortunately, due to a procedural error the data cannot be used. Given the results obtained for the larger size fraction showed procedural contamination, it is not likely that the lower fraction would have presented any further data.

2.2.4. Fish feed

Fish feed samples were received from all three RAS. 20 g of each fish feed was weighed into clean glass flasks. Each sample was crushed into a fine powder using a pestle and mortar inside a laboratory fume hood. The pestle and mortar were cleaned using filtered RO water between each sample. Subsequently, 5 g triplicates of each sample were weighed into new, clean glass beakers. Next, 150 mL of 20% KOH (aq) was added to each sample, and they were left in an incubator overnight at 50°C at 100 RPM. The following day, the samples were sieved through a 50 µm sieve and rinsed into new, clean glass beakers using 10% acetic acid (aq). The samples were topped off with acid so that each one had a volume of 150 mL. They were then left overnight in an incubator at 40°C at 100 RPM. The next day, the samples were sieved at 50 µm again, then transferred into clean glass beakers using a squirt bottle filled with 10% Alcojet (biological detergent). The samples were filtered to 150 mL with 10% Alcojet and left in the incubator overnight at 40°C with 100 RPM. On the final day, the samples were split into two fractions using two sieves with different mesh sizes (300 µm, 50 µm).

The large size fraction (> 300 µm) was subsequently filtered using a 47 mm GF/A filter for visual inspection before µFTIR analysis. Whereas the small size fraction (50-300 µm) was subsequently filtered using a 13mm silver filter for µFTIR analysis. Since there was a lot of material in the small size fraction, each sample was split into two different silver filters to reduce overload on the filters.

2.2.5. Microplastic lab QA/QC

Optimized contamination control procedures were used throughout sample processing and analysis. In short, all personal with access to the NIVA microplastic laboratory wore cotton clothing and cotton lab coats. A lint roller is used on all coats and any exposed clothing items before entering the laboratory. All solutions were filtered through 0.2 µm filters ahead of use. Consumables were made of glass where possible, and all equipment and materials were cleaned before use with filter RO water. Petri dishes used to hold filter papers were cleaned by direct and strong ventilation and sealed before use. Nitrile gloves were worn throughout processing. Positive controls were performed to ensure maximum recovery and procedural blanks were performed along with all samples per batch processing. An overview of all samples processed is included in Table 4.

Table 4 Summary of method validation, performed as recovery tests, and number of processed samples for microplastic analysis.

Sample type	Method validation			Number of processed samples and procedural controls		
	Spiked particles	Replicates	Average recovery	Large fraction (size):	Small fraction (size):	Procedural control (blanks)
Water	30 PS beads (100 µm)	3	28/30 (94%)	32 (>300 µm)	32 (300-20 µm)	21 (x2 fractions)
Sludge	60 PS beads (100 µm)	3	57/60 (95%)	9 (>300 µm)	9 (300-50 µm)	4 (x2 fractions)
Fish feed	60 PS beads (100 µm)	3	54/60 (90%)	21 (>300 µm)	21 (300-20 µm)	3 (x2 fractions)
Fish samples	30 PE fragments (100 µm)	3	35/30 (117%)*	55 (>100 µm)	n.a.	18

*Reference materials used for recovery fragmented (multi-layer fragments).

2.3. Chemical analysis of plastic polymers in RAS

An initial systematic survey of potential sources of microplastics was performed in collaboration with industry partners who provided information on the RAS operations. All samples were photographed and described according to visual characteristics and the information provided by the facilities. Each plastic sample was tested for plastic composition, and additives at NIVA to generate a known plastics database.

2.3.1. FTIR analysis of known plastics

A small fraction of each plastic was cut away using a scalpel and ATR-FTIR (Spotlight 400, Perkin Elmer) was performed to determine the polymer composition of each sample. The instrument is used together with the Spectrum 10 software (v. 10.6.2), and each spectrum is compared to several different libraries: PerkinElmer ATR Polymers library, STJapan Polymers ATR library, BASEMAN library (Primpke et al., 2018), and in-house libraries including reference material, various textiles, and potential sources of contamination from the lab. All spectra were manually inspected to ensure that each library match is acceptable. Particles were accepted as plastics if they fell into the categories as assigned by AMAP (2021). A QA/QC step was performed using OpenSpecy (https://openanalysis.org/openspecy/). following the standard procedure, with correction for baselines[1]. The results are presented in Section 3.1. The spectra from all three RAS facilities were compiled into a reference library to be used when the environmental samples were analysed. The reference library was created for use with siMPle (version 1.1.β) which contained the ATR-FTIR spectra of the known plastics.

[1] http://wincowger.com/OpenSpecy-package/articles/app.html

2.3.2. FTIR analysis of unknown plastics

Large plastics fraction (>100 µm / >300 µm): The GF/A filters with sample material from the larger fraction (>300 µm) were visually inspected using a stereomicroscope (Nikon SMZ745T, magnification x20), and potential microplastic particles and other anthropogenic particles (such as cellulose) were sorted out. The shape (fibre, fragment, film, sphere) and colour were noted. The length and width (µm) of the particles were measured using Infinity Analyze and Capture 6.5 software (Lusher et al., 2020). Visual analysis was followed by chemical characterization using FTIR. A PerkinElmer Spotlight 400 µFTIR spectrometer was used, and the particles were transferred and compressed with a diamond compression cell to achieve uniform thickness. The particles were analysed using Spectrum 10 (v. 10.6.2) in transmission mode with a resolution of 4 cm^{-1} and a wavelength range of 4000 to 600 cm^{-1}. Each spectrum was manually inspected and compared to libraries available at NIVA: PerkinElmer ATR Polymers library, STJapan Polymers ATR library, BASEMAN library (Primpke et al., 2018), and several in-house libraries including reference polymers, various textiles, and potential sources of contamination from the lab. The results from chemical characterization were used to determine concentration of microplastics in each sample.

Small plastics fraction (20-300 µm): The samples which were filtered onto silver membrane filters (5 µm pore size, 13 mm diameter) were analysed using the automatic imaging mode the µFTIR imaging reflectance mode (Spotlight 400, PerkinElmer). The method limits particle loss linked to the physical handling of particles. In short, µFTIR imaging involves analysing spectra for each defined pixel within a defined area, and building a chemical map of the entire sample, i.e., all identified particles.

The reference library generated from the plastics collected from the RAS was used to evaluate the presence of particles in the small fraction in siMPle (version 1.3.2.1β). The Perkin Elmer .fsm files were converted into the .spe form, allowing for automated comparison to the REMIRA polymer database. Using the standard pathway, each spectrum is compared twice with the database (REMIRA_database_v1), first using the untreated spectra and a second time using the 1st derivatives for spectral correlation calculation. Only if both processes determine the spectrum of the same polymer type, it is labelled as correctly identified for later image analysis, following the approach from (Primpke et al. 2017). The data processing in siMPle was followed by image analysis via particle analysis pipeline (APA, Primpke et al. 2017, Primpke et al. 2019). Here, the determined image containing the x,y coordinates on the filter, the combined hit quality and assigned polymer type are first analysed against a threshold of 600 for each polymer type. See Primpke et al., (2017) for the details of the procedure. The final analysis using APA provided information on numbers, sizes, and polymer composition of particle MP items.

2.4. Chemical analysis of additives in RAS

In development of this project, we began with the scope to focus on plastic softeners, however as the research developed, we focused our analysis on plasticizers, antioxidants, flame retardants, and UV-filters. As such, we refer to this group of chemicals as plastic additives throughout this report. Some of these compounds have multiple uses and are not necessarily only used as plastic additives.

2.4.1. Screening for major additives present in plastics used in RAS systems

Prior to optimising the target GC-MSMS method for the quantification of plastic additives, a thermal desorption-GC-MS (with pyrolysis unit) screening of plastic samples from the RAS systems was conducted. A pyrolysis-GCMS method in full scan mode was set up for screening for plastic additives from mg of plastic collected at two RAS systems. Samples of various plastics were obtained from RAS#1 and RAS#3 and analysed by pyrolysis-GCMS at the end of June/beginning of July 2023. Full scan spectra were interpreted

with help of the NIST library and Frontier's F-search library (through a collaboration with CNRS/University of Laval, Canada) for further tentative identification of additives in sourced plastics.

An instrumental analytical method was set up using pyrolysis-GC-MS (thermal desorption mode, including cryotrap) to evaluate the presence of volatile and semi-volatile compounds in plastic samples. In short, a small amount of plastic samples are placed in pyrolysis sample cup after a rapid rinse with acetone to remove possible surface contamination with adsorbed compounds. During the thermal desorption analysis, the pyro-cup is heated to a temperature of 330 °C. Chemicals present inside the plastic evaporate and a re-focused on a cryotrap prior to injection onto the GC column. A full scan mass spectrometric method was used to evaluate the presence of plastic additives (amenable to GC-MS analysis). Tentative identification of chemicals is then conducted using two libraries of MS spectra, the NIST library and the F-search (the latter is specific to the Frontier pyrolysis-GCMS analysis).

A search for possible correspondence between GC/MS chromatogram features and spectral data in the NIST library was conducted. Search results include retention times (Rt), match factor indicative of the agreement of the mass spectral data from the sample and that from the NIST library, and deconvoluted peak height which represents the intensity of the signal observed. Features with match factor over 50 % and highest peak heights were included. The objective was to identify major potential plastic additives amenable to pyrolysis-GCMS analysis and present in our plastic samples. We then checked whether some of these features/compounds correspond to potential plastic additives.

Samples of plastics sourced as shown in Table 5 were analysed. Between 0.5 to 5 mg of selected plastics were analysed. We also analysed two reference materials that were PE and PVC particles containing plasticizers (phthalates). These can be considered as positive control to ensure we are able to detect and identify these compounds with our screening method. Empty pyrolysis sample cups were also analysed in order to check blank conditions (x5 in total).

Table 5 Overview of plastic samples received from RAS for additive screening.

Sample ID	Sample amount analysed [mg]	Description /including polymer confirmed by FTIR
RAS#1		
nr5	2.07	"Kjetting trekkforsystem" / Polyamide from within the feeding system
nr6	1.69	"Forsekk hvit plast" / Polypropylene fish feed bag
nr7	4.00	"Saltsekk hvit og blå plast" /Polyethylene and polypropylene salt storage bag
nr8	2.36	"karliner" / Polyethylene tank liner
nr9	3.01	"Svart PE rør" / Polyethylene pipe
nr10	4.53	Polypropylene biofilter media
RAS#3		
nr1	3.77	Polyethylene liner
nr2	2.71	Polyurethane retention plates on top of fixed bed
nr3	3.55	Polyethylene pipe to/from water tank
nr4	2.01	Polyethylene fish tank
nr5	4.29	Polyvinyl chloride aeration pipe/fixed bed
nr6	2.18	Polyethylene feeding system pipe using air
nr7	3.70	Polypropylene fixed bed biomedia
nr8	3.64	Polyethylene/polyamide mix CO_2 degasser media
nr9	2.88	Polyvinyl chloride diffuser hose emergency O2
nr11	1.90	Polyethylene feed bag
QA/QC samples		
PVC/phthalates	0.53	Analogue to a reference material
PE/phthalates	1.35	Analogue to a reference material
Empty sample cups x 5	-	

2.4.2. Passive sampling of additives in water of RAS systems

Silicone rubber passive samplers (Figure 5) were used to sample plastic additives (plasticisers and UV filters) in the dissolved form in aqueous matrices. Absorption-based passive samplers such as those using silicone rubber or low-density polyethylene are well suited to the sampling of non-polar non-ionised chemicals such as those of interest in this study (Booij et al., 2016; Pintado-Herrera et al., 2020).

Here, we used SSP silicone rubber (purchased from Shielding Solutions Ltd, UK) cut into 1 m long and 2.5 cm wide strips. One batch of samplers was prepared for the entire study. The first step in the preparation of the samplers was to clean the silicone. First, silicone strips were rinsed in a laboratory dishwasher, prior to Soxhlet extraction with ethyl acetate for 24 h. This step aims to remove contamination, impurities and silicone oligomers. Further Soxhlet extraction was conducted using methanol. Silicone membranes were then removed from the Soxhlet extractor and transferred to a solvent-rinsed 10 L glass jar. Silicone strips (x60) were then soaked further in methanol prior to spiking with performance reference compounds (PRCs). These compounds (deuterated polycyclic aromatic hydrocarbons) are non-naturally occurring chemicals spiked into the samplers prior to exposure. The kinetics of dissipation from the samplers during exposure informs us of the kinetics of uptake of compounds of interest since the exchange of chemicals between the samplers and water is isotropic (Booij and Smedes, 2010). Homogenous loading of PRCs into all silicone strips is conducted using a co-solvent approach where silicones are initially in a small amount of methanol spiked with PRCs. The methanol is subsequently diluted with ultrapure water, thereby decreasing the solubility of the PRCs in solution and transferring them into the silicone (Booij et al., 2002). Once spiking completed, silicone strips were packed into two solvent-rinsed metal tins and placed at -20 °C until fieldwork. A sufficiently large batch of silicone passive samplers was prepared to ensure deployments could be conducted in triplicate at each sampling location.

A simple silicone sampler exposure cell was set up in order to expose the samplers to inlet water (seawater and freshwater) and to water in the recirculating systems. Polyethylene PEX tubing (15 mm diameter) with ball valve connectors were used to supply water to a 40 L stainless steel bucket containing triplicate silicone samplers placed onto spider holders. Buckets were equipped with an overflow and the exposure water went to waste through drainpipes. The water flow into the buckets was chosen to be sufficiently large to avoid any depletion of the water as a result of the uptake of the chemicals into the samplers. Exposure cells were set-up and left to run for 20 min prior to installing silicone samplers in order to flush the system and minimise contamination (Figure 6 and Figure 7). Once silicone samplers installed the exposure system was covered with a layer of aluminium foil to prevent possible dust in the air settling onto the water in bucket.

Manipulation of the silicone samplers during deployment/retrieval operations was conducted using the same type of nitrile gloves. Silicone was collected from the metal tins and rapidly mounted onto the spider holder and place in the buckets already filled with exposure water. This aimed to minimise the amount of time samplers were exposed to the air. Field blanks, with samplers exposed to the air during manipulation, were used to assess possible contamination during exposure operations. Laboratory blanks were used to assess possible contamination from sampler preparation and laboratory extraction and analysis. Field blanks were not used at every sampling point, but their use was representative all sampling locations.

At one specific location, one silicone sampler was mounted onto a spider holder and left exposed to the air in the RAS system. These samplers are as effective to sample air as they are to sample water.

Retrieval operations mimicked deployment procedure involving the use of clean surface with aluminium foil, the use of nitrile gloves. Once the exposure completed, samplers were removed, the surface rinsed, and these were placed individually in separate solvent-rinsed metal tins. Metal tins were then placed in a freezer at -20 °C until sampler extraction in the laboratory.

2.4.3. Passive sampler processing

Back in the laboratory, exposed silicone rubber samplers, field blanks, laboratory blanks, solvent blanks and spike/recovery samples were extracted by soaking in pentane overnight in 250 mL glass jars. Pentane extracts (approx. 200 mL) were then evaporated down to 1-2 mL under a gentle flow of nitrogen. Samples were split into two with one half for the quantification of plastic additives and the remaining portion for PRC determination.

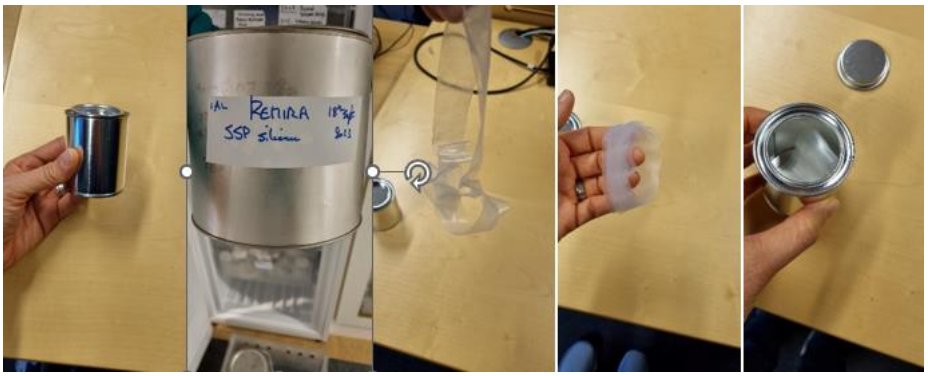


Figure 5 Silicone rubber samplers in metal tins.

Figure 6 Typical installation of silicone passive samplers mounted on spider holders placed in exposure cells made of a stainless-steel bucket and covered with aluminium.

Figure 7 Installation of silicone samplers onto spider holders.

2.4.4. Additive extraction from sludge, fish and selected plastic samples

Sludge samples (with different proportions of water) were first left to settle, and the overlying water decanted in order to obtain more concentrated sludge samples. These samples were subsequently frozen and freeze dried. For each RAS, one sample was prepared, homogenised and triplicate samples were extracted. Soxhlet extraction was conducted using 200 mL dichloromethane to extract 2 g dry weight of sludge placed in glass fibre extraction thimbles (triplicate)

Sludge samples (with different proportions of water) were first left to settle, and the overlying water decanted in order to obtain more concentrated sludge samples. The concentrated sludge was centrifuged to remove as much water as possible before they were subsequently frozen and freeze dried. For each RAS, one sample was prepared, homogenised and triplicate samples were extracted. Liquid extraction with ultrasonic and orbital shaker was used to extract 2 g dry weight of sludge with a mixture of acetonitrile, cyclohexane, and ethyl acetate. Prior to extraction, internal standards were added to the samples. The extraction procedure was performed two times, and the combined extracts were concentrated under nitrogen. Extracts (1-2 mL) were then ready for further clean-up and analysis for plastic additives.

Soxhlet extraction was conducted for 8 hours using 200 mL dichloromethane to extract 0.5-1 g of selected plastic parts from the RAS and placed in glass fibre extraction thimbles (previously cleaned by placing overnight in a muffle furnace at 550 °C). Recovery standards were added to the plastic parts/glass fibre thimble prior to starting the extractions. Upon completion, dichloromethane extracts were transferred to zymark glass tubes for further evaporation of the solvent. Extracts (1-2 mL) were then ready for further clean-up and analysis for plastic additives. Before extraction the fish samples, their lipid content was determined to extract equivalent amounts of samples (on lipid basis). The method used for lipid determination is based on Allan et al. (2013) by cyclohexane and isopropanol solvent extraction using the ultra-sonic bath and orbital shaker. Sample extraction for plastic additives was similar to the extraction of sludge samples, using an equivalent of 160 mg of lipids for each fish sample. During all extractions, laboratory blanks, and spike/ recovery samples were extracted for QA/QC.

2.4.5. Passive sampler, sludge, fish and fish feed extract clean-up and instrumental analysis

2.4.5.1. Extract clean-up

The solvent of the extracts (dichloromethane or pentane) was changed to ethyl acetate: cyclohexane (80:20, v:v) and interfering matrix and sulphur was removed by GPC (gel permeation chromatography). All extracts were clean by GPC prior GC-MS\MS analysis. The fraction representative for the plastic additives was collected and further concentrated.

2.4.5.2. Analysis for performance reference compounds (PRCs) in silicone membranes

Analysis for PRCs (deuterated PAHs) was performed on an Agilent 7890A gas chromatograph coupled to an Agilent 5975c inert XL EI/CI quadrupole mass spectrometer operated in single-ion monitoring mode (SIM) with electron impact ionisation (70 eV). Analyte separation was on a DB-5MS column (30 m, 0.25 mm inside diameter and 0.25 µm film thickness; Agilent JW Scientific) with a 1 µL pulsed split-less injection (pulse pressure 25 psi for 0.5 min and injector temperature of 280 °C). Helium was used as carrier gas with flow set to 1.2 mL min^{-1}. The oven temperature program for the GC consisted of a step at 60 °C (held for 2 min) before an increase to 250 °C (at the rate of 7 °C min^{-1}) and a final increase to 310 °C (at the rate of 15 °C min^{-1}, held for 5 min). Temperatures for the ion source, quadrupole, and transfer line were 230, 150 and 280 °C, respectively. Quantification was performed using the relative response of surrogate internal standards and 7-point calibration curves. Deviation (<20%) of the qualifier ion response relative to that of the quantifier ion was used for identification. Internal standards were naphthalene-d_8, biphenyl-d_{10}, acenaphthylene-d_8, dibenzothiophene-d_8, pyrene-d_{10}, benz[a]anthracene-d_{12}, and perylene-d_{12}.

2.4.5.3. Analysis for plastic additives

Analysis for chemical additives and other personal care products was performed on an Agilent 7890B gas chromatograph coupled to an Agilent 7010B Triple Quad mass spectrometer operated in multiple reaction mode (MRM) with electron impact ionisation (70 eV). Analyte separation was on a HP-5MS UI column (2x 15 m, 0.25 mm inside diameter and 0.25 µm film thickness; Agilent JW Scientific) with a 1 µL pulsed split-less injection (pulse pressure 25 psi for 0.6 min and injector temperature of 280 °C). Helium was used as carrier gas with flow set to 1 mL min^{-1}. The oven temperature program for the GC consisted of a step at 60 °C (held for 1 min) before an increase to 120 °C (at the rate of 40 °C min^{-1}), followed by an increase to 280 °C (at the rate of 5 °C min^{-1}) and a final increase to 300 °C (at the rate of 10 °C min^{-1}, held for 3 min). Temperatures for the ion source, quadrupole, and transfer line were 280, 150 and 280 °C, respectively. Quantification was performed using the relative response of surrogate internal standards and calibration curves. Deviation (<20%) of the qualifier ion response relative to that of the quantifier ion was used for identification (Table 6).

These compounds were quantified by isotopic dilution method. To ensure the validity of the calibration over the hole batch, QC samples were injected approx. after 10 samples. Internal standards were pipetted directly onto the sample once placed in the Soxhlet extractor or in the solvent for the extraction of silicone samplers. These included the deuterium-labelled DEHP (d_4-DEHP). For UV filters, internal standards were d_3-Benzophenone-3, d_{15}-ethylhexyl methoxycinnamate (Z/E), d_3-Bumetrizole and ^{13}C-labelled-Octocrylene spiked at various concentration levels. For the quantification of hexachlorobenzene, the polychlorinated biphenyl congener PCB 30 was added to the extraction vessel. These recovery standard concentration levels were the same for all sample types.

2.4.6. Passive sampler data interpretation

Models and procedures have been developed over the last two decades to translate masses of chemicals accumulated in absorption-based passive samplers (e.g., silicone rubber samplers) into freely dissolved concentrations in water the samplers were exposed to. If polymer-water partition coefficients (K_{sw} or K_{pw}) are available for the compounds of interest and PRCs, it is possible to use a non-linear least square method to gauge sampler-water exchange kinetics and sampling rates for the chemicals under study (Booij and Smedes, 2010). This uses a boundary layer-limited uptake model as described in Rusina et al. (2010). This procedure has been standardised and has regularly been tested in Quasimeme interlaboratory testing schemes (Booij et al., 2017). The compound- and exposure-specific Rs is calculated from:

$$R_s = \beta_{sil} K_{sw}^{-0.08}$$

With K_{sw} the sampler-water partition coefficient and b_{sil} an exposure-dependent parameter obtained from the PRC dissipation data. Freely dissolved concentrations are then calculated through:

$$C_{free} = \frac{m_{acc}}{(K_{sw} m(1 - \exp\left(\frac{-R_s t}{K_{sw} m}\right))}$$

With m_{acc} the mass of chemical accumulated (ng), m the mass of the silicone sampler (g), and t the exposure time (d).

*Table 6 Chemicals determined with target analysis of passive samplers, sludge, and fish samples. The additives added following screening are indicated with *.*

Compounds	logKow	logKsw-AlteSil**	logKsw-SSP**	Final logKsw-SSP***	
2,4-Di-tert-butylphenol (2,4-DTBT)*	4.9			4.7	Antioxidant
Butylated hydroxytoluene (BHT)*	5.3			5.1	Antioxidant
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*	6.6			6.4	Antioxidant
Hexachlorobenzene (HCB)	5.2	5.00	4.96	4.96	POP
Dibutylphthalate (DBP)	4.7	4.64		4.44	Plasticiser
Benzophenone (BP3)	3.6	3.08		2.88	UV filter
2,4-Dihydroxybenzophenone (2,4-DHBP)	3.2	3.20		3.00	UV filter
Ethylhexyl methoxycinnamate (EHMC z)	5.3	5.27	5.01	5.01	UV filter also used in sunscreen / cosmetics
Pentabromotoluene (PBT)	5.7	5.68		5.48	Flame retardant
Tributyl-O-acetylcitrate (ATBC)	3.3	3.3		3.1	Plasticiser also found in cosmetics
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)	5	3.80	3.71	3.71	UV filter used in sunscreen/cosmetics
Ethylhexyl methoxycinnamate (EHMC-E)	5.3	5.89	5.58	5.58	UV filter used in sunscreen/cosmetics
Benzylbutylphthalate (BBP)	4.9	5.2		5	Plasticiser
Triphenyl phosphate (TPhP)*		5		4.8	Flame retardant/ plasticiser
Hexabromobenzene (HBB)	6.1	5.74		5.54	Flame retardant
2-Benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320)	7.3	6.70	6.72	6.72	UV filter
2-(2H-Benzotriazol-2-yl)-4-(tert-butyl)-6-(sec-butyl)phenol (UV-350)	6.3			6.1	UV filter
Bis(2-ethylhexyl) phthalate (DEHP)	7.4	4.61		4.41	Plasticiser
2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (UV-326)	5.6	6.46	6.5	6.5	UV filter
2-(2-Hydroxy-5-tert-octylphenyl)benzotriazole (UV-329)	6.21	6.31	6.3	6.3	UV filter
2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)	7.3	6.87	6.93	6.93	UV filter
2,4-Di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (UV-327)	6.91	7.04	7.11	7.11	UV filter
Octocrylene (OC)	6.1	5.51	5.31	5.31	UV filter also used in cosmetics
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)	7.6	7.6		7.4	UV filter
Diiso-nonyl-phtalate (DINP)	9.6	5.11		4.91	Plasticiser
Diiso-decyl-phtalate (DIDP)	10.6	5.22		5.02	Plasticiser
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*	5.5			5.3	UV filter
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (Plastic additive-11)	13.8	13.8		13.6	Antioxidant

**Silicone-water partition coefficients ($logK_{sw}$) for AlteSil and SSP silicones reported in the literature.
***Final logKsw for SSP silicone used in the interpretation of the passive sampling data acquired here. These combine literature values when available and values predicted from relationships of $logK_{sw}$ for AlteSil and SSP or from $logK_{ow}$ when $logK_{sw}$ values for AlteSil were also not available.

3 Results - Quantification of microplastics in water and fish in RAS

3.1. Occurrence and origin of microplastics in RAS

All three RAS systems were inventoried for plastic materials. Figure 8 provides an overview on the location of water samples and the location of plastics used within the facility. The plastic materials were run through FTIR to confirm their polymer identity and develop a reference library. The most common plastics were polyethylene, followed by polypropylene (Table 7). The detailed overview of the tested plastic materials is presented in the Appendix.

Table 7 Plastic materials inventoried for each RAS facility.

	RAS#1	RAS#2	RAS#3
Feeding system	Polyamide	Polyamide	Polyethylene
Feed bags	Polypropylene	Polyethylene	Polyethylene
Tank liner Pipes (water)	Polyethylene Polyethylene	Polyurethane Polyethylene	Polyethylene Polyethylene
Water treatment unit	n.a.	n.a.	Polyethylene
Biofilter media	Polypropylene	Polyethylene (MBBR) Polypropylene (FBBR)	Polypropylene
Salt storage bags	Polypropylene and polyethylene	Polyethylene	n.a.
Pipes (aeration)	n.a.	n.a.	Polyvinylchloride
Fixed bed retention unit	n.a.	n.a	Polyester /polyethylene terephthalate
Drum filter	n.a.	Polyester and polyethylene	n.a.
Fish transport tube	n.a.	Polyurethane	n.a
Degasser media	n.a.	n.a.	Polyethylene/polyamide

MBBR – mixed bed bioreactor, FBBR – fixed bed bioreactor

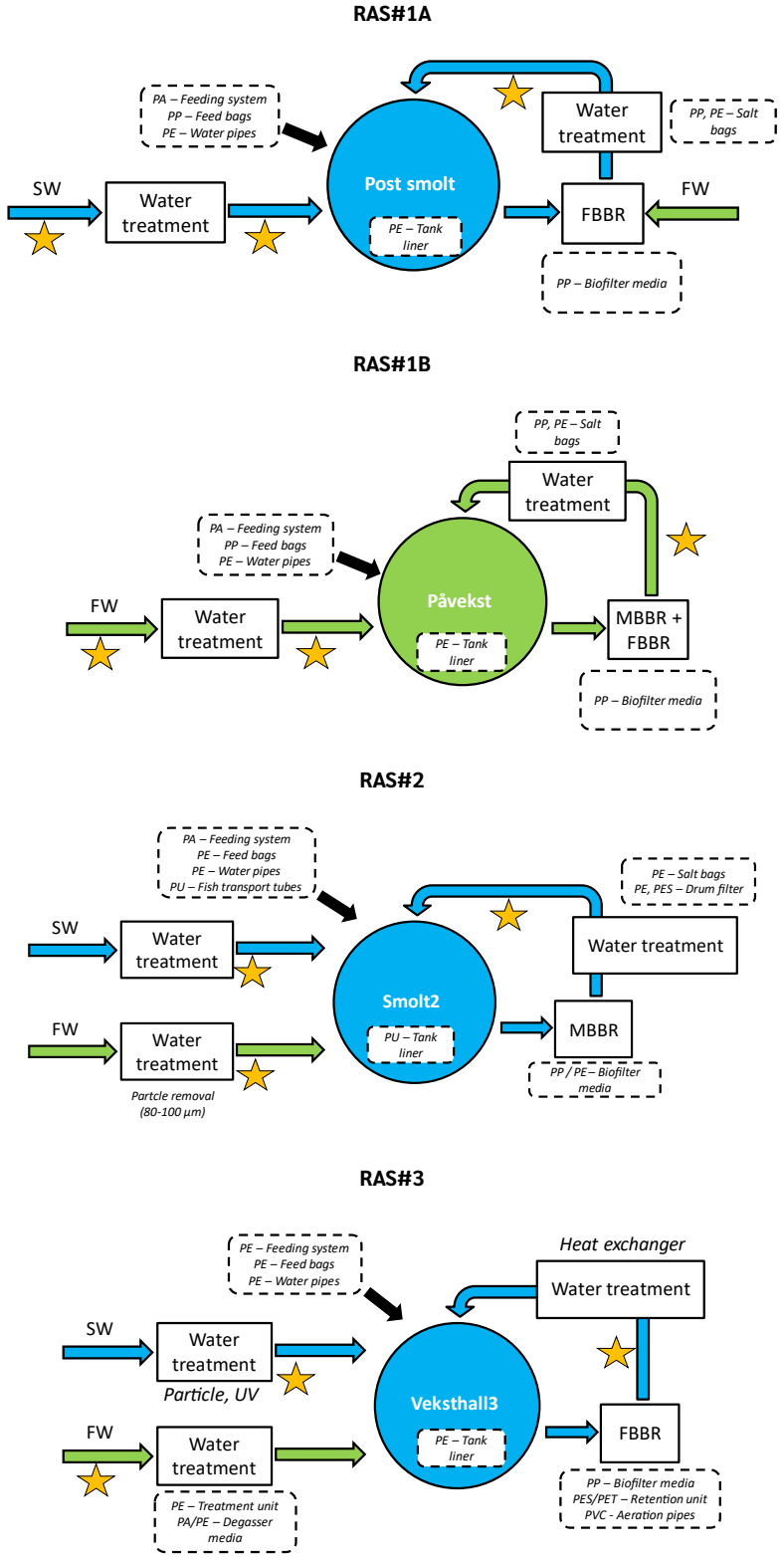


Figure 8 Schematics of RAS systems sampled for the REMIRA project. Stars identify the location of sampling points. Known sources of plastics are in checkered boxes. SW – seawater, FW – freshwater, FBBR – fixed bed bioreactor, MBBR – moving bed bioreactor, PA – polyamide, PE – polyethylene, PP – polypropylene, PU – polyurethane, PES/PET – polyester/polyethylene terephthalate, PVC – polyvinyl chloride.

3.2. Microplastics in water samples

Samples of water were collected from all three RAS, although the proportions of samples varied between the sites. Samples were split for processing and thus data is displayed according to size fraction. All data analysis refers to particles that were confirmed as being plastic polymers.

Summary: No relation with any RAS sources could be established for the large size fraction (>300 µm, Table 8) because of the low number and the random distribution of particles. Particle analysis of the smaller size fraction suggests some microplastic generation from within the RAS. Polyamide and polypropylene particles may have originated from the RAS infrastructure, such as the FBBR, MBBR, and biomedia. Data is limited and there were some inconsistencies between polymers, sample types, replicates, and RAS systems.

3.2.1. Large size fraction (>300 µm)

RAS#1A: All fibres identified within the RAS were excluded because they did not return a positive polymer identification or because they matched the particles identified the procedural controls. Microplastic fragments (n=3) were only observed in the replicates collected after the seawater filter. These were a single black polyethylene fragment (Figure 9A) and two acrylic (paint) fragments (Figure 9B). A single paint fragment was identified in the atmospheric blanks – but not the one corresponding to the seawater samples. Considering the number of replicates was low (n=2) it is not possible to draw conclusions from this data.

RAS#1B: Fibres were only identified in the freshwater samples collected before and after the water treatment. The fibres (n=10) were identified as cellulose acetate (a modified cellulose polymer). The atmospheric blanks performed also contained fibres (polyester, n=3, and cellulose acetate, n=1). Therefore, all fibres were excluded from further analysis because the source was likely atmospheric deposition. No fragments were observed in RAS#1B.

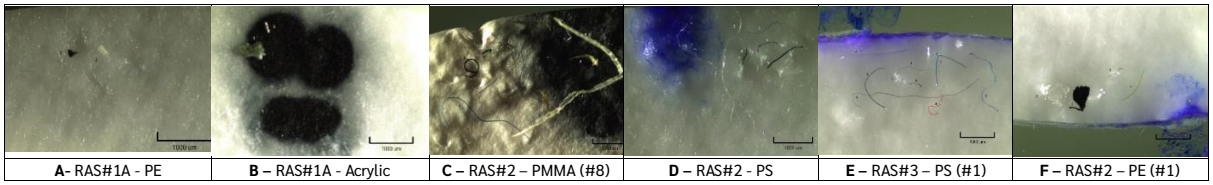
RAS#2: Fibres were identified in all field samples including the atmospheric blanks. No fibres were identified in the laboratory procedural controls. The fibres were identified as cellulose acetate (n=7) and polyester (n=3). All fibres were excluded from further analysis because the source was likely atmospheric deposition. A single fragment was identified as PMMA in a seawater intake sample (Figure 9C). The source of the PMMA remains unknown.

RAS#3: Fibres were identified in all field samples including the atmospheric blanks. No fibres were identified in the laboratory procedural controls. The fibres were identified as polyester (n=11), cellulose acetate (n=8), polyamide (n=1), and polystyrene (n=1). All fibres were excluded from further analysis because the source was like atmospheric deposition. Two polystyrene fragments were identified in a single freshwater (Figure 9D) and saltwater (Figure 9E) inlet water. A single polyethylene fragment was identified in a freshwater inlet sample (Figure 9F).

Table 8 Overview of total plastic fragments identified in water samples (>300 µm). Fibres were excluded from final counts. Data displayed as particles per 100 L. Samples that were not collected are indicated as not applicable, n.a.

Sampling location	SW inlet (before filter)	FW inlet (before filter)	SW (after filter)	FW (after filter)	FBBR	MBBR	Procedural controls
RAS#1A	**0**	n.a.	**0.69**	n.a.	**0**	n.a.	**0**
RAS#1B	n.a.	**0.74**	n.a.	**0.73**	n.a.	**0.**	**0**
RAS#2	n.a.	n.a.	**0.25**	**0**	n.a.	**0**	**0**
RAS#3	n.a.	**0.33**	**0.17**	n.a.	**0**	n.a.	**0**

Given the low numbers of particles found in the large size fraction it is not possible to draw any conclusions as to the source of the particles. There is no indication of particle introduction from the RAS systems.



A- RAS#1A - PE	**B** – RAS#1A - Acrylic	**C** – RAS#2 – PMMA (#8)	**D** – RAS#2 - PS	**E** – RAS#3 – PS (#1)	**F** – RAS#2 – PE (#1)

Figure 9 Examples of particles identified in the water samples >300 µm.

3.2.2. Small size fraction (<300 µm)

All data presented for the small size fraction (300 – 20 µm) were compared to our polymer database (polymers identified within the RAS facilities). Any additional materials are excluded from this analysis. Data is presented in Table 9 as averages of replicates whereas the graphs contain values by replicates (Figure 10-12) Limit of Detection (LOD) was calculated as 3xSD of the blanks collected in the field, separately for each site, by polymer type.

RAS#1A: There appeared to be some level of procedural contamination in the samples since the atmospheric blanks contained PA and PES. This was not found in the laboratory blanks. However, many of the samples had larger particle counts overall with many exceeding the limits of detection in the blanks (Figure 10A). None of the polymers identified provide any clear indication that the microplastics are specifically generated within the RAS. For example, the positive PA signals may be linked to the PA feeding system, two of the replicates were above the LOD. PP was identified in higher numbers after filtration but not after the FBBR (which was identified as having PP biomedia), it is not possible to identify the source. PES/PET was found throughout the samples from RAS#1 but not identified as a source within the RAS system. The elevate PE after the seawater filtration might indicate PE from pipes, however the single replicate limits this interpretation. It is not possible to distinguish whether the source may be linked to the inlet water or other contamination of the system. There were too few positive records of PVC to draw any conclusions.

RAS#1B: There appeared to be some level of procedural contamination in the samples since the atmospheric blanks contained PA and PES. This was not found in the laboratory blanks. However, many of the samples had larger particle counts overall with many exceeding the limits of detection in the blanks (Figure 10B). None of the polymers identified provide any clear indication that the microplastics are specifically generated within the RAS. For example, the positive PA signals may be linked to the PA feeding system but there was only a single replicate from the MBBR above the LOD. Similarly, PP showed elevated numbers after the MBBR, which was identified as having PP biomedia. PES/PET was found throughout the samples from RAS#1 but not identified as a source within the RAS system. It is not possible to distinguish whether the source may be linked to the inlet water or other contamination of the system. There were too few positive records of PVC and PE to draw any conclusions.

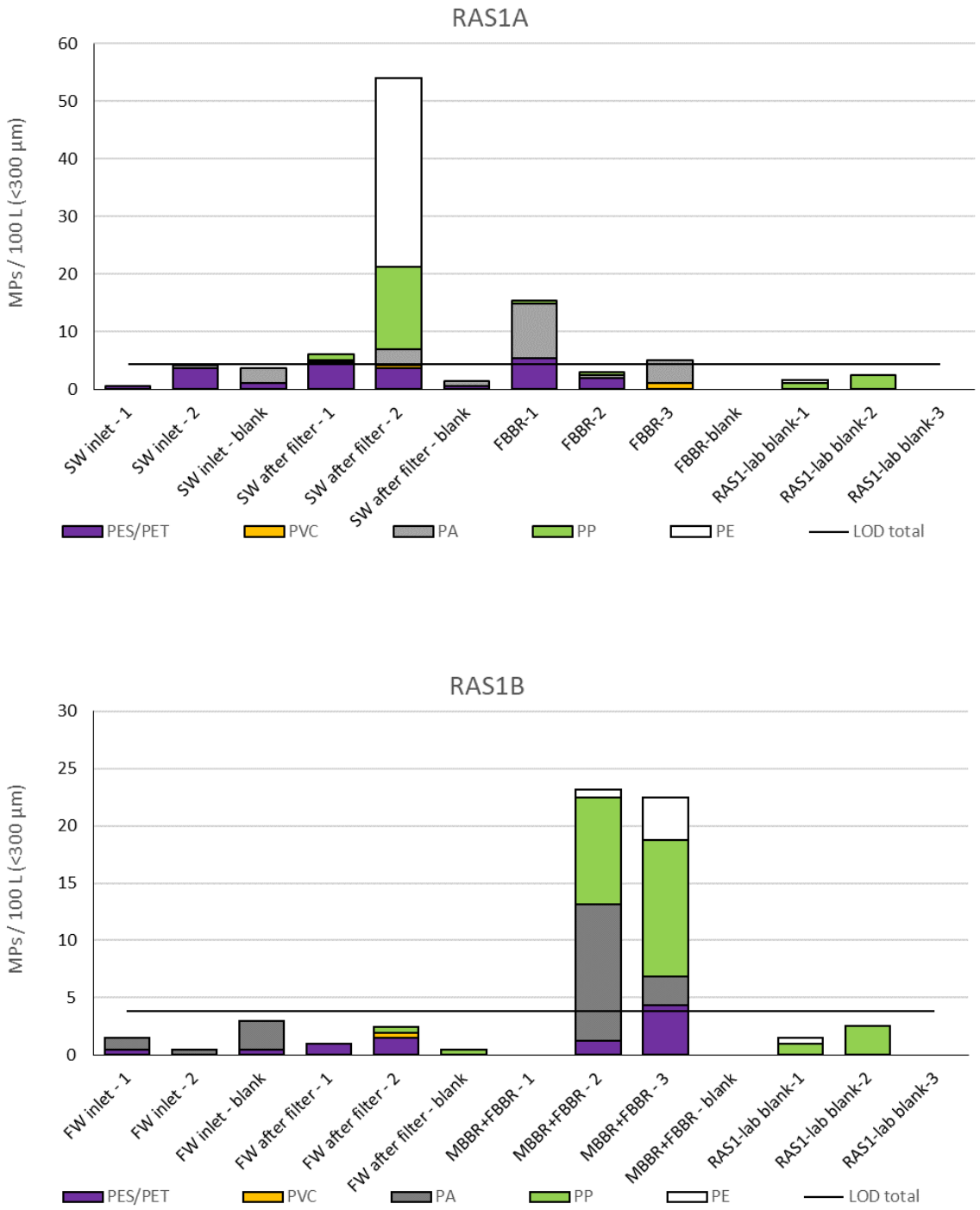


Figure 10. Microplastics (300 - 20 µm) which gave positive results when compared against reference library created from the known plastics at RAS#1. Top panel is all data compiled for RAS#1A(top) and RAS#1B(bottom). Limit of Detection (LOD) compared to the blanks is presented as a line. A detailed breakdown by polymer type is provided in the Appendix.

RAS#2: No procedural contamination (field or lab) was observed in any samples RAS#2. All the samples had larger particle counts exceeding the limits of detection in the blanks (Figure 11). PA showed elevated numbers after the MBBR, the positive PA signals may be linked to the PA feeding system. On the other hand, PP was found throughout the samples from RAS#2 and is used in the biomedia however it is not possible to distinguish whether the source may be linked to the inlet water or other contamination of the system. PE appeared to peak after the freshwater inlet water (following the water treatment) although this was only linked to a single replicate. There were too few positive records of PES/PET and PVC to draw any conclusions.

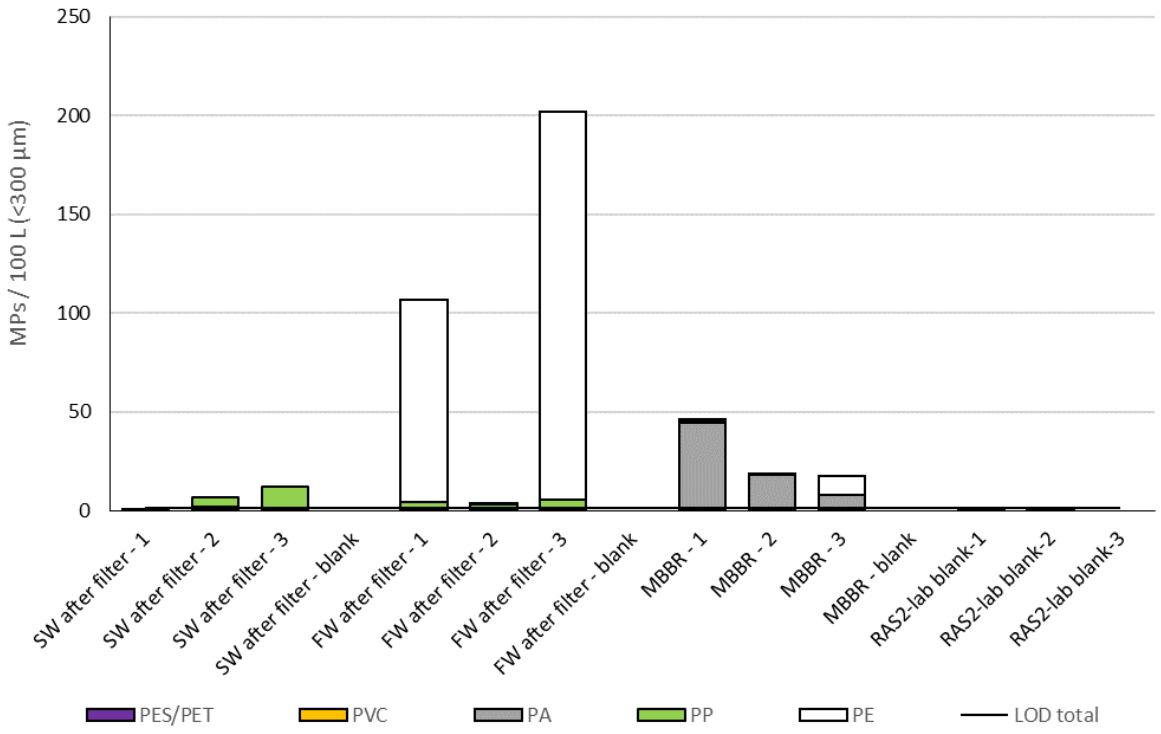


Figure 11 Microplastics (300 - 20 µm) which gave positive results when compared against reference library created from the known plastics at RAS#2. Limit of Detection (LOD) compared to the blanks is presented as a line. A detailed breakdown by polymer type is provided in the Appendix.

RAS#3: No procedural contamination (field or lab) was observed in any samples from RAS#3. All the samples had larger particle counts exceeding the LOD in the blanks (Figure 12). PA showed elevated numbers after the FBBR, the positive PA suggests a source within the RAS, PA was identified as a mixed polymer in the degasser media. PE was identified in both FW and SW inlet however no particles were observed further in the system. Even though it is used in the water treatment unit, tank liner, feed bags and feeding system and water papers pipes, no signal of contamination from these materials could be seen. PVC was only observed in FW inlet water. PES/PET was found consistently in samples including the inlet waters. PES/PET appeared to peak after the freshwater inlet water however it is not possible to link it to a source. The only known source of the PES/PET is the retention unit which the water passes through before the samples were taken. On the other hand, PP was found in low numbers throughout, not possible to distinguish source.

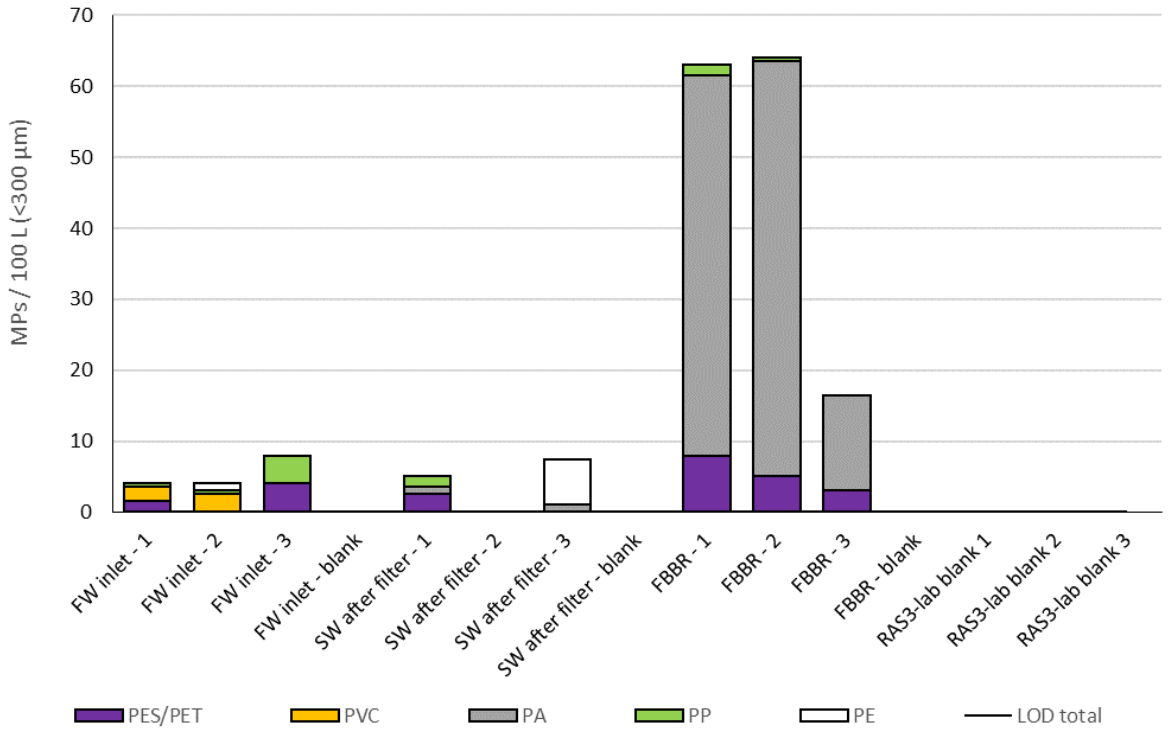


Figure 12. Microplastics (300 - 20 µm) which gave positive results when compared against reference library created from the known plastics at RAS#3. Limit of Detection (LOD) compared to the blanks is presented as a line. A detailed breakdown by polymer type is provided in the Appendix.

Table 9 Overview of total microplastics identified in water samples (300 – 20 µm), data displayed as particles per 100 L (averaged by replicates). Samples that were not collected are indicated as not applicable, n.a.

Sampling location	SW inlet (before filter)	FW inlet (before filter)	SW (after filter)	FW (after filter)	FBBR	MBBR	MBBR+FBBR	Procedural controls
RAS#1A	**2.3**	n.a.	**23**	n.a.	**7.7**	n.a.	n.a.	**1.5**
RAS#1B	n.a.	**0.99**	n.a.	1.7	n.a.	n.a.	15	**1.2**
RAS#2	n.a.	n.a.	**6.5**	**104.3***	n.a.	**28**	n.a.	0
RAS#3	n.a.	**5.3**	**4.2**	n.a.	**48**	n.a.	n.a.	0

**value driven by two replicates each containing >100 PE particles*

3.3. Microplastics in sludge

Samples of water were collected from two RAS facilities. Two tanks were tested at RAS #1, and one tank was tested at RAS#2. Three replicates of sludge were processed for each tank. Samples were split for processing and thus data is displayed according to size fraction. All data analysis refers to particles that were confirmed as being plastic polymers. The % dry weight varied between samples: RAS#1A -12%, RAS#1B - 20% (), RAS#2- 38%. Data is presented by dry weight.

Summary: Fibres dominated the larger size fraction (>300 µm). Particle analysis of the smaller size fraction suggests some microplastic generation from within the RAS although data is limited.

3.3.1. Large size fraction (>300 µm)

Microplastic fibres were kept in the data analysis because there was no evidence of field or procedural contamination. 80 putative plastic particles were isolated from the >300 µm size fraction. Following FTIR analysis, 32 particles were identified as microplastic (40% of original count, Figure 13A). No plastic particles were identified in the field or procedural controls. All confirmed microplastics were fibres (Figure 13B). Cellulose acetate (modified cellulose, often found in clothing) was the most observed polymer (78%) followed by polyester (16%), polypropylene (3%), and polyamide (3%). No plastic fragments were observed in the RAS sludge samples. The fragments identified in samples were organic cellulose or chitinous material, likely fish scales or other biological material. **None of the particles matched plastics inventoried at the RAS facilities in polymer and colour.**

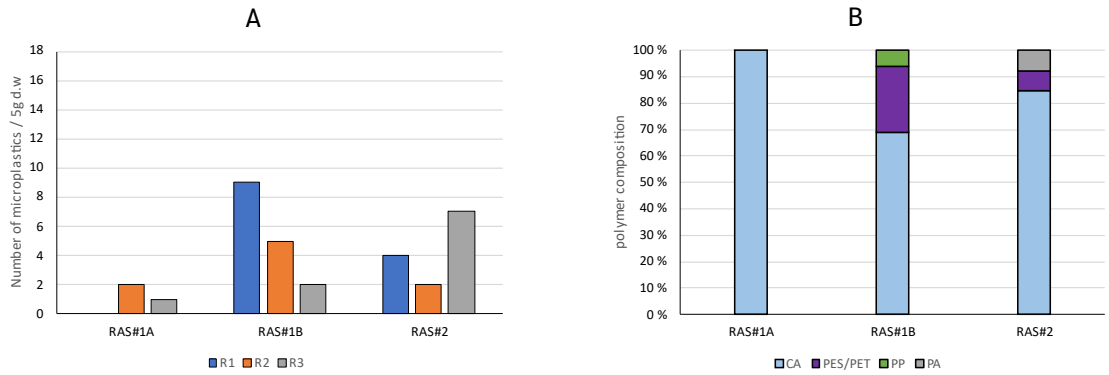


Figure 13 Microplastic fibres in REMIRA sludge samples. Displayed as (A) total counts in replicates; and (B) polymer composition per RAS.

3.3.2. Small size fraction (<300 µm)

Similar to the large size fraction limited information can be drawn from the sludge samples. However, some comparisons can be drawn between the small size fraction analysis for the water samples (Figure 14). PES occurred in low numbers in RAS#1 but there was no known source. PA occurred in individual replicates but not consistent. PA feeding systems may contribute as source for both RAS#1 and RAS#2. PP was isolated to sludge from RAS#1 – The biomedia material may contribute as source. **It was observed that some of the filters may have been overloaded with undigested sample which may contribute to an underestimation of particles.**

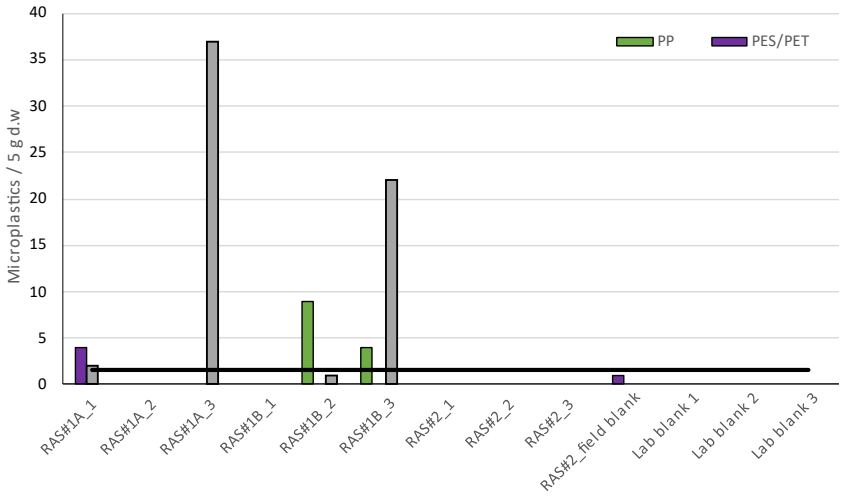


Figure 14 Microplastics in REMIRA sludge samples (<300 µm). Displayed as total counts in replicates according to polymer composition.

3.4. Microplastics in fish feed and supplementary RAS processes

3.4.1. Fish feed: Large size fraction (>300 µm)

Fish feed was received from all three RAS. RAS#1 had two types of feed, RAS#2 – had 1 type of feed, whilst RAS#3 had 4 types of feed. Fish feed was processed in triplicate (Figure 15). Samples were processed for both the large (>300 µm) and small size fractions (<300 µm). It was not possible to scan the smaller fraction due to overloading of the filters.

There was no evidence of procedural contamination, fibres and fragments are therefore included in the results. A total of 33 particles were confirmed as microplastics. Triplicates had between 0 and 3 microplastics (Figure 16). No significant difference between the different fish feeds (KW: χ^2=7.076, p=0.314). Note the power of the test is low due to the low sample size.

In general, CA fibres dominated all samples (n= 20, 61% of all particles). It does not appear that the fibres are a source of contamination since the procedural blanks were free of fibres.

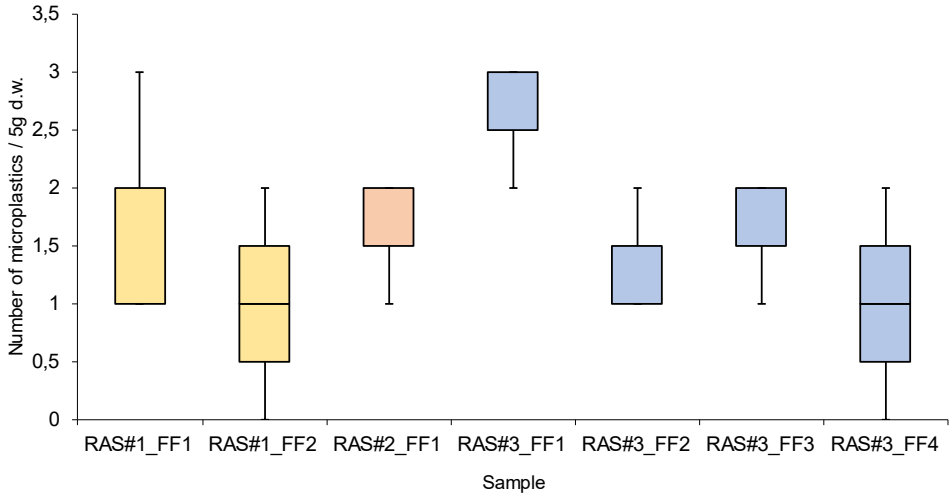


Figure 15 Microplastics in REMIRA fish feed samples (>300 µm). Replicates are presented together.

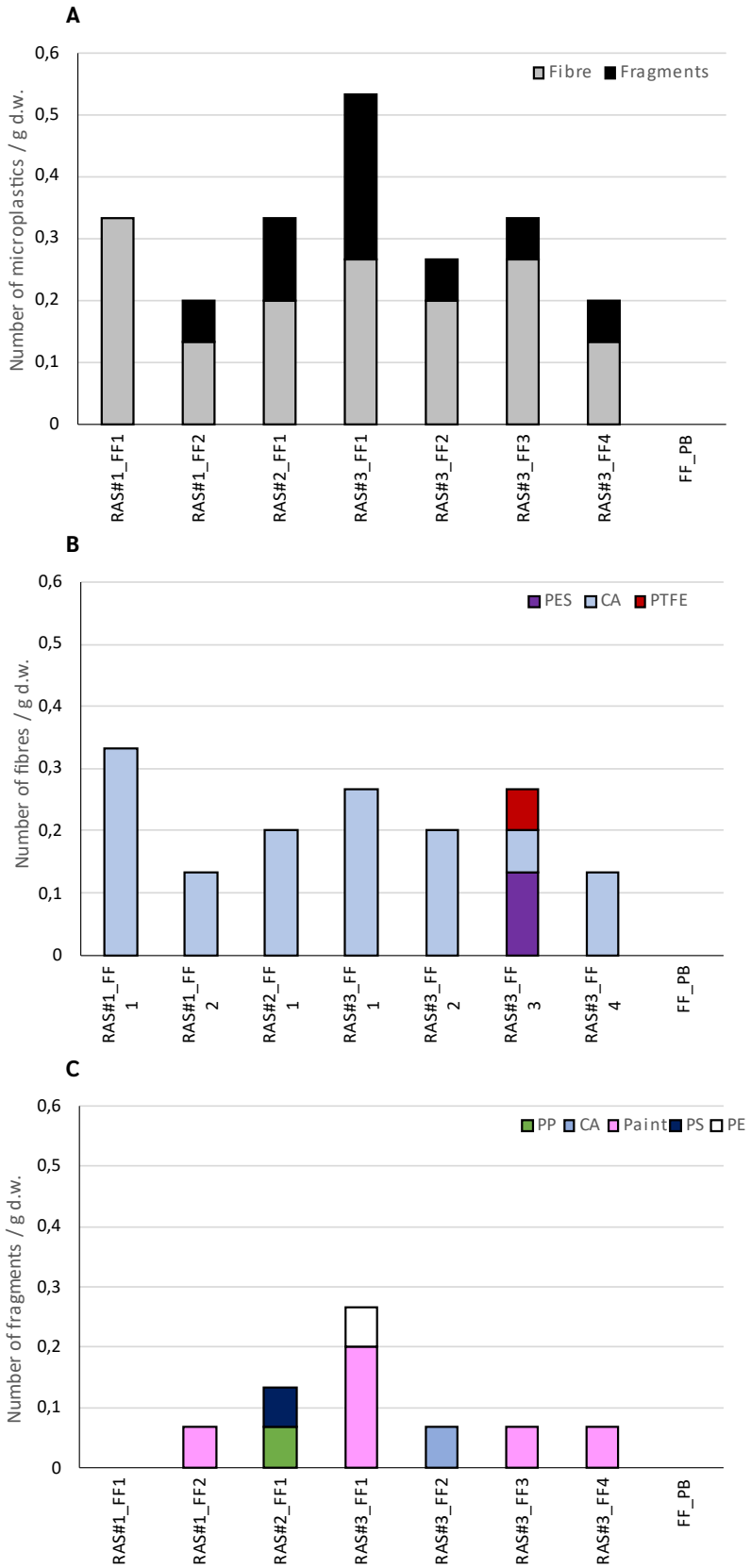


Figure 16 Microplastics in REMIRA fish feed (FF) samples (>300 µm). (A) division by particle morphology, (B) polymer composition of fibres, (C) polymer composition of fragments. Replicates are presented together.

3.4.2. Salt and chalk samples

Samples from RAS#1 were tested for the presence of microplastics. The volumes used were too little to provide any conclusive information on salt and chalk as a source. Given that salt and chalk are mixed and added to RAS in different quantities it was no included in further analysis. Both salt and chalk could be considered further if significant differences in microplastic quantities were observed after the water treatment steps.

3.5. Microplastics in fish

Fibers were excluded from fish results. There are two reasons for this: first, our airborne and procedural blanks showed the presence of several fibres, and second, the occurrence of fibres within the muscle tissue is extremely unlikely. Therefore, a high proportion of the fibres found in both stomach content and muscle were most likely coming from contamination. Consequently, only data on fragments have been presented. Date presented refer to particles >100 µm in size. Procedural error with the <100 µm fraction prevents the use of this data.

3.5.1. Fish stomach (>100 µm)

Among the 9 procedural blanks, only 1 contained 1 fragment, which was not made of plastic. The stomach content samples were therefore not contaminated during laboratory processing.

In total, 10 plastic fragments were found in 7 out of 55 stomachs (frequency of occurrence: 13%). The average number of plastic fragments was 0.18 fragment per fish. Three different plastic polymers and one semi-artificial polymers were found: PP, polyurethane (PUR), PVC and cellulose acetate. Five different colours were observed: red, white, orange, transparent and blue. The global polymer distribution, the fragment distribution per station and the polymer distribution per station are shown in Table 10. Proportionally, RAS#2 fish contained more plastic fragments than the other fish.

It was not possible to link the plastic polymers found in fish stomachs to the known plastics in the facilities. At RAS#1, PUR and PVC were not identified as plastic infrastructure but were found in the fish. At RAS#2 and RAS#3, PP was identified as present in the biomedia but there the colour of the PP fragments observed in the fish did not match the biomedia.

Table 10 Overview of the plastic fragments found in the stomach content samples per station. LD: longest dimension, SD: shortest dimension, PP: polypropylene, PUR: polyurethane, PVC: polyvinylchloride, CA: cellulose acetate. Transp.- transparent.

Sampling location	Number of fish	Total	PP	PUR	PVC	CA	Red	White	Orange	Transp.	Blue	Average LD (µm)	Median LD (µm)	Average SD (µm)
RAS#1A	15	2	0	1	1	0	1	1	0	0	0	265.5	265.5	157.5
RAS#1B	10	0	0	0	0	0	0	0	0	0	0	-	-	-
RAS#2A	10	5	5	0	0	0	1	0	0	1	3	449	430	217.2
RAS#2B	10	2	0	0	1	1	0	1	1	0	0	331.5	331.5	149.5
RAS#3	10	1	1	0	0	0	0	0	0	1	0	399	399	136

3.5.2. Fish fillet (>100 µm)

Among the 9 procedural blanks, only 3 contained fragments, with a total number of 5. Two out of those five fragments were made of plastic, i.e. red polyvinylchloride (PVC) and black polypropylene (PP). The blank showing a red PVC fragment was performed along with RAS#1 samples, where we occasionally found red PVC particles. The red PVC particles were matched to the forceps used to weight the tissues. Therefore, data was blank corrected to exclude the red PVC fragments in the results. The samples from other locations and tanks were found not contaminated.

In total, 7 plastic fragments were found in 4 out of 55 fillets (frequency of occurrence: 7%). The average number of plastic fragments in fillet was 0.13 fragment per sample. Four different plastic polymers were found: PP, polyester, polyethylene (PE) and rubber. Three different colours were observed: red, black, and blue. The global polymer distribution, the fragment distribution per station and the polymer distribution per station are shown in Table 11.

NOTE: Even though 7 fragments were observed, the occurrence of fragments is surprising since particles this size should not have entered the fish fillets. **No conclusions on plastic contamination from within the RAS could be drawn from this data.**

Table 11 Overview of the plastic fragments (MP) found in the fish fillet samples per station. LD: longest dimension, SD: shortest dimension, PE: polyethylene, PP: polypropylene.

Sampling location	Fish (n)	Average tissue weight (g, d.w.)	Total	PP	PES	PE	Rubber	Red	Black	Blue	Average LD (µm)	Median LD (µm)	Average SD (µm)
RAS#1	15	4.5 (2.4-7.6)	3	0	0	1	2	1	2	0	293.3	291.0	265.0
RAS#1B	10	0.4 (0.3-0.6)	0	0	0	0	0	0	0	0	-	-	-
RAS#2A	10	4.6 (3.1-6.1)	0	0	0	0	0	0	0	0	-	-	-
RAS#2B	10	7.4 (5.3-11.6)	3	3	0	0	0	0	0	3	435.7	416.0	127.3
RAS#3	10	1.4 (1.0-2.1)	1	0	1	0	0	1	0	0	NA	NA	NA

4 Results - Quantification of plastic additives and other contaminants in RAS systems

4.1. Screening for potential plastic additives with pyrolysis-GCMS

A total of 16 plastics were sources from RAS#1 and RAS#3. Two samples of PVC and PE loaded with a series of phthalates were used to check whether our procedure could identify the presence of these chemicals in the plastics.

A list of suspect chemicals identified in the different plastic is shown in Table 12. These chemicals are plastic additives, impurities, or chemicals possibly associated with the synthesis of the polymers themselves. Note that these are suspect chemicals, and these identifications are not definite since definitive identification requires the use of a standard of the chemical potentially identified, which was not done here. Compounds are ordered according to retention times and not in terms of importance. It is also possible that a chemical is present in a plastic in a significant amount but not detected or identified through our methodology.

The analysis of phthalate-spiked PE material showed it was possible to identify phthalates in this plastic with our methodology. Match factors were mostly over 90 % and deconvoluted peak heights were highest. Retention times/elution order for the different phthalate plasticisers were according to theory. This retention time information can then be used to check specifically for the presence of these phthalates in our samples. Levels of phthalates in the PE sample range between 0.3 % to 3 % in weight for individual phthalates.

Using this information, Table 13 provides chemical that have been short-listed for inclusion in our target analysis method with GC-MSMS. Their general uses are also given in the table.

Table 12 Chemicals tentatively identified in plastic samples from the RAS facilities. Green cells indicate a potential detection/identification. Tentative retention times (Rt in min) are also given.

Rt (min)	Supected compounds	PE phthalates	PVC phthalates	RAS 1 nr5	RAS 1 nr6	RAS 1 nr7	RAS 1 nr8	RAS 1 nr9	RAS 1 nr10	RAS3 nr1	RAS3 nr2	RAS3 nr3	RAS3 nr4	RAS3 nr5	RAS3 nr6	RAS3 nr7	RAS3 nr8	RAS3 nr9	RAS3 nr11	Empty cups
4.55	Fumaronitrile					■		■												
6.35	4-tert-Octylphenol, TMS derivative								■				■							
7.26	Cyclohexylamine																	■		
7.65	Phenol, 4-ethyl-														■					
8.42	Mesitylene										■									
8.67	D-Limonene							■												
9.63	Isophorone										■									
10.66	Benzothiazole																	■		
10.84	Thymol									■										
10.88	Phenol, 4-propyl-					■				■										
10.84	Phenol, p-tert-butyl-	■									■							■		■
10.87	Caprolactam			■	■						■									
11.37	Phthalic anhydride	■																		
11.40	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester										■									
12.14	Dimethyl phthalate	■																		
12.25	Di-tert-butyl peroxide					■														
12.26	2,4-Di-tert-butylphenol			■	■	■	■	■	■				■			■			■	
12.32	Butylated Hydroxytoluene								■									■		
12.56	Diethyl Phthalate																			■
12.64	Ethylparaben					■														
12.79	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate										■									
12.96	Diethyl Phthalate																			
13.04	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-													■						
13.98	3,5-di-tert-Butyl-4-hydroxybenzaldehyde																	■		
14.12	2-Propanol, 1-chloro-, phosphate (3:1)																			
14.22	3,5-di-tert-Butyl-4-hydroxyacetophenone								■			■								
14.23	Phenol, 3-ethoxy-																■			
14.40	Dimethyl palmitamine										■									
14.76	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione							■	■			■			■					
14.92	Dibutyl phthalate	■	■							■										

Rt (min)	Supected compounds	PE phthalates	PVC phthalates	RAS 1 nr5	RAS 1 nr6	RAS 1 nr7	RAS 1 nr8	RAS 1 nr9	RAS 1 nr10	RAS3 nr1	RAS3 nr2	RAS3 nr3	RAS3 nr4	RAS3 nr5	RAS3 nr6	RAS3 nr7	RAS3 nr8	RAS3 nr9	RAS3 nr11	Empty cups
15.33	Methyl stearate											■								
15.43	Oleic Acid					■										■	■	■		■
15.45	4-Nonylphenol																■			
15.46	Phenol, 4-dodecyl-												■							■
15.58	Butyl 2-butoxyacetate												■							
15.60	Hexadecanoic acid, butyl ester		■							■		■	■		■	■			■	■
15.66	Bis(2-ethylhexyl) maleate										■									
15.81	Diamyl phthalate		■																	
16.48	Octadecanoic acid, butyl ester		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
16.66	1,2-Benzenedicarboxylic acid, dihexyl ester		■																	
16.92	Benzyl butyl phthalate	■	■							■										
16.94	1,8-Diazacyclotetradecane-2,9-dione			■	■															
16.95	1-Decanol, 2-hexyl-																		■	■
17.29	Triphenyl phosphate								■											
17.29	Di-n-octyl phthalate			■		■		■					■	■			■			
17.29	Dicyclohexyl phthalate																		■	■
17.29	Phthalic acid, di(2-propylpentyl) ester									■					■					
17.58	Bis(2-ethylhexyl) phthalate	■	■																	
17.57	Diethylene glycol dibenzoate										■									
18.63	Di-n-octyl phthalate	■																		■
18.84	Di-isononyl phthlate	■																		
18.94	Di-isononyl phthlate	■																		
19.04	Di-isononyl phthlate	■																		
19.15	Di-isononyl phthlate	■																		
19.18	5-.beta.-cholestan-3.alpha.-ol, propionate											■								
19.22	Di-isononyl phthlate	■																		
19.33	Di-isononyl phthlate	■	■																	
19.42	Di-isononyl phthlate	■																		
19.53	Di-isononyl phthlate	■	■																	
19.62	4,4'-Ethylenebis(2,6-di-tert-butylphenol)																	■		
19.61	Di-isononyl phthlate	■																		
19.81	Di-isononyl phthlate	■																		
20.02	Diisodecyl phthalate	■																		
21.76	Decanedioic acid, bis(2,2,6,6-tetramethyl-4-piperidinyl) ester				■	■	■		■											
23.74	4-tert-Octylphenol, TMS derivative			■											■	■			■	
26.05	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)				■	■	■			■						■			■	

Table 13 Summary list of chemicals identified through pyrolysis-GCMS screening of plastics from RAS facilities.

Chemicals	Uses
2,4-Di-tert-butylphenol (2,4-DTBT)	UV filter/antioxidant in fuel, in PEX tubing
Butylated Hydroxytoluene	Synthetic antioxidant uses in personal care products and plastics/rubber
Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	Antioxidant and stabiliser in plastics such as PVC or polyurethanes
3,5-Di-tert-butyl-4-hydroxyacetophenone	Possible degradation product of antioxidant
Triphenyl phosphate (TPhP)	Flame retardant and plasticiser
Decanedioic acid, bis(2,2,6,6-tetramethyl-4-piperidinyl) ester	UV stabiliser in plastics, adhesive sealants coatings or rubber
Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Antioxidant or stabiliser in polymers

4.2. Passive sampling of plastic additives and other contaminants in water of RAS systems

4.2.1. Sampling rates

Sampling rates, R_s, expressed in equivalent volumes of water extracted by a sampler per unit of time, were estimated from the dissipation of performance reference compounds (PRCs) for each sampler deployed at each location (Table 14-16). Mean R_s values are in the range of 3.6 to 14.4 L d^{-1} depending on the exposure location. Relative standard deviations for triplicate measurements range from 1.4 to 37 %. Estimated Rs values are in the range of those expected for standard deployments silicone rubber passive samplers. Some variability between replicate silicone samplers can be expected since these were exposed to differences in turbulence level in the exposure buckets. Sampling rates obtained for intake waters at RAS#3 are generally lower than at other locations. This may be the result of the low water temperature during the winter (rather than autumn) exposure. The particularly low R_s values for intake seawater may have also been the result of low turbulence levels in the bucket.

Table 14 Mean sampling rates (L d^{-1}) estimated for triplicate silicone passive sampler exposed at each sampling location at RAS#1.

Sampling site	Sampling R_s (L d^{-1})*
Freshwater intake, before filtration	14.4 (37)
Freshwater intake, after filtration	14.3 (6.3)
Seawater intake, before filtration	3.6 (1.4)
Seawater intake, after filtration	8.7 (33)
RAS system, Påvekst	8.7 (15)
RAS system, Post smolt	10.6 (4.3)

*Mean of triplicate passive sampling measurements with % relative standard deviation (%RSD) in brackets; R_s estimated for $\log K_{sw}$ = 5

Table 15 Mean sampling rates (L d^{-1}) estimated for triplicate silicone passive sampler exposed at each sampling location at RAS#2.

Sampling site	Sampling R_s (L d^{-1})*
Freshwater intake	7.0 (26)
Seawater intake	7.9 (22)
RAS system, smolt 2	6.4 (22)

*Mean of triplicate passive sampling measurements with % relative standard deviation (%RSD) in brackets; R_s estimated for $\log K_{sw}$ = 5

Table 16 Mean sampling rates (L d^{-1}) estimated for triplicate silicone passive sampler exposed at each sampling location at RAS#3.

Sampling site	Sampling R_s (L d^{-1})*
Freshwater intake	2.3 (13)
Seawater intake	1.3 (13)
RAS system	8.0 (20)

*Mean of triplicate passive sampling measurements with % relative standard deviation (%RSD) in brackets; R_s estimated for $logK_{sw}$ = 5

4.2.1.1. Freely dissolved concentrations of plastic additives in waters of RAS systems

Freely dissolved concentrations were calculated from masses accumulated in the samplers and sampler-water exchange kinetics (Table 17-19). Concentrations were estimated for each sampler at each sampling location and means and standard deviations were calculated from the triplicate samplers deployed at each sampling point. When compounds were below limits of quantifications, these were used to calculate limits of quantification on a concentration in water basis. In most cases, data were consistent, i.e., when a compound was detected a t a site, it was detected in quantified in all replicates. On a few occasions only, a chemical was detected in 2 out of three replicate samplers. The limit of quantification was used in that case to calculate mean and standard deviations. In rare occasions, a chemical was found in one out of three samplers. In these cases, the data is reported as detected but without any standard deviations. Relative standard deviations for freely dissolved concentrations in most cases are equivalent or below the standard deviations obtained for R_s values.

Table 17 Mean of triplicate freely dissolved concentrations (ng L^{-1}) measure in intake freshwater, intake seawater and RAS system water (% relative standard deviation in brackets) at RAS#1. Full compound names are presented in Table 6.

Compound	Freshwater intake	Freshwater after filtration	Seawater intake	Seawater after filtration	RAS#1, Påvekst	RAS#1, Post smolt
2,4-DTBT	<1.8	<1.8	<4.0	<2.3	<2.3	<2.0
BHT	<0.066	<0.063	<0.19	<0.096	**5.1 (12%)**	**9.9 (5.1%)**
DTBSBP	**0.0038 (22%)**	**0.00023**	<0.00081	**0.0037 (12%)**	**0.0030 (19%)**	**0.011 (10%)**
HCB	**0.010 (11%)**	**0.0065 (3%)**	**0.013 (9%)**	**0.0047 (23%)**	**0.013 (7%)**	**0.0072 (7%)**
DBP	0.069	**0.070**	**0.14 (21%)**	**0.078**	**0.27 (24%)**	**0.076**
BP3	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
2,4-DHBP	<21	<21	<21	<20	<22	<21
EHMC z	<0.001	**0.0011**	<0.0028	<0.0014	<0.0013	<0.0012
PBT	<0.0002	<0.0002	<0.0007	<0.0003	<0.0003	<0.0003
ATBC	**4.8**	**34 (44%)**	<2.9	<2.9	**3.5 (11)**	**15 (59%)**
ODPABA	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
EHMC-E	<0.0004	**0.00084(18%)**	<0.0014	**0.00072(27%)**	**0.0010 (18%)**	<0.0005
BBP	**0.080 (10%)**	**0.013 (15%)**	<0.009	<0.005	**0.017 (17%)**	**0.0069 (8%)**
TPhP	**0.13 (8%)**	**0.041 (7%)**	**0.030 (17%)**	**0.013 (9%)**	**0.66 (12%)**	**0.20 (15%)**
HBB	<0.0002	<0.0002	<0.0007	<0.0003	<0.0003	<0.0003
UV-320	<0.0017	<0.0015	<0.006	<0.0027	<0.0026	<0.0021
UV-350	<0.003	<0.003	<0.010	<0.005	<0.005	<0.004
DEHP	<0.16	**0.20**	<0.25	**0.37**	**2.1 (18)**	<0.17
UV-326	<0.007	<0.007	<0.025	<0.011	**0.066 (14%)**	**0.076 (9%)**
UV-329	<0.017	<0.017	<0.064	<0.029	<0.027	<0.022
UV-328	<0.002	<0.002	<0.008	<0.004	<0.003	<0.003
UV-327	<0.0015	<0.0014	<0.0056	<0.0025	**0.0072 (16%)**	**0.048 (6%)**
OC	**0.016 (32%)**	**0.033 (81%)**	**0.036 (11%)**	**0.015**	**0.017 (19%)**	**0.024 (45%)**
HOBP	<0.13	<0.12	<0.49	<0.22	<0.21	<0.17
DINP	<1.5	<1.4	<3.9	<2.0	<2.0	<1.7
DIDP	<1.4	<1.3	<3.9	<1.9	<1.9	<1.6
BTMPS	<0.11	<0.10	<0.35	<0.17	<0.16	<0.13
Plastic additive 11	**5.5 (38%)**	**8.1 (13%)**	<4.4	<2.0	**22 (30%)**	**18 (12%)**

Hexachlorobenzene (HCB), a persistent organic pollutant listed on the Stockholm Convention is ubiquitously distributed in the aquatic environment but not related to plastics. It is therefore not surprising to find it in the intake of fresh- and seawater at the RAS facilities. As expected, HCB was consistently detected in all waters. Concentrations drop by half upon filtration of both intake waters at RAS#1. Concentrations in the two RAS systems increased slightly compared with levels in intake waters after filtration. At RAS#2, the concentration was slightly higher in RAS water (25 pg L^{-1}) than in intake freshwater (20 pg L^{-1}) and much higher than those found in intake seawater (7 pg L^{-1}).

Levels of circulating ***butylated hydroxytoluene (BHT)*** in RAS systems at RAS#1 are similar to those measured at RAS#2 (5-11 ng L^{-1}). BHT was found in RAS#2 circulating water but below limits of quantification in intake waters.

Benzophenone (BP3) was found in RAS#2 circulating water (concentrations ranging from 8 pg L^{-1} to 40 ng L^{-1}) but were below limits of quantification in intake waters.

4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP) was not detected in intake seawater it was present after filtration of RAS#1. Detectable levels are found in intake freshwater but not after filtration in RAS#1. Freely dissolved concentrations tend to increase in the RAS systems when compared with intake waters. Conversely, DTBSBP was found in RAS#2 circulating water, but concentrations were below limits of quantification in intake waters.

Ethylhexyl methoxycinnamate (EHMC-E) was consistently measured after filtration of both intake waters, but only found in water of RAS#1A system at the pg L^{-1} level.

Benzylbutylphthalate (BBP) was only observed above limits of quantification in freshwater samples. Concentrations drop from 80 to 12 pg L^{-1} upon filtration. Concentrations of 7 and 17 pg L^{-1} can be observed in RAS waters.

Dibutylphthalate (DBP) was sparsely detected at the various sampling location but consistently measured in RAS waters of RAS#1A (Påvekst). Dibutyl phthalate was found in RAS#2 circulating water but concentrations were below limits of quantification in intake waters.

Triphenylphosphate (TPhP) a compound added to our list of chemicals after the first pyrolysis GC-MS screening step, is consistently found throughout the waters at RAS#1, as for waters of RAS#2 facility. Filtration of freshwater and seawater consistently decreases concentrations by a factor of 2-3. Concentrations of 20 and 60 pg L^{-1} are found in RAS#1 water. These are higher than in intake water after filtration, indicating possible sources in the RAS system. At RAS#2, TPhP was found in intake sea and freshwater at a concentration of 0.1 ng L-1 but was below limits of quantification in RAS water. A decrease in concentration could indicate minimal sources of TPhP in the RAS system and a sorption of TPhP to surfaces, feed/faeces, removal with sludge or degradation.

Octocrylene (OC) was consistently detected in intake freshwater with concentrations that tend to be as high after filtration. OC was consistently found in RAS waters at concentrations similar to those in intake waters.

Plastic additive 11 was consistently found in intake freshwater with no effect of filtration. It is found in all RAS waters at higher concentrations than in intake freshwater. Since no measured values of K_{sw} exist for silicone for this compound, we relied on some modelling based on $\log K_{ow}$ to infer a $\log K_{sw}$ value. Considering the unusually high $\log K_{ow}$ value, estimated concentrations are likely overestimating actual concentrations.

Tributyl-o-acetylcitrate (ATBC) was found above limits of quantification in one sample in intake freshwaters. The concentration was higher after filtration was after filtration of freshwater. It was found in RAS waters at concentration at the ng L^{-1} level. It was also found in RAS#2 circulating water but concentrations were below limits of quantification in intake waters.

Bis(2-ethylhexyl) phthalate (DEHP) was consistently measured at approx. 2 ng L^{-1} level in RAS waters of RAS#1A (Påvekst). The UV filters UV-326 and UV-327 were consistently measured at concentrations in the range of 50-80 pg L^{-1} in both RAS systems, but not in intake waters.

The UV filter **UV-326** is found in intake freshwater and seawater and at clearly higher concentration in RAS#2 re-circulating water (1.6 ng L^{-1}). This indicates a clear source of this compound within the RAS facility. Most other compounds are below limits of quantification, except for a few detections (but not in all replicate samplers).

Where concentrations were below limit of detection in intake waters, this tends to indicate the source of these compounds are in the RAS system, i.e. feed, plastics, air.

The proportion of chemicals detected in waters of RAS#3 is lower than at the other RAS but that may be attributed to the lower sampling rates observed for intake waters. Lower sampling rates in turn result in higher limits of quantification since the overall amount of water sampled is lower. A few compounds are consistently detected in the water systems. These include **HCB, BHT, EHMC, ATBC** and **triphenyl phosphate (TPhP).** The phthalates **DEHP** and **BBP** are consistently found in intake seawater.

Silicone samplers was exposed to the air in the RAS system at the RAS#2 and RAS#3 (Table 20). These samplers are as efficient to sample air as they are to sample water. Compounds detected in the air are reported in bold in the table below. The presence of **BHT, DTBSBP, HCB, DBP, BP3** and **UV 326** is in line with their presence in recirculating waters of the RAS system. A few other compounds are detected in the air including **ATBC, TPhP** and **DEHP**. This is generally in line with the signal observed in water. However, it is difficult at present to determine whether water and/or are sources or sink of these compounds.

Table 18 Mean of triplicate freely dissolved concentrations (ng L^{-1}) measure in intake freshwater, intake seawater and RAS system water (% RSD in brackets) at RAS#2.

Compound	Freshwater intake	Seawater intake	RAS#2, smolt 2
2,4-Di-tert-butylphenol (2,4-DTBT)*	<20	<18	<21
Butylated hydroxytoluene (BHT)*	<1.8	<1.6	**11 (20)**
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*	<0.0028	<0.0024	**0.0081 (30)**
Hexachlorobenzene (HCB)	**0.021 (16)**	**0.0072 (17)**	**0.025 (25)**
Dibutylphtalate (DBP)	<0.31	<0.28	**0.47 (22)**
Benzophenone (BP3)	<0.72	<0.76	**12 (4)**
2,4-Dihydroxybenzophenone (2,4-DHBP)	<21	<22	<20
Ethylhexyl methoxycinnamate (EHMC z)	<0.0091	<0.0081	<0.01
Pentabromotoluene (PBT)	<0.0012	<0.0010	<0.0013
Tributyl-O-acetylcitrate (ATBC)	<22	<23	**39 (10)**
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)	<0.003	**0.003**	<0.0029
Ethylhexyl methoxycinnamate (EHMC-E)	<0.006	**0.0056**	<0.0065
Benzylbutylphtalate (BBP)	**0.0066 (34)**	**0.0060**	**0.0099**
Triphenyl phosphate (TPhP)*	**0.10 (14)**	**0.11 (47)**	<0.061
Hexabromobenzene (HBB)	<0.0012	<0.0011	<0.0013
2-Benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320)	<0.0088	<0.0077	<0.0095
2-(2H-Benzotriazol-2-yl)-4-(tert-butyl)-6-(sec-butyl)phenol (UV-350)	<0.020	<0.017	<0.021
Bis(2-ethylhexyl) phthalate (DEHP)	<0.54	**0.55**	<0.57
2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (UV-326)	**0.31 (80)**	**0.29 (110)**	**1.5 (18)**
2-(2-Hydroxy-5-tert-octylphenyl)benzotriazole (UV-329)	<0.081	<0.071	<0.088
2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)	<0.009	<0.008	<0.0099
2,4-Di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (UV-327)	<0.006	<0.006	<0.0068
Octocrylene (OC)	<0.20	<0.18	<0.21
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)	<1.7	<1.5	<1.8
Diiso-nonyl-phtalate (DINP)	<6.2	<5.6	<6.7
Diiso-decyl-phtalate (DIDP)	<6.2	<5.6	<6.8
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*	<0.58	<0.51	<0.63
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Plastic additive-11)	<24	<21	<26

Table 19 Mean of triplicate freely dissolved concentrations (ng L^{-1}) measure in intake freshwater, intake seawater and RAS system water (% RSD in brackets) at RAS#3.

Compound	Freshwater intake	Seawater intake	RAS#3, smolt 2
2,4-Di-tert-butylphenol (2,4-DTBT)*	<257	<443	<84
Butylated hydroxytoluene (BHT)*	<0.9	**2.1 (9)**	**17 (27)**
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*	<0.06	<0.11	**0.020 (22)**
Hexachlorobenzene (HCB)	**0.042 (22)**	**0.019 (11)**	**0.011 (18)**
Dibutylphtalate (DBP)	<0.4	<0.6	<0.12
Benzophenone (BP3)	<0.7	<0.7	**6.4 (25)**
2,4-Dihydroxybenzophenone (2,4-DHBP)	<29	<30	<29
Ethylhexyl methoxycinnamate (EHMC z)	**0.013**	**0.023 (19)**	<0.004
Pentabromotoluene (PBT)	<0.004	<0.006	<0.001
Tributyl-O-acetylcitrate (ATBC)	**2.7 (102)**	**8.8 (110)**	**12.7 (24)**
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)	<0.004	<0.006	<0.003
Ethylhexyl methoxycinnamate (EHMC-E)	**0.034 (120)**	**0.022 (13)**	**0.0038 (22)**
Benzylbutylphtalate (BBP)	<0.005	**0.048 (42)**	<0.003
Triphenyl phosphate (TPhP)*	**0.38 (26)**	**0.63 (15)**	**0.29 (20)**
Hexabromobenzene (HBB)	<0.003	<0.006	<0.001
2-Benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320)	0.008149015	0.014352297	0.002365312
2-(2H-Benzotriazol-2-yl)-4-(tert-butyl)-6-(sec-butyl)phenol (UV-350)	0.007278387	0.012812192	0.002118917
Bis(2-ethylhexyl) phthalate (DEHP)	<0.08	**0.57 (44)**	<0.05
2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (UV-326)	<0.2	<0.3	**0.055**

2-(2-Hydroxy-5-tert-octylphenyl)benzotriazole (UV-329)	<0.1	<0.2	<0.03
2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)	0.008469278	0.014917222	**0.0033**
2,4-Di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (UV-327)	<0.009	<0.016	**0.0025**
Octocrylene (OC)	NA	NA	NA
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)	<3	<4	<0.7
Diiso-nonyl-phtalate (DINP)	<17	<30	<6
Diiso-decyl-phtalate (DIDP)	<17	<30	<6
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*	<1.6	<2.8	<0.5
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Plastic additive-11)	<1040	<1820	<300

Table 20 Masses of chemical absorbed in the silicone sampler exposed to the air at RAS#2 and RAS#3 (ng sampler^{-1}).

Compound	RAS#2	RAS#3	RAS#3 intake water area
2,4-Di-tert-butylphenol (2,4-DTBT)*	<1700	<8700	<8700
Butylated hydroxytoluene (BHT)*	**2120**	**969**	**164**
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*	**13**	**40**	**16**
Hexachlorobenzene (HCB)	**6.6**	**2.8**	**3.1**
Dibutylphtalate (DBP)	**74**	<11	**91**
Benzophenone (BP3)	**29**	**5.4**	<3.5
2,4-Dihydroxybenzophenone (2,4-DHBP)	<150	<200	<200
Ethylhexyl methoxycinnamate (EHMC z)	<0.8	**0.5**	**1**
Pentabromotoluene (PBT)	<0.1	**0.2**	**0.7**
Tributyl-O-acetylcitrate (ATBC)	<196	**116**	**92**
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)	<0.1	<0.1	<0.1
Ethylhexyl methoxycinnamate (EHMC-E)	**0.77**	**0.5**	**0.9**
Benzylbutylphtalate (BBP)	**0.73**	**3.1**	**7.8**
Triphenyl phosphate (TPhP)*	<5	**21**	**72**
Hexabromobenzene (HBB)	<0.1	<0.1	**0.3**
2-Benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320)	<0.6	<0.2	<0.2
2-(2H-Benzotriazol-2-yl)-4-(tert-butyl)-6-(sec-butyl)phenol (UV-350)	**2.6**	<0.2	<0.2
Bis(2-ethylhexyl) phthalate (DEHP)	**51.4**	**48**	**201**
2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (UV-326)	**147**	**20**	**32**
2-(2-Hydroxy-5-tert-octylphenyl)benzotriazole (UV-329)	**15**	<2.6	<2.6
2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)	<0.6	0.5	**0.8**
2,4-Di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (UV-327)	<0.4	<0.2	<0.2
Octocrylene (OC)	<17	n.A.	n.A.
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)	<100	<50	<50
Diiso-nonyl-phtalate (DINP)	<550	<550	<550
Diiso-decyl-phtalate (DIDP)	<550	<550	<550
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*	<50	<50	<50
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Plastic additive-11)	<1300	<20000	<20000

4.3. Plastic additives and other contaminants in sludge samples

Some compounds were detected in sludge samples (Table 21). However, in general, less compounds tend to be detected in the sludge than in RAS water with passive samplers. **Hexachlorobenzene (HCB)** was found above limits of quantification in sludge from RAS#1A only. A sludge-water distribution coefficient $\log K_{sludge\text{-}water}$ of 4.20 is in line with the $\log K_{ow}$ for this chemical.

A few compounds are consistently found in sludge samples irrespective of location. Concentrations of **Butylated hydroxytoluene (BHT),** detected in all sludge samples, are in the hundreds of ng g^{-1} with calculated $\log K_{sludge\text{-}water}$ values close to 5.0. **Triphenyl phosphate (TPhP)** was also found in most sludge samples at the low ng g^{-1} level. Plastic additive 11 was also consistently found in sludge samples at the highest concentrations (at the mg g^{-1} level).

Some compounds such as **tributyl-o-acetylcitrate (ATBC), benzylbutylphthalate (BBP), UV-326, UV-328, 2,4-Di-tert-butylphenol (2,4-DTBT), and 4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)** were sparsely detected in sludge samples from both facilities. Some compounds such as octocrylene or some phthalates consistently detected in RAS waters were not found above limits of detection in sludges.

UV-327 was only found above limits of quantification in samples of sludge from RAS#1. This is in line with this chemical consistently measured in recirculating RAS water of RAS#1 but not RAS#2.

Table 21 Concentration in sludge at RAS#1 and RAS#2 facilities (ng g^{-1}). Relative standard deviations in brackets (%) based on triplicate measurements.

Compound	RAS#2	RAS#1A	RAS#1B
2,4-Di-tert-butylphenol (2,4-DTBT)*	<224	**362 (18)**	<224
Butylated hydroxytoluene (BHT)*	**1213 (30)**	**624 (4)**	**345 (9)**
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*	<0.1	**0.2 (12)**	<0.1
Hexachlorobenzene (HCB)	<0.4	<0.4	**0.56 (12)**
Dibutylphtalate (DBP)	<13	<13	<13
Benzophenone (BP3)	<3	<3	<3
2,4-Dihydroxybenzophenone (2,4-DHBP)	<75	<75	<75
Ethylhexyl methoxycinnamate (EHMC z)	<0.05	<0.05	<0.05
Pentabromotoluene (PBT)	<0.05	<0.05	<0.05
Tributyl-O-acetylcitrate (ATBC)	**20 (100)**	<6.4	**6.5**
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)	<0.1	<0.1	<0.1
Ethylhexyl methoxycinnamate (EHMC-E)	<0.3	<0.05	**0.05**
Benzylbutylphtalate (BBP)	**0.5 (46)**	<0.3	0.30
Triphenyl phosphate (TPhP)*	**2.0 (27)**	**8.5 (3)**	**3.0**
Hexabromobenzene (HBB)	<0.05	<0.05	<0.05
2-Benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320)	<0.2	<0.2	<0.2
2-(2H-Benzotriazol-2-yl)-4-(tert-butyl)-6-(sec-butyl)phenol (UV-350)	<0.7	<0.7	<0.7
Bis(2-ethylhexyl) phthalate (DEHP)	<40	<40	<40
2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (UV-326)	<0.4	0.6	**4.1 (5)**
2-(2-Hydroxy-5-tert-octylphenyl)benzotriazole (UV-329)	<2.5	<2.5	<2.5
2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)	<0.30	<0.3	**0.39 (8)**
2,4-Di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (UV-327)	<0.20	**0.92 (3)**	**2.1 (5)**
Octocrylene (OC)	<1.3	<0.88	<0.9
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)	<25	<25	<25
Diiso-nonyl-phtalate (DINP)	<275	<270	<275
Diiso-decyl-phtalate (DIDP)	<275	<270	<275
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*	<25	<25	<25
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Plastic additive-11)	**3322 (18)**	**1831 (37)**	

4.4. Plastic additives and other contaminants in fish samples

Only a few compounds are detected in fish samples (Table 22). **Butylated hydroxytoluene (BHT), Hexachlorobenzene (HCB), Ethylhexyl methoxycinnamate (EHMC),** and **2-hydroxy-4-(octyloxy)benzophenone (HOBP)** are found above limits of quantification in all fish samples. No major differences in concentrations can be observed between different fish samples and facilities. A few other chemicals such as **tributyl-o-acetylcitrate (ATBC), benzylbutylphthalate (BBP) and triphenyl phosphate (TPhP)** are found in some but not all samples.

Table 22 Contaminant concentration in fish fillet for fish from RAS #1, 2 and 3 (ng g^{-1} ww). Relative standard deviations in brackets (%) based on replicate measurements. Relative percent differences (% RPD) were calculated for duplicates.

Compound	RAS#1A	RAS#1B	RAS#2A	RAS#2B	RAS#3
2,4-Di-tert-butylphenol (2,4-DTBT)*	<715	<665	<975	<562	<520
Butylated hydroxytoluene (BHT)*	**29 (24)**	**92 (14)**	**39 (64)**	**30.6 (39)**	**53 (97)**
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*	<0.08	<0.07	<0.11	<0.06	<0.1
Hexachlorobenzene (HCB)	**0.033 (20)**	**0.049 (1.5)**	**0.08 (42)**	**0.06 (90)**	**0.025 (62)**
Dibutylphtalate (DBP)	<4	<4	<6	<3.2	<3
Benzophenone (BP3)	<1.6	<1.5	<3	<1.3	<1.2
2,4-Dihydroxybenzophenone (2,4-DHBP)	<40	<38	<55	<32	<30
Ethylhexyl methoxycinnamate (EHMC z)	**0.032 (16)**	**0.056 (36)**	**0.04**	**0.026 (10)**	**0.037**
Pentabromotoluene (PBT)	<0.03	<0.02	<0.04	<0.02	<0.02
Tributyl-O-acetylcitrate (ATBC)	<0.3	**1.9 (98)**	**1.7 (29)**	**0.36**	**0.85 (91)**
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)	<0.03	0.02	<0.04	<0.02	<0.02
Ethylhexyl methoxycinnamate (EHMC-E)	**0.028**	**0.11 (89)**	<0.04	**0.02**	**0.13 (140)**
Benzylbutylphtalate (BBP)	<0.03	**0.12 (70)**	**0.15 (78)**	<0.02	**0.50 (37)**
Triphenyl phosphate (TPhP)*	<0.9	**0.96**	<1.5	<0.7	**1.9**
Hexabromobenzene (HBB)	<0.03	<0.02	<0.04	<0.02	<0.02
2-Benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320)	<0.2	<0.2	<0.3	<0.2	<0.2
2-(2H-Benzotriazol-2-yl)-4-(tert-butyl)-6-(sec-butyl)phenol (UV-350)	<0.8	<0.7	<1	<0.6	<0.5
Bis(2-ethylhexyl) phthalate (DEHP)	<0.4	<0.4	<2	<0.3	<0.3
2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (UV-326)	<0.2	<0.2	<0.3	<0.2	<0.1
2-(2-Hydroxy-5-tert-octylphenyl)benzotriazole (UV-329)	<3.2	<3	<5	<3	<3
2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)	<3	<3	<4	<2.3	<2.1
2,4-Di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (UV-327)	<0.2	<0.2	<0.3	<0.2	<0.1
Octocrylene (OC)	<6	<6	7.99	<4.6	<5
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)	**3.6 (10)**	**24 (5)**	**9.2 (35)**	**2.5 (20)**	**16 (113)**
Diiso-nonyl-phtalate (DINP)	<147	<137	<200	<115	<107
Diiso-decyl-phtalate (DIDP)	<147	<137	<200	<115	<107
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*	<0.2	<0.15	<0.22	0.13	<0.1
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Plastic additive-11)	<932	<867	<1280	<732	<678

4.5. Additional analyses for additives in selected samples

The Soxhlet extraction procedure used for fish and sludge samples was applied to selected plastics from the RAS systems as well as to the PEX tubing used in our sampling set-up and to two feed stuff samples (Table 23). PEX tubing testing was to ensure that measurements made with passive samplers in water are not influenced by a possible release of substances from the PEX tubing.

The most significant chemicals found in PEX tubing are **2,4-di-tert-butylphenol (2,4-DTBT)** and **plastic additive 11** with concentration levels in the μg g^{-1} of plastic. Residual amounts of **benzylbutylphthalate (BBP), dibutylphthalate (DBP) and Bis(2-ethylhexyl) phthalate (DEHP)** are also measured. Considering the results above, the presence of these compounds in PEX tubing (not necessarily able to leach out of the tubing) is unlikely to have influenced our results.

The chemicals **2,4-DTBT, BHT, plastic additive 11 and bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate (BTMPS)** are consistently detected in most plastic tested.

Investigating the content of chemicals in the other plastic samples generally show the presence of the highest number of chemicals in biofilter media. The "tank liner" and "PE pipe" plastics generally contain the lowest amounts of the chemicals measured here. **Triphenyl phosphate (TPhP), 2-hydroxy-4-(octyloxy)benzophenone (HOBP) and plastic additive 11** are found at concentrations of 10-100s of μg g^{-1} in one of the biofilter media.

Since the extraction procedure applied here was relatively harsh and exhaustive, the presence of these compounds in the plastic does not mean they are necessarily easily released from the plastic materials. In addition, some of the chemicals found in lower concentrations may be contamination accumulated by the plastic during its lifetime rather than originally added as additives. Only one sample per plastic type were analysed and this also needs to be taken into account and these may not be truly representative.

Table 23 Contaminant concentrations in selected plastic samples (ng g^{-1}).

Compound	PEX tubing	RAS#1 Tank liner	RAS#1 PE pipe	RAS#1 Biofilter Media	RAS#2 Biofilter media	RAS#3 Biofilter media
2,4-Di-tert-butylphenol (2,4-DTBT)*	**12344**	**13582**	**12124**	**14148**	**23267**	**13175**
Butylated hydroxytoluene (BHT)*	**84**	**129**	**89**	**2694**	**580**	**136**
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*	<1.4	**19**	**12**	**4.7**	<4	<2
Hexachlorobenzene (HCB)	<0.3	<4	<4	<3	**2.0**	**1.2**
Dibutylphtalate (DBP)	**586**	<540	<520	<466	**1452**	**624**
Benzophenone (BP3)	<4	<8	<7	423	**12**	**8.6**
2,4-Dihydroxybenzophenone (2,4-DHBP)	<92	<300	<290	<259	<205	<107
Ethylhexyl methoxycinnamate (EHMC z)	<2	<56	<54	**126**	**23**	<3
Pentabromotoluene (PBT)	<0.1	**0.5**	**15**	**30**	<0.2	**0.2**
Tributyl-O-acetylcitrate (ATBC)	**38**	<764	<735	**1371**	**1192**	**57**
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)	**0.3**	<0.6	<0.6	**7.4**	**1.0**	**0.4**
Ethylhexyl methoxycinnamate (EHMC-E)	<4.6	<58	<56	**200**	**31**	**5.4**
Benzylbutylphtalate (BBP)	**6.8**	<11	<11	**1240**	**14**	**24**
Triphenyl phosphate (TPhP)*	<23.0	<12	<11	**26126**	**240**	**74**
Hexabromobenzene (HBB)	<0.1	**0.2**	**1.0**	**151**	<0.2	**0.3**
2-Benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320)	<0.3	<2	<2	**56**	<0.6	**1.0**
2-(2H-Benzotriazol-2-yl)-4-(tert-butyl)-6-(sec-butyl)phenol (UV-350)	<0.7	<4	<4	**75**	<2	<0.9
Bis(2-ethylhexyl) phthalate (DEHP)	**171**	<2660	<2560	**3711**	**799**	**1057**
2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (UV-326)	<3.7	15.9	9.2	**2372**	**3992**	**35**

2-(2-Hydroxy-5-tert-octylphenyl)benzotriazole (UV-329)	<3.2	17.8	17.1	**268**	<8	<4
2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)	<3.7	9.6	9.2	**590**	**8.6**	**37**
2,4-Di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (UV-327)	<0.2	0.8	**1.4**	**2539**	**2.5**	**13**
Octocrylene (OC)	n.a.	116.0	<1112	**2356**	n.a.	n.a.
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)	<46	<300	**297**	**531922**	<103	<54
Diiso-nonyl-phtalate (DINP)	<735	<1100	<1100	<950	**3198**	**5242**
Diiso-decyl-phtalate (DIDP)	<506	<1100	<1100	**37478**	**1372**	**1425**
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*	<46	**22666**	**23**	**42**	<103	**89**
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Plastic additive-11)	**86221**	**1995**	**7860**	**22701**	**55397**	<21347

Two feed stuff samples were also extracted and analysed for our list of target chemicals (Table 24). The data for the two samples are consistent. As for the plastic samples, **2,4-DTBT, BHT, BTMPS** and plastic **additive 11** are found in the fish feed. Two additives, namely **2,4-DTBT** and **HOBP** are also found in the feed stuff. Concentrations of the latter are in the range of 60-120 μg g^{-1} feed.

Table 24 Contaminant concentration in feed samples (ng g^{-1}).

Compound	R3 For1	R3 For2
2,4-Di-tert-butylphenol (2,4-DTBT)*	**5065**	**4249**
Butylated hydroxytoluene (BHT)*	**198**	**409**
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*	<1	<2
Hexachlorobenzene (HCB)	<2	<2
Dibutylphtalate (DBP)	<246	<263
Benzophenone (BP3)	<4	<4
2,4-Dihydroxybenzophenone (2,4-DHBP)	**60743**	**120705**
Ethylhexyl methoxycinnamate (EHMC z)	<26	<27
Pentabromotoluene (PBT)	<0.1	<0.1
Tributyl-O-acetylcitrate (ATBC)	<348	<371
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)	<0.3	<0.3
Ethylhexyl methoxycinnamate (EHMC-E)	<27	<30
Benzylbutylphtalate (BBP)	<5	<6
Triphenyl phosphate (TPhP)*	<6	**6.8**
Hexabromobenzene (HBB)	<0.1	<0.1
2-Benzotriazol-2-yl-4,6-di-tert-butylphenol (UV-320)	<0.8	<0.9
2-(2H-Benzotriazol-2-yl)-4-(tert-butyl)-6-(sec-butyl)phenol (UV-350)	<2	**4.3**
Bis(2-ethylhexyl) phthalate (DEHP)	<1210	<1292
2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (UV-326)	<5	<5
2-(2-Hydroxy-5-tert-octylphenyl)benzotriazole (UV-329)	<8.1	**12.3**
2-(2H-Benzotriazol-2-yl)-4,6-ditertpentylphenol (UV-328)	<5	<5
2,4-Di-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)phenol (UV-327)	<0.4	**0.7**
Octocrylene (OC)	<53	<57
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)	**2215**	**4651**
Diiso-nonyl-phtalate (DINP)	<500	<534
Diiso-decyl-phtalate (DIDP)	<500	<534
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*	**1026**	**878**
Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Plastic additive-11)	**7315**	**9502**

5 Discussion

This study presents a first combined exploration of the presence of microplastics and plastic additives in RAS facilities by investigating inlet waters, tank waters, sludge, fish feed and the fish themselves. We utilize a novel approach to first "capture" and explore the known sources within the RAS facilities and use a fingerprinting approach to trace the same materials within the RAS. Our results suggest that there may be generation of microplastics and the release of plastic additives within the RAS system, although within our dataset it was not possible to elaborate on the extent of microplastic generation. In the following section we explore our findings in context with the wider scientific literature, expand on caveats and limitations of our approach, and present a way forward for future research.

5.1. Presence and sources of microplastics in RAS systems

Microplastics were detected across RAS facilities in different sample types. The data obtained from the microplastic assessments was divided into size fractions to aid analysis. Generally, the larger size fractions were easier to process but contained less particles. This is not unexpected since smaller sized microplastics are normally found in greater numbers (e.g., Haave et al., 2019; Lindeque et al., 2020). Due to the low numbers of microplastics found in the larger size fractions comparative analysis could not be performed between the sample types. Table 25 presents a brief overview of the RAS and the positive matches from known plastic infrastructure.

Table 25 Summary of positive matches from the plastic reference library generated for the project.

Polymer	RAS#1	RAS#2	RAS#3
Polyester / polyethylene terephthalate (PES/PET)	No known sources identified from RAS. Not possible to identify source – also in inlet waters.	Low number of particles	Peak after water retention unit but environmental levels from inlet cannot be ruled out.
Polyvinyl chloride (PVC)	Low number of particles	Low number of particles	Only observed in inlet water
Polyamide (PA)	Slightly elevated numbers at after the MBBR/FBBR in both systems. PA feeding systems may contribute as source.	Elevated numbers after the MBBR. PA feeding systems may contribute as source.	Elevated after FBBR – unknown source in RAS
Polypropylene (PP)	Slightly elevated numbers at after the MBBR+FBBR in Påvekst. Biomedia may contribute as source.	PP was found throughout the samples, biomedia made of PP, not possible to distinguish source. Biomedia may contribute as source.	Low number of particles
Polyethylene (PE)* also used in PEX tubing	Low number of particles	PE appeared to peak after the freshwater inlet water (following the water treatment). Data linked to a single replicate.	Only observed in inlet water.

**note: PE used for PEX tubing during sampling. If contamination from the pipe was evident, we would expect to see consistent values throughout all samples. This is not the case.*

To visualise the differences between sample types and RAS systems, Table 26 to 29 present overviews for each RAS and the different plastic polymers identified across sample types. Generally, where polymers were found in the system they were also found in the samples from the facilities (e.g., PP. PA and PE). We reiterate that inlet waters as well as the atmosphere may have contributed to the observed microplastic particles. This is evidence by the presence of PES/PET and PVC in RAS#1A and RAS#1B where particles were not identified in the plastic infrastructure but were found in the samples.

The presence of microplastics in the sludge samples suggest there may be some particle removal from the system (RAS#1 and RAS#2). Unfortunately, we were unable to quantify this. We did observe increases in microplastic counts after the biomedia (RAS#1B and RAS#3) but since there were no samples taken after subsequent water treatment (particle removal or before biofilter), it is not possible to say whether the values are similar or reduced when water returns to the tanks. The same is true for the samples taken after the water treatment (generally low microplastic count), we do not have corresponding samples after the MBBR (RAS#2) and FBBR (RAS#1A) to see if there was a change in particle counts.

Microplastics do not seem to be exclusively related to RAS components, as seen with the prevalence of fibres. These are not likely to come from the RAS infrastructure. Three potential sources include atmospheric deposition, influent water, and fish meal. In our study, the influent did not appear to contribute significantly, and it is likely that the water treatment (which includes particle filtration) prevents a significant influx of microplastics. That said, it is not expected that influent waters will have high numbers of microplastics since the RAS are located in relatively secluded fjords with little to no sources of microplastics. Generally, the Norwegian fjord systems have low numbers of microplastics compared to the other RAS studies (Spain, Portugal, and China). Fish feeds have been identified to contain up to 139 MP/ g (e.g., Thiele et al., 2021) although according to the current study few MPs may be coming from the fish feed when compared to other elements in the RAS.

Table 26 Plastic polymers (both large and small fractions) identified in RAS infrastructure and samples taken from within RAS#1A. Yes – >5 particles per sample, low signal - 5 particles per sample or similar values to those identified in blanks, n.d. – not detected.

RAS#1A	PP	PES/PET	PA	PVC	PE	PU
Plastic infrastructure	Yes	n.d.	Yes	n.d.	Yes	n.d.
Inlet water (SW)	n.d.	Yes	Low signal	Low signal	Yes	n.d.
After filter (SW)	Yes	Yes	Low signal	Low signal	n.d.	n.d.
FBBR	Low signal	Yes	Yes	n.d.	n.d.	n.d.
Sludge	n.d.	Low signal	Yes	n.d.	n.d.	n.d.
Fish	n.d.	n.d.	n.d.	Low signal	n.d.	Low signal
Blank(s)	Yes	Low signal	Low signal	n.d.	Low signal	n.d.

Table 27 Plastic polymers (both large and small fractions) identified in RAS infrastructure and samples taken from within RAS#1B. Yes – >5 particles per sample, low signal - <5 particles per sample or similar values to those identified in blanks, n.d. – not detected.

RAS#1B	PP	PES/PET	PA	PVC	PE	PU
Plastic infrastructure	Yes	n.d.	Yes	n.d.	Yes	n.d.
Inlet water (FW)	Low signal	Low signal	Low signal	Low signal	n.d.	n.d.
After filter (FW)	Yes	Low signal	n.d.	Low signal	Low signal	n.d.
MBBR + FBBR	Yes	Yes	n.d.	n.d.	n.d.	n.d.
Sludge	Yes	Low signal	Yes	n.d.	n.d.	n.d.
Fish	Low signal	n.d.	n.d.	n.d.	n.d.	n.d.
Blank(s)	Low signal	Low signal	Low signal	n.d.	Low signal	n.d.

Table 28 Plastic polymers (both large and small fractions) identified in RAS infrastructure and samples taken from within RAS#2. Yes – >5 particles per sample, low signal - 5 particles per sample or similar values to those identified in blanks, n.d. – not detected.

RAS#2	PP	PES/PET	PA	PVC	PE	PU
Plastic infrastructure	Yes	Yes	Yes	Yes	Yes	Yes
After filter (SW)	Yes	Low signal	Low signal	Low signal	n.d.	n.d.
After filter (FW)	Yes	Low signal	Low signal	n.d.	Yes	n.d.
MBBR	Low signal	Low signal	Yes	n.d.	Yes	n.d.
Sludge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fish	Low signal	n.d.	n.d.	n.d.	n.d.	n.d.
Blank(s)	n.d.	n.d.	n.d.	n.d.	Low signal	n.d.

Table 29 Plastic polymers (both large and small fractions) identified in RAS infrastructure and samples taken from within RAS#2. Yes – >5 particles per sample, low signal - 5 particles per sample or similar values to those identified in blanks, n.d. – not detected.

RAS#3	PP	PES/PET	PA	PVC	PE	PU
Plastic infrastructure	Yes	Yes	Yes	Yes	Yes	n.d.
Inlet water (FW)	Yes	Yes	n.d.	Yes	Low signal	n.d.
After filter (SW)	Low signal	Low signal	Low signal	n.d.	n.d.	n.d.
FBBR	Low signal	Yes	Yes	n.d.	Yes	n.d.
Sludge						
Fish	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Blank(s)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

5.1.1. Challenges in gathering dataset of microplastic presence

Microplastics are still a developing research field and many of the methods are still in the development phase. This presents several challenges when designing and implementing research projects and monitoring campaigns (Aliani et al., 2023). This project is not alone in the challenges it faced.

Sample locations: The position of sampling replicates was not entirely similar between the RAS systems which makes it challenging to compare the data from one RAS to another. Even though the project aimed to perform similar sampling at each RAS, once samples were collected it transpired that sampling of inlet water was both performed before and after water treatment (including filtration), this was not consistent across RAS facilities for the inlet waters. The same is true for the position of sampling after the BBR's.

Number of samples: Sample replicates were limited to triplicate (and duplicate at some locations in RAS1) because of restrictions in the sampling time on site. Our data was highly variable, it would be necessary to increase the number of replicates to reduce this variability. ***We propose higher resolution with a minimum of 10 replicates per sampling location.***

Sample volumes: Sample volumes of water appear to be sufficient for this current work, although greater volumes would likely have allowed us to achieve better LODs, above those of the blanks. Sample volumes for sludge and fish feed appeared sufficient and comparable to other research, however further work is needed to clean up the smaller size fraction, more replicates should have been performed.

Sample processing: All sample types would have benefitted from a great number of replicates to reduce the variability in our data.

Target size fraction: In this study we focused on two size fractions, with some variation in the size fractions of different sample types related to processing methods. The large fraction (generally >300 µm) presented low numbers of particles across all sample types. If the research is repeated, we do not anticipate the numbers would increase unless sample volume is increased. This would have to be considered carefully with respect to the smaller size fraction, which should be the focus of this research. It might be possible to merge the large and smaller size fractions however using an upper boundary may allow us to eliminate most cases of procedural contamination or atmospheric particles (fibres). The smaller fraction presented better overall results, however there was an issue with processing fish tissues, sludge, and fish feed. The sludge and fish feed smaller fractions were compromised because the filters used were overloaded with content whilst the fish tissues were deemed contaminated during processing because of the presence of particles (>200 µm) in blanks and fish tissues where such particles cannot enter.

We did not investigate particles less than 20 µm in this study, methodology for this smaller fraction is still being optimised however, for further research we could consider including pyrolysis. Still remains is the required starting sample volume to achieve recoveries above limits of detection. It is hard to estimate at this stage.

Procedural contamination: was an issue from few samples although the inclusion of blanks from the field collection and during laboratory processing allowed us to identify the cause and take corrective action when necessary.

5.1.2. Comparison to published literature

There have been limited investigation of microplastics within RAS facilities before. Since the conceptualisation of this project, some similar investigations have become available (incl. Lu et al., 2019; Huong et al., 2022; Blonç et al., 2023; Egea-Corbacho et al., 2023; Matias et al., 2023) as well as a Master thesis from Norway (Eidsvik, 2023). The methods applied in our study are similar and comparable to published literature in many ways. Table 30 presents an overview of the similarities and differences between all studies. Polymer type/composition varies between studies, this is likely a reflectance of the different sample types and instrumentation employed. Our results are similar to the other studies where the prevalence of known polymers in the RAS seems relatively low.

Most of the studies encountered similar challenges to ours. In previous studies, fibres also the most prevalent material. We actively chose to eliminate fibres when procedural contamination was highlighted as concern, although this action was not taken in some of the previous investigations.

Lu et al. 2019 investigated microplastics in the larger size fraction (>300 µm) making it only comparable to the large fraction data from this study. The low numbers of microplastics are not surprising and resemble the values observed in this study. The purpose of Lu et al., 2019 as to investigate the presence of antibiotic resistance genes. There was no significant difference between the sites.

Huang et al., 2022 compared the generation of microplastics between four different aquaculture systems near to the Yangtze Estuary. The RAS contained the lowest concentration of microplastics compared to an aquarium, cement pond and earthen pond.

Blonç et al., 2023 utilized an orbitrap mass spectrometer to generate data on the mass of particles. This is not directly comparable to the present study since we utilized particle counts and quantitative FTIR. However, they did identify polyisoprene, PE, polysiloxene, perhydropolysilazane and poly-dimethylsiloxane in their samples of water and fish tissues. In addition to the stomach (polyethylene identified) and muscle (polyethylene, polysiloxane and poly-dimethylsiloxane identified), they looked at other fish tissues than our study, including the brain (perhydropolysilazane identified) and liver (no particles identified)

Egea-Corbacho et al., 2023 investigated water from inlets, purified water, culture tanks feed and fish in their experimental RAS. The authors saw a reduction in the number of different polymers before between inlet and purification but increased again at sampling point after the BBR. They suggested both fish feed and plastic materials in the system may be the contributing source.

Concerns surrounding the study by ***Matias et al., 2023*** include the use of high temperatures for processing which have been shown to cause the loss of colour and impact plastic polymers (Bråte et al., 2018) – this likely elevated the number of transparent items. The identification of fibres in both the liver and fish mussel (fillet) within the size range samples is not possible. No fibre this size would enter those tissues and they more than likely are a result of procedural contamination. The lack of procedural contamination is surprising given that these steps are a prerequisite for all microplastics studies (Brander et al., 2021), without these it is impossible to properly judge the outcomes of this work. Furthermore, where we were conservative and excluded fibres, and cellulose items, Matias et al., (2023) included these datapoints.

Eidsvik, 2023 – Sampled water in triplicates from three different RAS in Norway. Water sampled from effluent of fish tank, effluent of drum filter and effluent of biofilter, including make up water and sludge. The author found that the makeup water (comparable to the inlet water in this study) consistently contained lower values than other samples. Results found MPs in all RAS samples, and similar to this study, identified procedural contamination (although at far higher values than in our study, 10 MP/L). No significant difference between sample locations within a system but differences between systems. The use of Nile red for quantification should be taken with caution given the predisposition for false positives, especially when working with organic rich samples (Nel et al., 2021), such as the waters from the RAS. Sampling processing was not performed in accordance with microplastic contamination controls. Even though this study bares the most resemblance to our present study, the results should be viewed with the study limitations in mind.

*Table 30 Summary of available data on microplastics (MP) in RAS facilities. Samples that were not included in the studies are indicated as not applicable, n.a. * data presented here are calculated based on the small size fraction (< 300 µm).*

Study	Location	Water samples	Fish feed	Sludge	Fish	Additional information
Lu et al., 2019	Fujian Province, China	300 µm net, 3 x 100 L **0.58 – 0.72 MP / L** **100% fibres.**	n.a.	n.a.	n.a.	No mention of procedural controls. **PET fibres suggested to originate from biofilter materials**
Huang et al., 2022	Yangtze Estuary, China	3x 1 L using stainless steel water collector, 10% KOH **1.67 MP /L** **78% fibres**	n.a.	n.a.	n.a.	Most prevalent MP size: 50 – 1000 µm Polymer: cellulose acetate (61%)
Blonç et al., 2023	Spain	2 x 2L filtered onto GF/F (0.7 µm pore size) **1.56 – 257.7 ng/g (polyisoprene)**	n.a.	n.a.	8 fish, KOH 10%. HNO_3 30% **0.23-90.76 ng/g** *(brain, stomach, intestines, muscle)*	Used orbitrap mass spectrometer, low number of replicates.
Egea-Corbacho et al., 2023	Cadiz, Spain	5x 60-100L, Fenton's reagent (63µm sieve, 0.8 µm PC filter) **Average: 17.30 MP/ L.**	100g, density separation with NaCl (n=5) **2.8/ g *(cumulative total)***	n.a.	3 per sample, 10% KOH **9 MPs in total.**	Two size fractions 100µm and 63 µm. Most prevalent: MP size: 300 – 1000 µm Polymer: PE (28-50% in water), PA (36% in fish feed)
Matias et al., 2023	Portugal	10 x 1L grab samples – filtered directly onto GFF (1.2 µm pore size) **37.2 MP / L** **83% fibres**	6 replicates of 5g . 30% H_2O_2 **3.9 MP / g** **53% fragments**	n.a.	10% KOH – 60 degrees **Gills – 0.8 MP / g** **GIT - 1.0 MP / g** **Liver – 0.7 MP / g** **Fillet - 0.4 MP / g** **69% fibres**	No procedural controls, only atmospheric blanks. Most prevalent: MP size: 150-500 µm Polymer: cellulose acetate (59%, - water, 25% feed, 53% fish)
Eidsvik, 2023 – MSc thesis	Norway	10 L or 1L samples 10% KOH – 60 degrees **41-371 MP / L** **75% < 100 µm**	n.a.	45 mL in falcon tubes Estimated: **>1000 MP / L**	n.a.	Procedural controls showed contamination in waters and plastic containers used for sampling
Present study*	Norway	200 L sampled from multiple locations **0.24 MP / L***	3 replicates of 5g **0.4 / g**	3 replicates of 5g **1.76 MP / g**	10% KOH with alcojet Values not calculated due to low detection.	

5.1.2. Improvements for future work (sampling and analysis)

We have identified several next steps for the analysis of microplastics in RAS samples.

Sample locations and replicates: A high resolution investigation at a single RAS would be necessary to pin-point higher concentrations. We envision a minimum of 10 samples per water point within a RAS. If a comparative investigation is to be performed the same sample locations are necessary between RAS.

Sample volumes: Increased sample volume processing for sludge and fish feed.

Target size fraction: maintain the same size categories, although consider capturing small size fraction with pyrolysis. This would support increased comparison with additives as data can be presented as mass.

Procedural contamination: more procedural controls should be performed in the field.

Other potential sources of microplastic introduction to samples:

- ***Fish vaccinations*** – When fish are injected into their muscle this process may lead to the introduction of microplastics from the syringe equipment and vaccination liquid. This was not originally identified as a source and therefore has not been investigated. Fish from RAS#1B and RAS#3 were not vaccinated whereas the others were. Further research could investigate the possibility of microplastics being transferred directly into the fish tissues, if intramuscular vaccines are used.

- ***Backwashing of FBBRs*** - in this study we observed there were elevated levels of microplastics after the FBBR at RAS#3, further investigation has found that the backwashing of this system occurred in the two days preceding our sampling. These routine procedures should be taken into consideration when sampling in the future.

- ***Corrosion of plastics*** – the degradation of plastics materials was not taken into account for this present study. Some of the plastic components in the RAS were relatively new, whilst some were older infrastructure. Over time plastic become weak and prone to fragmentation, including degradation processes into discussion will support an understanding on the generation of microplastics and the release of additives. One approach to perform this would be a lifecycle assessment/mass balance, as well as experimental degradation studies on the plastic elements with the RAS. Such a study would also allow comparisons between new or older RAS.

5.2. Presence and sources of plastic additives and other contaminants in RAS systems

Detection of the different chemicals monitored in different matrices are mapped in the four tables below (Table 31-34).

Table 31 Chemicals detected in RAS#1A. yellow cells – not detected. Light blue cells – detected in one of triplicate samples, blue signal – Detected in all replicate samples (does not apply to fish feed since no replication).

RAS#1A	Inlet water (SW)	After filter (SW)	FBBR	Sludge	Fish	Feed
POPs						
Hexachlorobenzene (HCB)						
Antioxidants						
2,4-Di-tert-butylphenol (2,4-DTBT)*						
Butylated hydroxytoluene (BHT)*						
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*						
Plastic additive 11						
Plasticisers						
Dibutylphtalate (DBP)						
Tributyl-O-acetylcitrate (ATBC)						
Benzylbutylphtalate (BBP)						
Bis(2-ethylhexyl) phthalate (DEHP)						
Diiso-nonyl-phtalate (DINP)						
Diiso-decyl-phtalate (DIDP)						
Flame retardants						
Pentabromotoluene (PBT)						
Triphenyl phosphate (TPhP)**						
Hexabromobenzene (HBB)						
UV filters						
Benzophenone (BP3)						
2,4-Dihydroxybenzophenone (2,4-DHBP)						
Ethylhexyl methoxycinnamate (EHMC z)						
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)						
Ethylhexyl methoxycinnamate (EHMC-E)						
UV-320						
UV-350						
UV-326						
UV-329						
UV-328						
UV-327						
Octocrylene (OC)						
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)						
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*						

Table 32 Chemicals detected in RAS#1B. yellow cells – not detected. Light blue cells – detected in one of triplicate samples, blue signal – Detected in all replicate samples. (does not apply to fish feed since no replication).

RAS#1B	Inlet water (FW)	After filter (FW)	FBBR + MBBR	Sludge	Fish	Feed
POPs						
Hexachlorobenzene (HCB)						
Antioxidants						
2,4-Di-tert-butylphenol (2,4-DTBT)**						
Butylated hydroxytoluene (BHT)*						
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*						
Plastic additive 11						
Plasticisers						
Dibutylphtalate (DBP)						
Tributyl-O-acetylcitrate (ATBC)						
Benzylbutylphtalate (BBP)						
Bis(2-ethylhexyl) phthalate (DEHP)						
Diiso-nonyl-phtalate (DINP)						
Diiso-decyl-phtalate (DIDP)						
Flame retardants						
Pentabromotoluene (PBT)						
Triphenyl phosphate (TPhP)**						
Hexabromobenzene (HBB)						
UV filters						
Benzophenone (BP3)						
2,4-Dihydroxybenzophenone (2,4-DHBP)						
Ethylhexyl methoxycinnamate (EHMC z)						
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)						
Ethylhexyl methoxycinnamate (EHMC-E)						
UV-320						
UV-350						
UV-326						
UV-329						
UV-328						
UV-327						
Octocrylene (OC)						
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)						
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*						

Table 33 Chemicals detected in RAS#2. yellow cells – not detected. Light blue cells – detected in one of triplicate samples, blue signal – Detected in all replicate samples. (does not apply to fish feed since no replication).

RAS#2	After filter (FW)	After filter (SW)	MBBR	Sludge	Fish
POPs					
Hexachlorobenzene (HCB)					
Antioxidants					
2,4-Di-tert-butylphenol (2,4-DTBT)**					
Butylated hydroxytoluene (BHT)*					
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*					
Plastic additive 11					
Plasticisers					
Dibutylphtalate (DBP)					
Tributyl-O-acetylcitrate (ATBC)					
Benzylbutylphtalate (BBP)					
Bis(2-ethylhexyl) phthalate (DEHP)					
Diiso-nonyl-phtalate (DINP)					
Diiso-decyl-phtalate (DIDP)					
Flame retardants					
Pentabromotoluene (PBT)					
Triphenyl phosphate (TPhP)**					
Hexabromobenzene (HBB)					
UV filters					
Benzophenone (BP3)					
2,4-Dihydroxybenzophenone (2,4-DHBP)					
Ethylhexyl methoxycinnamate (EHMC z)					
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)					
Ethylhexyl methoxycinnamate (EHMC-E)					
UV-320					
UV-350					
UV-326					
UV-329					
UV-328					
UV-327					
Octocrylene (OC)					
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)					
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*					

Table 34 Chemicals detected in RAS#3. yellow cells – not detected. Light blue cells – detected in one of triplicate samples, blue signal – Detected in all replicate samples. (does not apply to fish feed since no replication).

RAS#3	Inlet water (FW)	After filter (SW)	FBBR	Fish
POPs				
Hexachlorobenzene (HCB)				
Antioxidants				
2,4-Di-tert-butylphenol (2,4-DTBT)**				
Butylated hydroxytoluene (BHT)*				
4-sec-Butyl-2,6-di-tert-butylphenol (DTBSBP)*				
Plastic additive 11				
Plasticisers				
Dibutylphtalate (DBP)				
Tributyl-O-acetylcitrate (ATBC)				
Benzylbutylphtalate (BBP)				
Bis(2-ethylhexyl) phthalate (DEHP)				
Diiso-nonyl-phtalate (DINP)				
Diiso-decyl-phtalate (DIDP)				
Flame retardants				
Pentabromotoluene (PBT)				
Triphenyl phosphate (TPhP)**				
Hexabromobenzene (HBB)				
UV filters				
Benzophenone (BP3)				
2,4-Dihydroxybenzophenone (2,4-DHBP)				
Ethylhexyl methoxycinnamate (EHMC z)				
Octyl-dimethyl-para-aminobenzoic acid (ODPABA)				
Ethylhexyl methoxycinnamate (EHMC-E)				
UV-320				
UV-350				
UV-326				
UV-329				
UV-328				
UV-327				
Octocrylene (OC)				
2-Hydroxy-4-(octyloxy)benzophenone (HOBP)				
Bis(2,2,6,6-tetramethyl-4-piperidyl) Sebacate (BTMPS)*				

5.2.1 Presence of globally distributed POP: Hexachlorobenzene (HCB)

Hexachlorobenzene (HCB) is a globally distributed legacy persistent organic pollutant present in most waters around the world (Allan et al., 2021; Lohmann et al., 2023). It is therefore not surprising that we were able to measure it in intake waters at all three RAS facilities. In addition, estimated HCB concentrations in the range of a few to tens of pg L^{-1} in the dissolved phase in agreement with expected levels tend to indicate the robustness of our PS measurements. Passive sampling measurements prior to and post filtration and UV treatment at RAS#1 indicated that these treatments reduced concentrations of this compound by a factor of 2-3. Treatment of the intake water can affect slightly the concentration of chemicals that are subsequently circulating in the RAS systems. Overall, circulating levels of HCB are

not substantially different from those measured in intake waters. HCB is a bioaccumulative chemical with low half-lives in the environment and is as expected found in fish fillet. Bioaccumulation factors calculated from lipid-normalised fillet concentrations and circulating dissolved concentrations of the chemical in water, logBAF, are in the range of 4.7-5.2 which is in line with the octanol-water partition coefficient, $\log K_{ow}$ of the HCB.

5.2.2 Consistent presence of certain plastic additives at all RAS facilities

Some plastic-related chemicals such as butylated hydroxytoluene (also an antioxidant in fish feed), 4-sec-butyl-2.6-di-tert-butylphenol, triphenyl phosphate (TPhP), tributyl-O-acetylcitrate (ATBC) or benzophenone were ubiquitous in RAS recirculating waters. Dissolved concentrations estimated here are generally at the lower range of values found for Cadiz Bay by Pintado Herrera et al. (2020). Circulating concentrations of BHT in RAS systems measured here are at the lower end of the range of whole water concentrations measured in German rivers (Fries and Püttmann, 2002). Very limited environmental data exist for 2,6-Di-tert-butyl-4-sec-butylphenol and comparisons with literature data are therefore difficult.

Other compounds are found at some but not all water sampling locations.

5.2.3 Additives generally not found in RAS systems

A few flame retardants were included on our target analytical method. While the organophosphorus flame retardant triphenyl phosphate (TPhP) was ubiquitous in RAS facilities, the brominated flame retardants pentabromotoluene (PBT) and hexabromobenzene (HBB) were not found above limits of quantification except for in the silicone samplers exposed to the air at RAS#3. These are current-use flame retardant but relatively little amounts of data on their levels in the environment are available (Arp et al., 2011). In the case of RAS facilities with a UV treatment of intake waters, these may contribute to lower levels of these compounds.

2,4-di-tert-butylphenol (2,4-DTBT) was added to the target list of chemicals in our initial screening/prioritisation step. Despite that, it was only found in sludge samples RAS#1A and plastics samples above limits of quantification. It was below limits of quantification in all other samples but with sometimes relatively high limits. These were mostly due to high levels of the chemical in blanks. More chemical-specific and rigorous procedures would be needed in order to lower blank values substantially.

Relatively high limits of quantification were also obtained for two plasticisers, di-iso-nonyl-phthalate and di-iso-decyl-phthalate. Analysis is particularly challenging for these compounds since these include many isomers. These two plasticisers were not found in any of the samples, except for one detection in biofilter plastic media.

The UV filter 2,4-dihydroxy benzophenone was not found above limits of quantification in any samples except for the two samples of fish feed. Levels in the fish feed from RAS#1 were 66-124 $\mu g\ g^{-1}$. As for the compounds above, limits of quantification are relatively high. It is therefore difficult to distinguish whether it is not found because concentrations are low or whether it is not stable in RAS systems.

5.2.4 Butylated hydroxytoluene (BHT)

As mentioned above, BHT was consistently detected in recirculating RAS waters at all three facilities. It was however only detected above limits of quantification in intake seawater at RAS#3 (at a concentration an order of magnitude below the circulating levels). Estimated dissolved water concentrations for BHT were also consistent for the three RAS facilities and in the range of 4-20 $ng\ L^{-1}$. A measurement of polymer-water distribution coefficient, not currently available for this compound would be beneficial to ensure the

precision of the data. The whole water concentration of 4.5 ng L^{-1} measured through liquid-liquid extraction of recirculating water from RAS#1 sampled in March 2023 is very close to the PS-based values. In general, these results clearly indicate that intake water (fresh or seawater) is not the source of BHT to RAS waters. Further evidence of this circulation is the consistent bioaccumulation observed in fish. This is not surprising considering the hydrophobicity of BHT ($logK_{ow}$ of 5.3). The resulting fish-water bioaccumulation factor ($logBAF = log(C_{fish}/C_{water})$) for this dataset is 4.9 on a lipid basis and generally in line with its $logK_{ow}$. Source of BHT to the water may be the plastics in contact with the water since BHT was indeed detected in the different plastic samples analysed by target analysis. A major source of BHT is fish feed since concentrations of 200-400 ng g^{-1} of fish feed were measured here. While plastics within RAS facilities may contribute to circulating levels of BHT, the major source of BHT is that associated with fish feed. Indeed, BHT is used as antioxidant in fish feed to protect oil/lipids from oxidation (Lundebye et al., 2010; Lee et al., 2023). Silicone samplers exposed to the air inside the RAS facilities accumulated close to or more than 1000 ng of BHT indicating possible air-water exchange.

5.2.5 Distribution of 2-hydroxy-4-(octyloxy)benzophenone (HOBP)

The UV filter 2-hydroxy-4-(octyloxy)benzophenone was not found in intake or recirculating waters at any of the three RAS facilities or sludge samples. Surprisingly, it was found in all fish samples and also at the 2-5 μg g^{-1} level in fish feed from RAS#1. The highest concentration reported here is over 300 μg g^{-1} and for one of the biofilter plastic. Overall, these data would tend to indicate that the source of the UV filter is the fish feed. It bioaccumulates in fish as a result of its hydrophobicity ($logK_{ow} > 6$) but is not found above limits of quantification in waters. There can be different reasons to explain this. Dissolved concentrations may have been too low for us to measure with passive samplers. It is also possible that the chemical is degraded relatively rapidly once release into the recirculating water.

5.2.6 Presence and distribution of benzotriazole UV filters

Six benzotriazole UV filters were tracked throughout the RAS facilities. Four of them were generally not found in any of the water, sludge, or fish samples.

UV-326 was found in recirculating water of RAS#2 and was found in both intake fresh and sea waters. This is further confirmed by its detection in sludge from RAS#1. An increase in concentration from intake water to recirculating water was observed indicating a possible additional source of the compound inside the RAS facility. UV-326 was also consistently found circulating in waters of RAS#1 but not in intake waters. This tends to support the possibility of a source of UV-326 inside the RAS#1 facility too. For RAS#3 facility, this compound was barely detectable in recirculating waters.

UV-327 was found in recirculating waters and sludge of RAS#1 but not in intake waters. This, as was the case for UV-326, suggests a source of the chemical in the RAS facility. The extraction and analysis of biofilter plastic showed the presence of both UV filters. UV-326 was also detected in the air at both RAS.

No of any of these benzotriazole UV filters were detected in fish but in some cases high limits of quantification were obtained rendering any conclusion on bioaccumulation difficult. Out of the benzotriazole UV filters, UV-326 is usually part of those consistently detected in the environment (Wick et al., 2016). It is therefore not surprising to find this chemical above limits of quantification in this study.

5.2.7 Presence and distribution of phthalate plasticisers

Dibutylphthalate (DBP) was found circulating in waters of two of the RAS facilities. Levels in RAS 1A tend to be higher than in intake waters and this would point towards additional sources within RAS facilities. Benzylbutylphthalate was also measured above limits of quantification in two RAS systems and in intake

waters. Bis(2-ethylhexyl) phthalate (DEHP) was only consistently found in triplicate samplers from RAS#1A and sparsely at other sampling locations. Concentrations were generally in the ten-hundreds pg L^{-1} up to ng L^{-1} level for DEHP in RAS#1A. Bearing in mind some relatively high limits of quantification, these compounds were however not found in sludge or fish samples. These three compounds were consistently found in biofilter media samples at concentrations 10-100s ng g^{-1}. DEHP is listed as a priority substance under the European Union's Water Framework Directive with an environmental quality standard (EQS) set at 1.3 μg L^{-1}. Levels measured here are well below this EQS value.

5.2.8 Removal from water through filtration/UV treatment

Intake waters at RAS#1 were filtered and UV treatment before further use in the facility. Treatment of freshwater and seawater reduced concentrations of a number of compounds in water. These included 4-se-butyl-2,6-di-tert-butylphenol (in freshwater), hexachlorobenzene, dibutylphthalate (in seawater) benzylbutylphthalate (in seawater), and triphenyl phosphate (TPhP). Some compounds such as plastic additive 11 (in freshwater), octocrylene (in freshwater), DEHP, tributyl-o-acetylcitrate (in freshwater), 4-sec-butyl-2,6-di-tert-butylphenol (in seawater) do not show any significant drop in concentration upon treatment.

5.3. Future work

This work has provided the foundations for further investigations on plastics and plastic additives in RAS systems. A quantitative approach should now be taken to establish a **dynamic mass balance or life cycle assessment** for plastics and additives with a RAS. Quantifying the flux of water. into and out of the RAS, coupled with a mass calculation of the input and removal of plastics from RAS is necessary. These numbers are important for the industry to put the scale of the potential problem into context.

Furthermore, the **behaviour of microplastics** is likely influenced by RAS processes, such as backwashing and sedimentation in FBBR. The former may introduce particles to the RAS whilst the later may concentrate particles for removal. Similarly, drum filters in MBBR system, including the size of the filter cloths can support the removal of particles (40-60 μm).

Looking further at the endpoint of **microplastics and additives in sludge**, even though our analysis did not identify proportionally higher numbers using current methods, there is evidence that microplastics accumulate in sludge samples from terrestrial wastewater treatment plants (e.g., Harley-Nyang et al., 2023, Hooge et al., 2023). Given this, it would be necessary to look at sludge in more detail, including where the sludge is repurposed. For example, aquaculture derived sludge can be repurposed for additives to produce fertiliser and other agricultural products (e.g., Del Campo et al., 2010). This discussion should also extend to the use of RAS waters in **aquaponics** (e.g., Monsees et al., 2017, Lunda et al., 2019).

Lastly, the **impact and effects** of microplastics and additives derived from RAS could be established in the form of a **risk assessment.** The data used in this report could act as a starting point for comparing water concentrations with PNEC values of additives. It was beyond the scope of the current work to look deeply at the effects of microplastic, although our does not suggest a significant contribution, in the size range investigated, reached the animal tissues. Similarly, knowledge on the impact of additives on fish physiology would be important to explore.

6 Conclusion

The data obtained from the microplastic analysis provided a good overview of the presence, and absence, of microplastic particles in a variety of matrices within RAS. Even though the number of replicates was low, and the microplastics levels were low, there were some indications of microplastic originating from the RAS. To solidify this assessment, it would be necessary to increase the sample size across all matrix types.

The primary step involving screening of plastics for the presence of major additives with a view to supplement the target analytical method proved particularly useful. Some of the substances added through this step were consistently detected in RAS facilities investigated. Two substances analysed here, the legacy POP hexachlorobenzene (HCB) and the fish feed antioxidant butylated hydroxytoluene (BHT) generally demonstrated the robustness of the sampling performed here. HCB was found at levels that would be expected for the natural aquatic environment in Norway. For BHT, circulating levels in the ng L^{-1} were high in comparison with result for many of the other substances and was not found in intake waters. This together with levels in fish feed, sludge and in fish are consistent with its use as antioxidant in fish feed.

Overall, different chemicals had different presence, fate, and distribution in the different RAS facilities. Some compounds like the plasticiser/flame retardant triphenyl phosphate (TPhP) was found at all facilities. Other substances were not found in intake freshwater and/or seawater but were present in recirculating waters. Fresh and seawater filtration/UV treatment at RAS#1 contributed to decreasing dissolved concentrations of certain chemicals by a factor of 2-3. For other compounds, the effect was not seen. The number of and identity of chemicals measured in recirculating RAS waters generally agreed with detections in passive samplers exposed to the air at these facilities. This is not surprising since many of these chemicals are semi-volatile and air-water exchange can take place. The lowest numbers of chemicals were found in sludge and fish. This is partly the result of the difficulty in analysing these chemicals in complex matrices such as sludge. While these chemicals are hydrophobic, this does not necessarily mean they bioaccumulate in fish. Analysis of selected plastic materials and fish feed resulted in the detection of a number of these chemicals.

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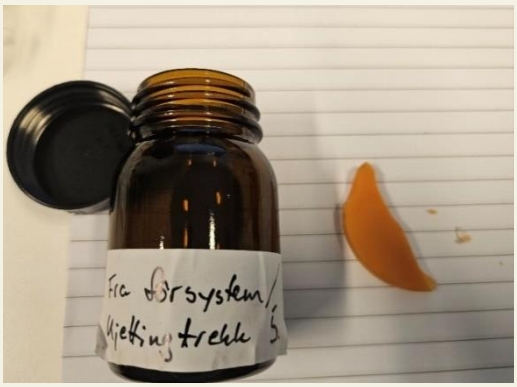
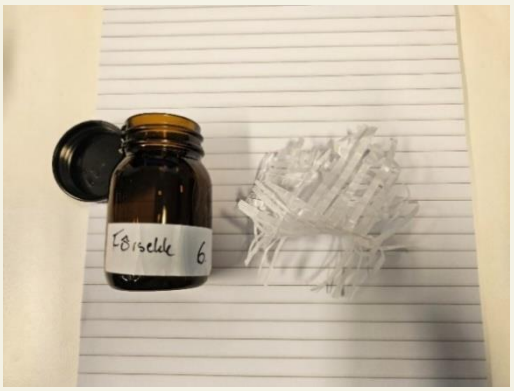
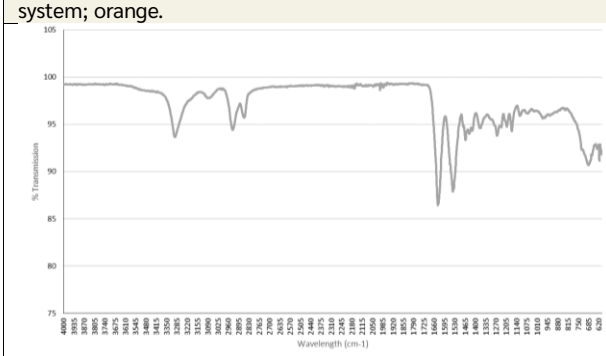
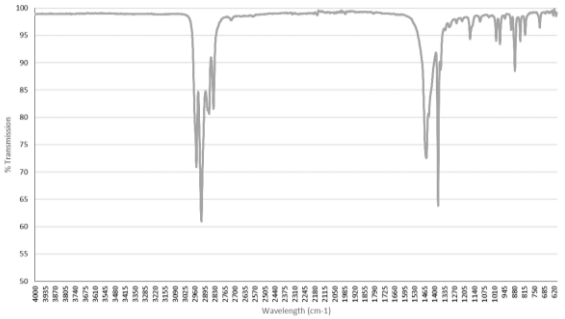
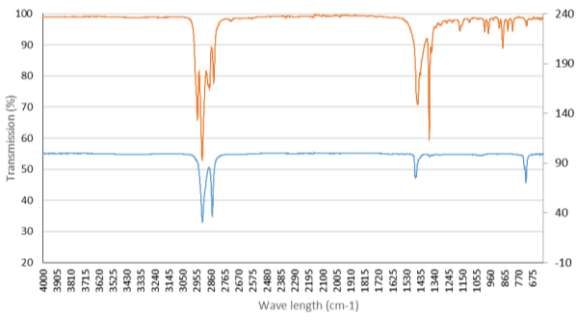
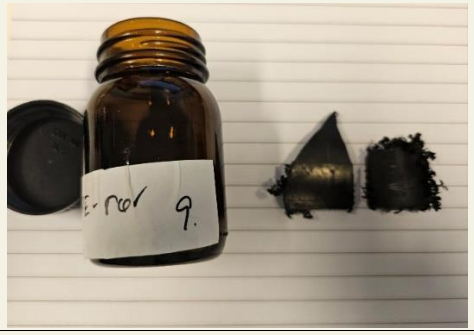
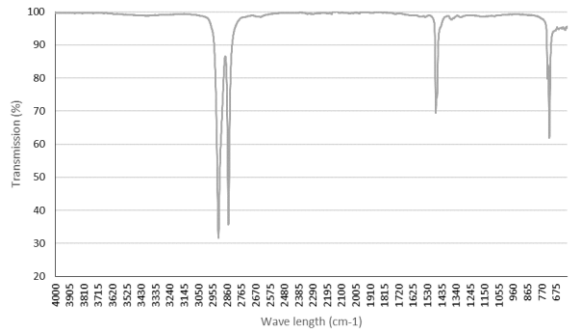
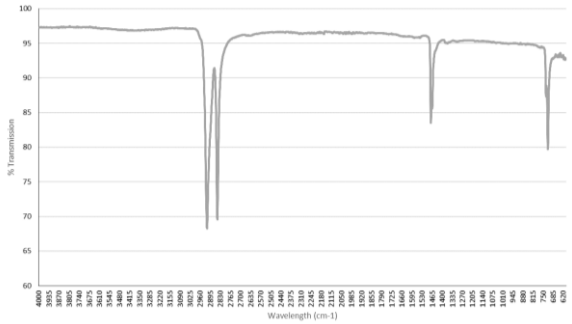
8 Appendix

8.1 Overview of plastics inventoried at RAS

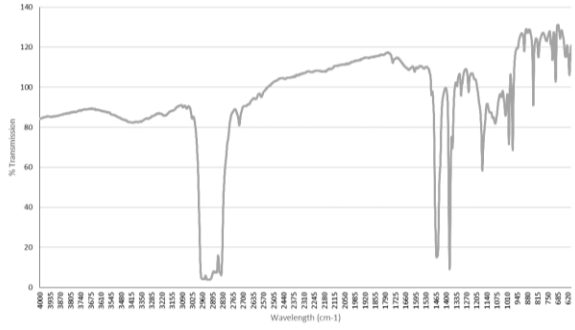
8.1.1. Overview of plastics inventoried at RAS#1

RAS#1 5	RAS#1 6
	
Description: Hard material taken from the inside of the feeding system; orange.	Description: Flexible, woven fibres/film used for transporting and storing fish feed pellets; white.
	
Plastic polymer: Polyamide	Plastic polymer: Polypropylene

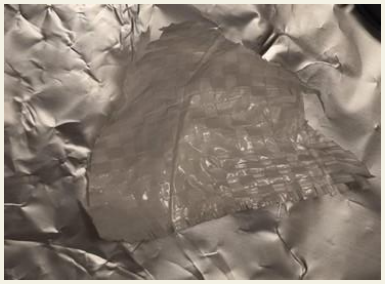
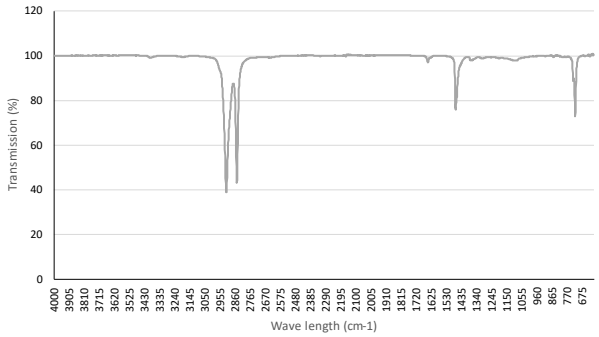
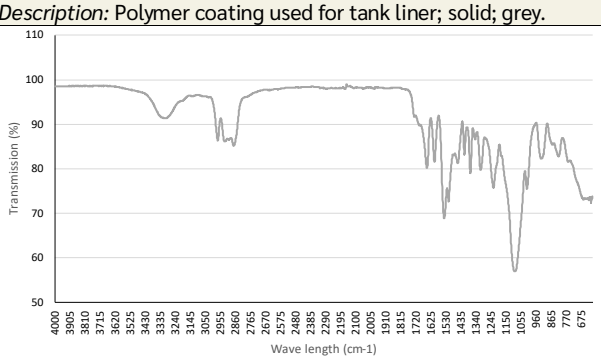
RAS#1_7	
	
Description: Flexible, woven fibres/film used for transporting and storing salt; white. Flexible, film used for transporting and storing salt; blue.	Plastic polymer: Polypropylene (white fibres – orange on figure) Polyethylene (blue film – blue on figure)

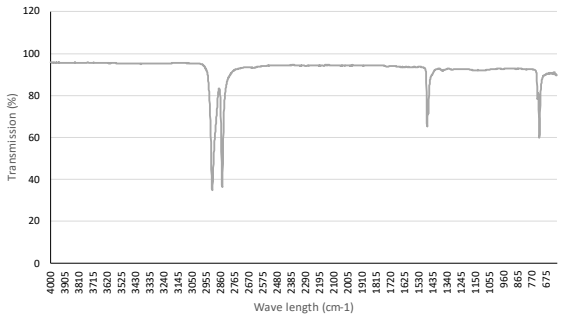
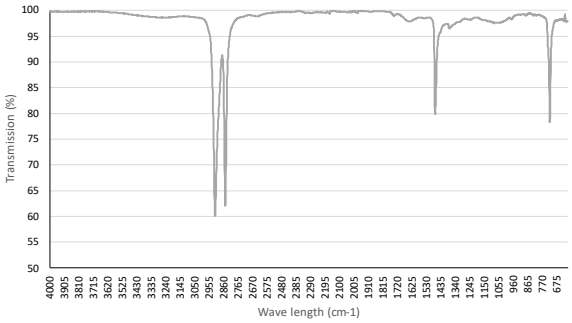
RAS#1_8	RAS#1_9
	
Description: Tank liners; Hard plastic cutting from lines; grey.	Description: Cutting of hard plastic using in the pipe systems; black.
	
Plastic polymer: Polyethylene	Plastic polymer: Polyethylene

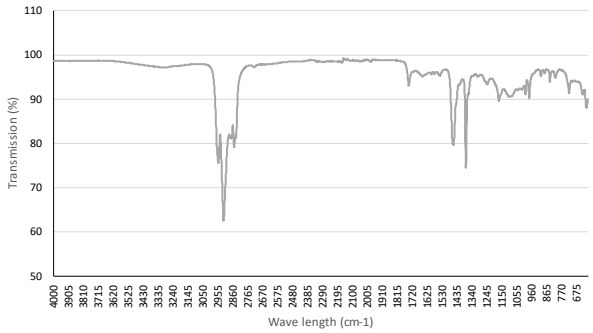
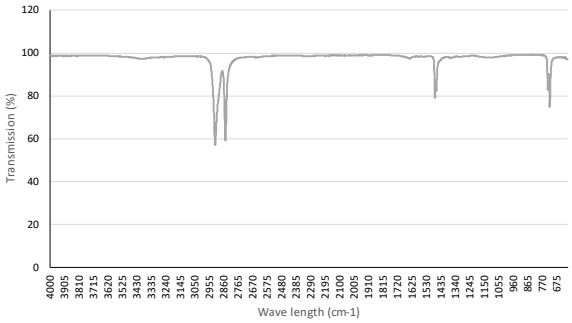
RAS#1_10

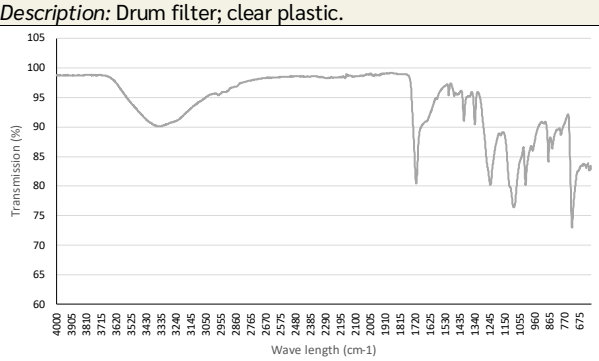
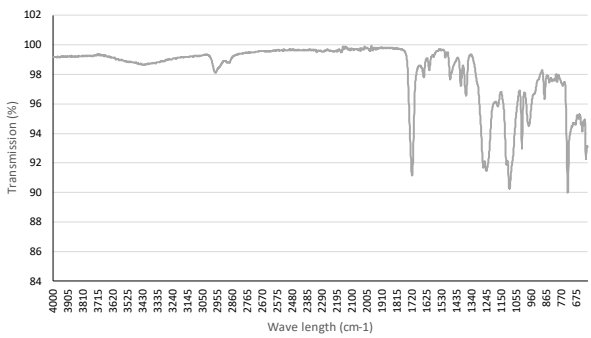
Description: Biofilter; Hard, black plastic.

Plastic polymer: Polypropylene

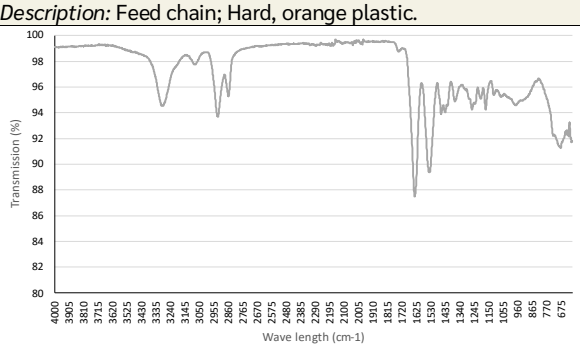
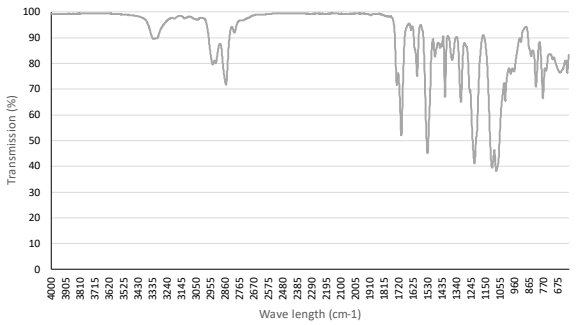
8.1.2. Overview of plastics inventoried at RAS#2

RAS#2 7	RAS#2 8
	
Description: Feed bag (Ewos); Flexible plastic; translucent.	*Description:* Polymer coating used for tank liner; solid; grey.
	
Plastic polymer: Polyethylene	*Plastic polymer:* Polyurethane

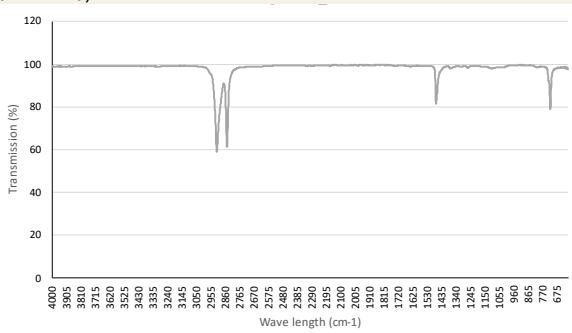
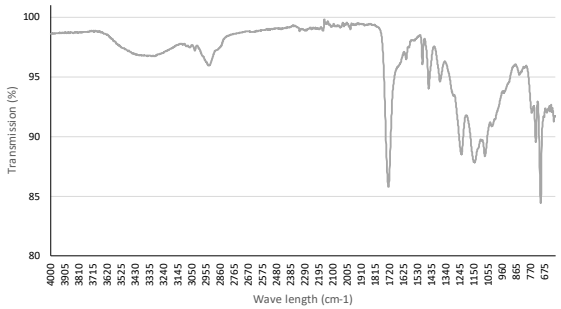
RAS#2 9	RAS#2 10
	
Description: Pipes used around the facility; black	*Description:* Biomedia from MBBR; black; solid.
	
Plastic polymer: Polyethylene	*Plastic polymer:* Polyethylene

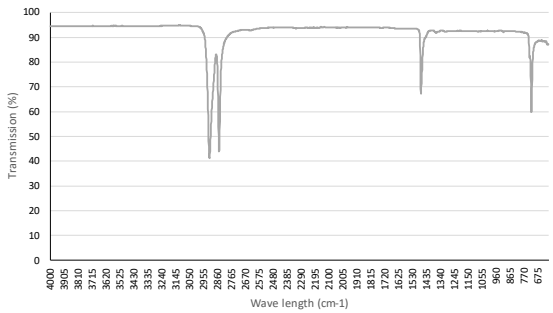
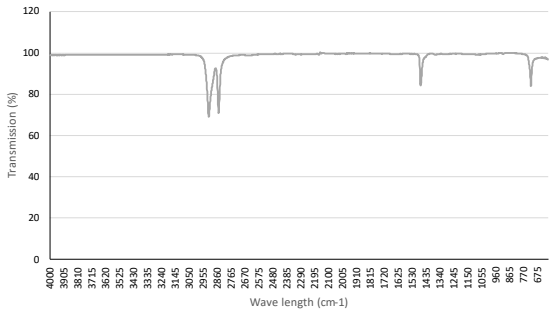
RAS#2_11	RAS#2_12
	
Description: Biomedia from FBBR; black; solid.	*Description:* Bag used to transport ammonium chloride; clear, flexible plastic.
	
Plastic polymer: Polypropylene	*Plastic polymer:* Polyethylene

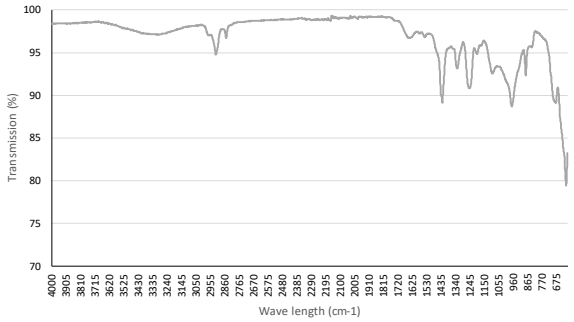
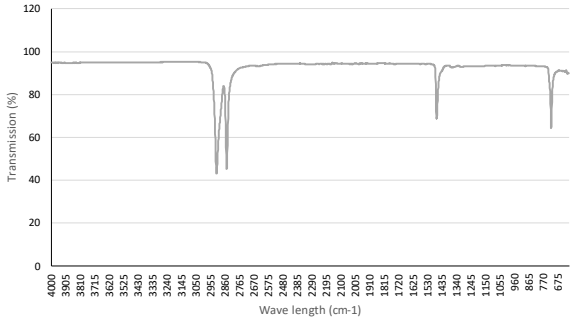
RAS#2 13	RAS#2 14
	
Description: Drum filter; clear plastic.	*Description:* Paint drum filter cover; red.
	
Plastic polymer: Polyester/PET	*Plastic polymer:* Polyester/PET

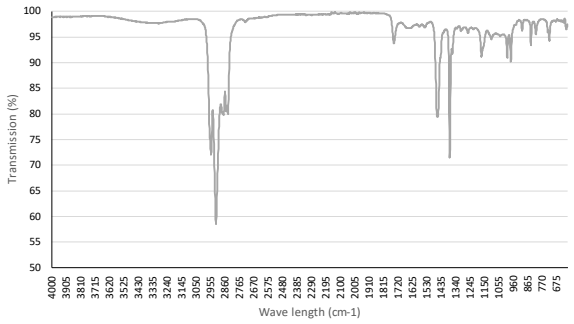
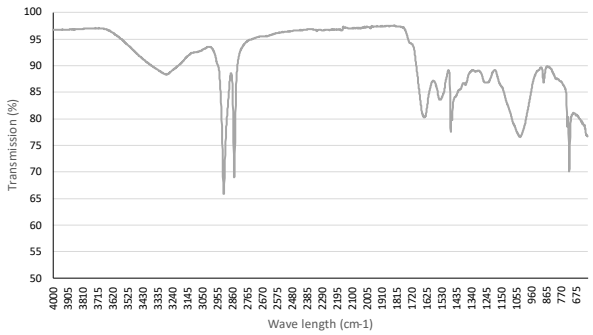
RAS#2_15	RAS#2_16
	
Description: Feed chain; Hard, orange plastic.	*Description:* Fish transport tube; Hard, black plastic.
	
Plastic polymer: Polyamide	*Plastic polymer:* Polyurethane

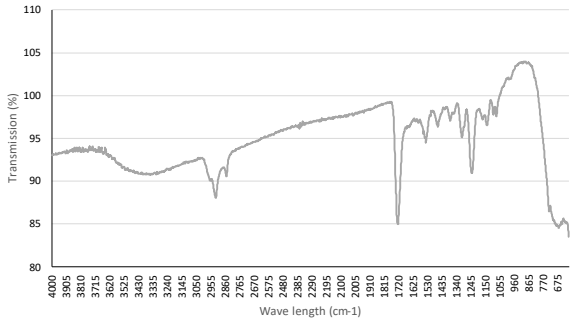
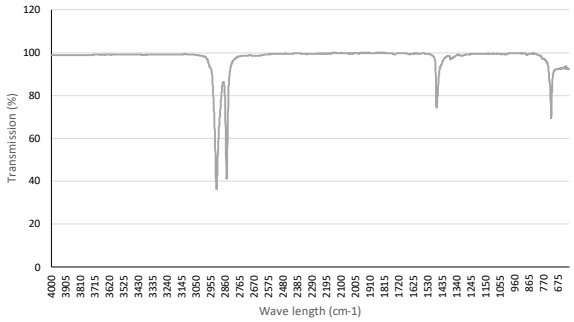
8.1.3. Overview of plastics inventoried at RAS#3

RAS#3 1	RAS#3 2
	
Description: Hard material taken from the water treatment unit (PE-liner); white.	Description: Film taken from retention plate on top of the fixed bed; white.
	
Plastic polymer: Polyethylene	Plastic polymer: Polyurethane

RAS#3 3	RAS#3 4
	
‘ Description: Solid black materials. Tubing pipes to and from the tanks; black.	Description: Tank liners; Hard plastic cutting from liner; grey.
	
Plastic polymer: Polyethylene	Plastic polymer: Polyethylene

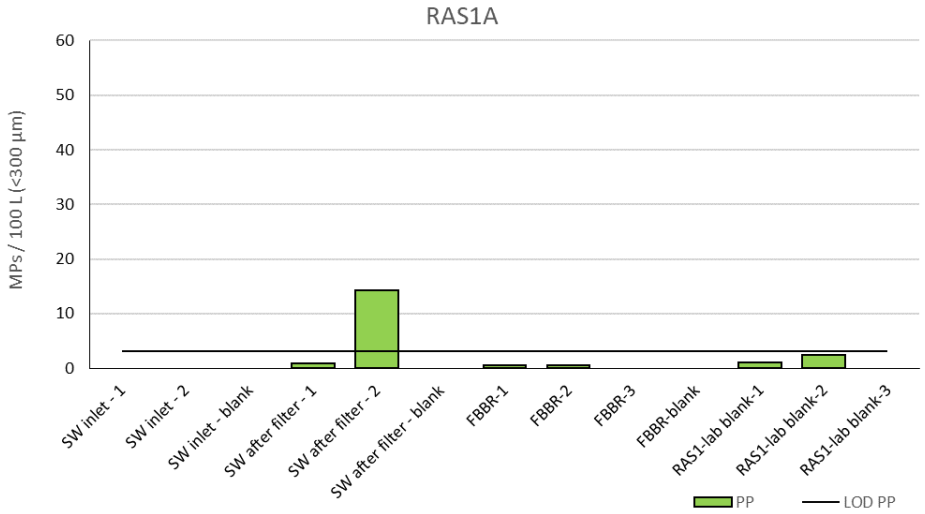
RAS#3 *5*	RAS#3 *6*
	
Description: Cutting of hard plastic used in the aeration pipe systems into the fixed bed; black.	*Description:* Feeding system pipes using air; Black, hard plastic.
	
Plastic polymer: Polyvinylchloride	*Plastic polymer:* Polyethylene

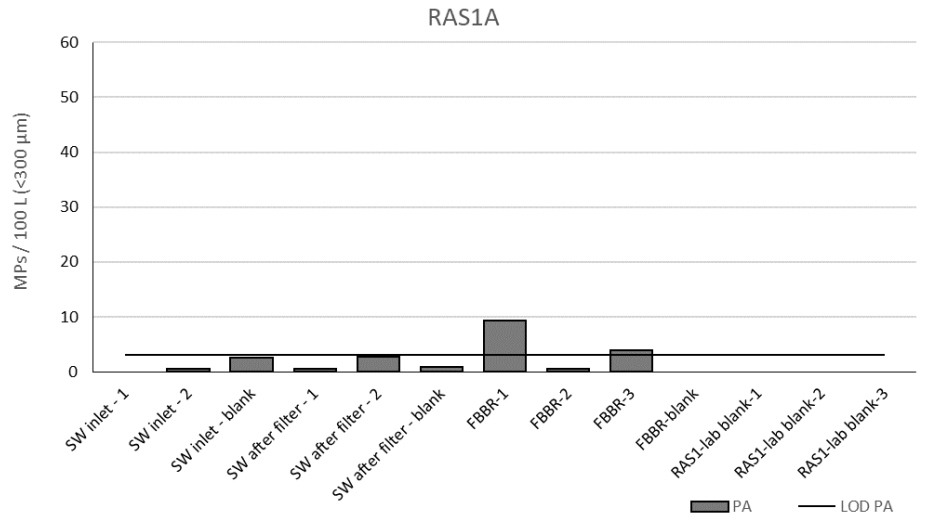
RAS#3 *7*	RAS#3 *8*
	
Description: Fixed bed biomedia; black, hard plastic.	*Description:* Degasser media, CO2; black; hard plastic.
	
Plastic polymer: Polypropylene	*Plastic polymer:* High density polyethylene / Polyamide mix

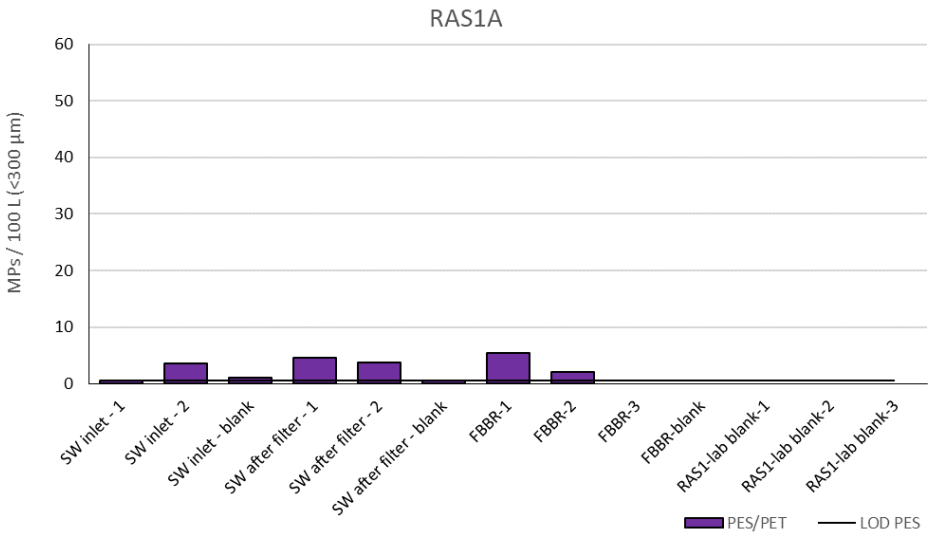
RAS#3 *9*	RAS#3 *11*
	
Description: Cutting of hard plastic used from the diffuser hose for emergency oxygen to the fish tank; black.	*Description:* Fish feed bag; white; flexible plastic.
	
Plastic polymer: Polyvinylchloride	*Plastic polymer:* Polyethylene

8.2 Detailed results of microplastics in water samples

8.2.1. RAS#1A







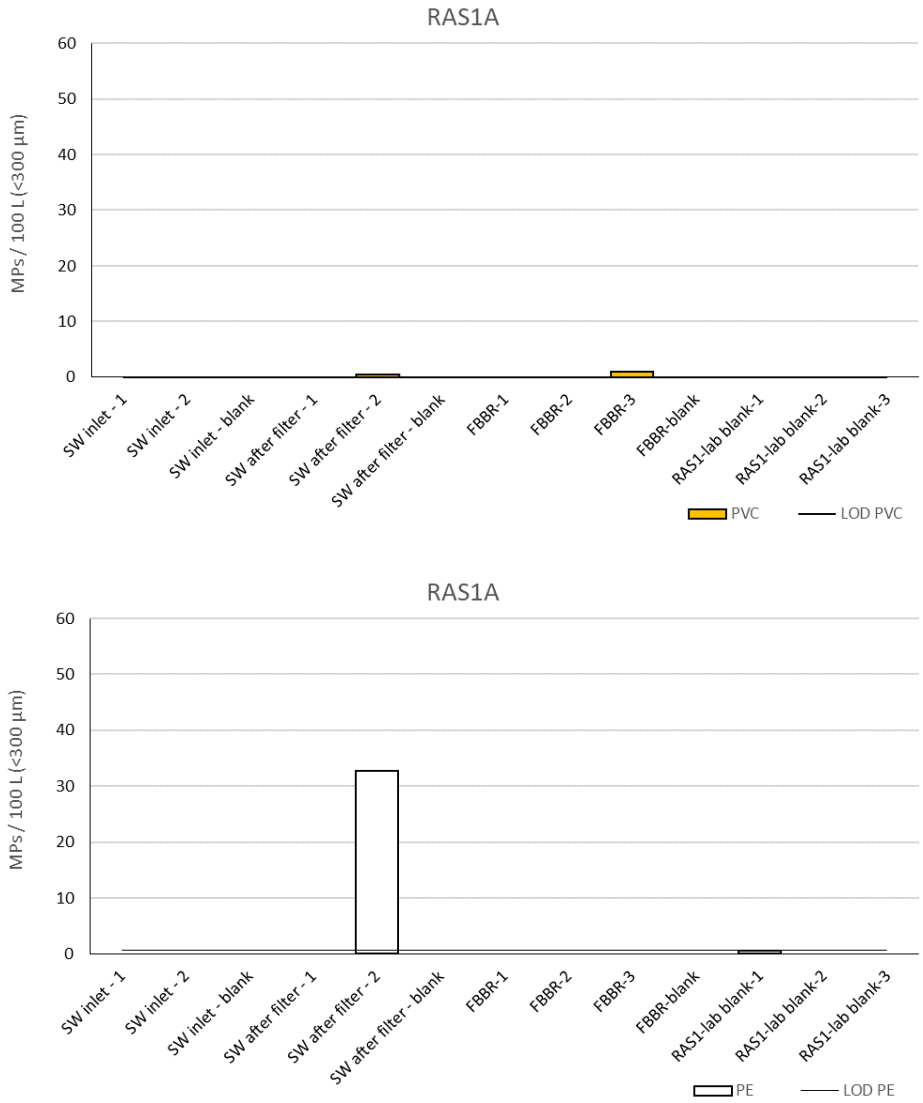
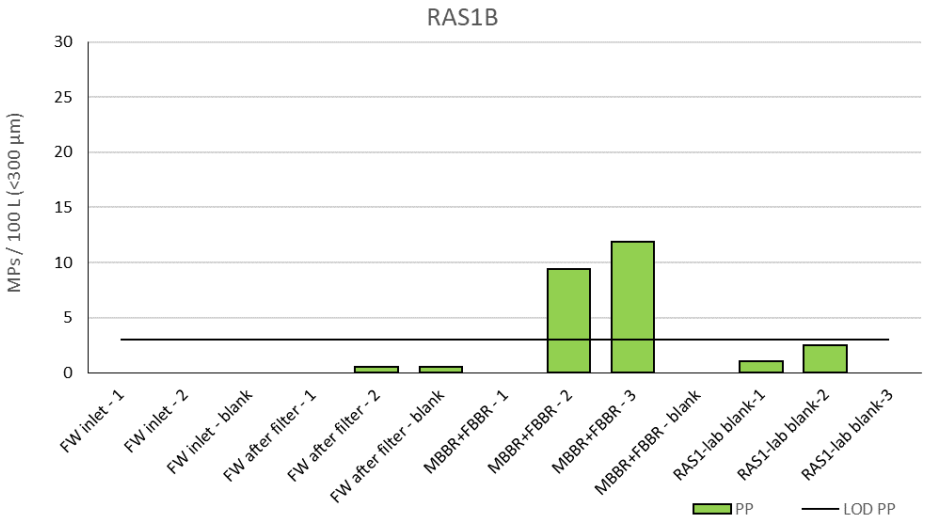
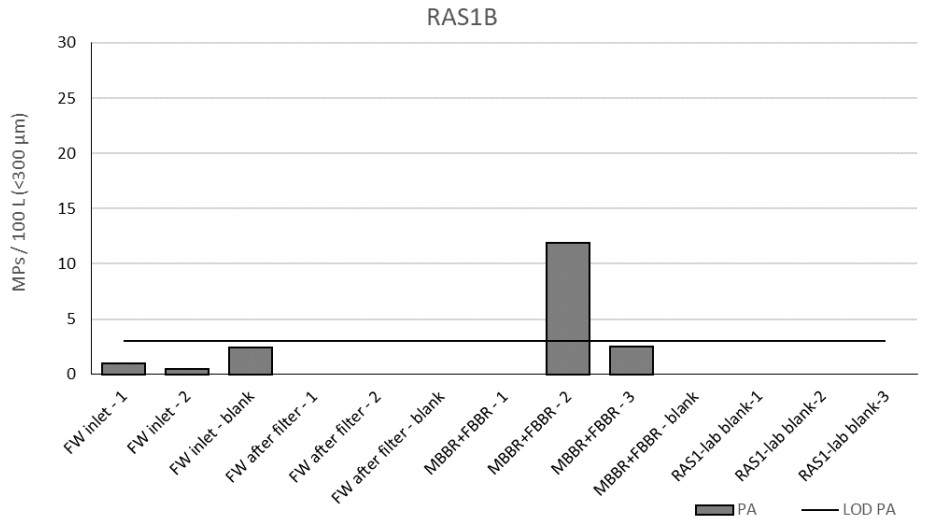
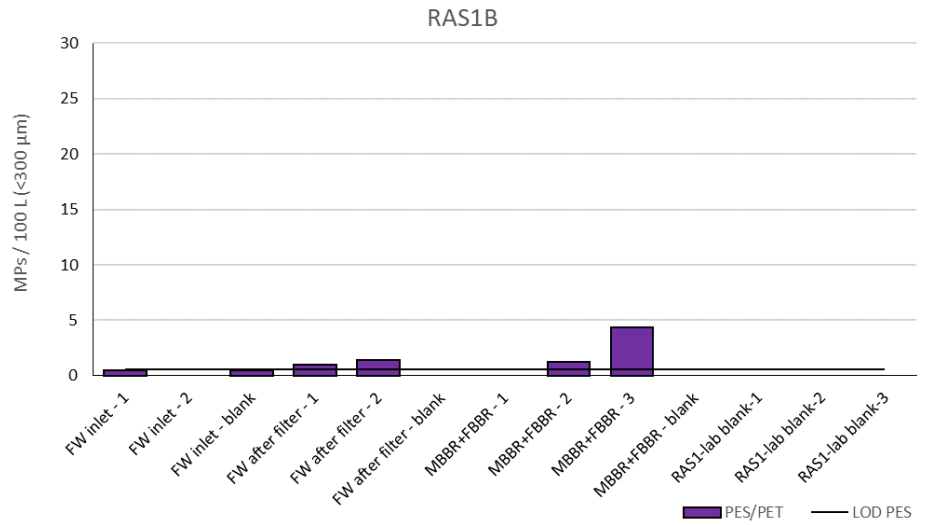


Figure 17. Microplastics (300 - 20 µm), broken down by polymer type, which gave positive results when compared against reference library created from the known plastics at RAS#1A. Limit of Detection (LOD) compared to the blanks is presented as a line.

8.2.2. RAS#1B


RAS1B
MPs / 100 L (<300 µm)
30
25
20
15
10
5
0
FW inlet - 1
FW inlet - 2
FW inlet - blank
FW after filter - 1
FW after filter - 2
FW after filter - blank
MBBR+FBBR - 1
MBBR+FBBR - 2
MBBR+FBBR - 3
MBBR+FBBR - blank
RAS1-lab blank-1
RAS1-lab blank-2
RAS1-lab blank-3
PP
LOD PP


RAS1B
MPs / 100 L (<300 µm)
30
25
20
15
10
5
0
FW inlet - 1
FW inlet - 2
FW inlet - blank
FW after filter - 1
FW after filter - 2
FW after filter - blank
MBBR+FBBR - 1
MBBR+FBBR - 2
MBBR+FBBR - 3
MBBR+FBBR - blank
RAS1-lab blank-1
RAS1-lab blank-2
RAS1-lab blank-3
PA
LOD PA


RAS1B
MPs / 100 L (<300 µm)
30
25
20
15
10
5
0
FW inlet - 1
FW inlet - 2
FW inlet - blank
FW after filter - 1
FW after filter - 2
FW after filter - blank
MBBR+FBBR - 1
MBBR+FBBR - 2
MBBR+FBBR - 3
MBBR+FBBR - blank
RAS1-lab blank-1
RAS1-lab blank-2
RAS1-lab blank-3
PES/PET
LOD PES

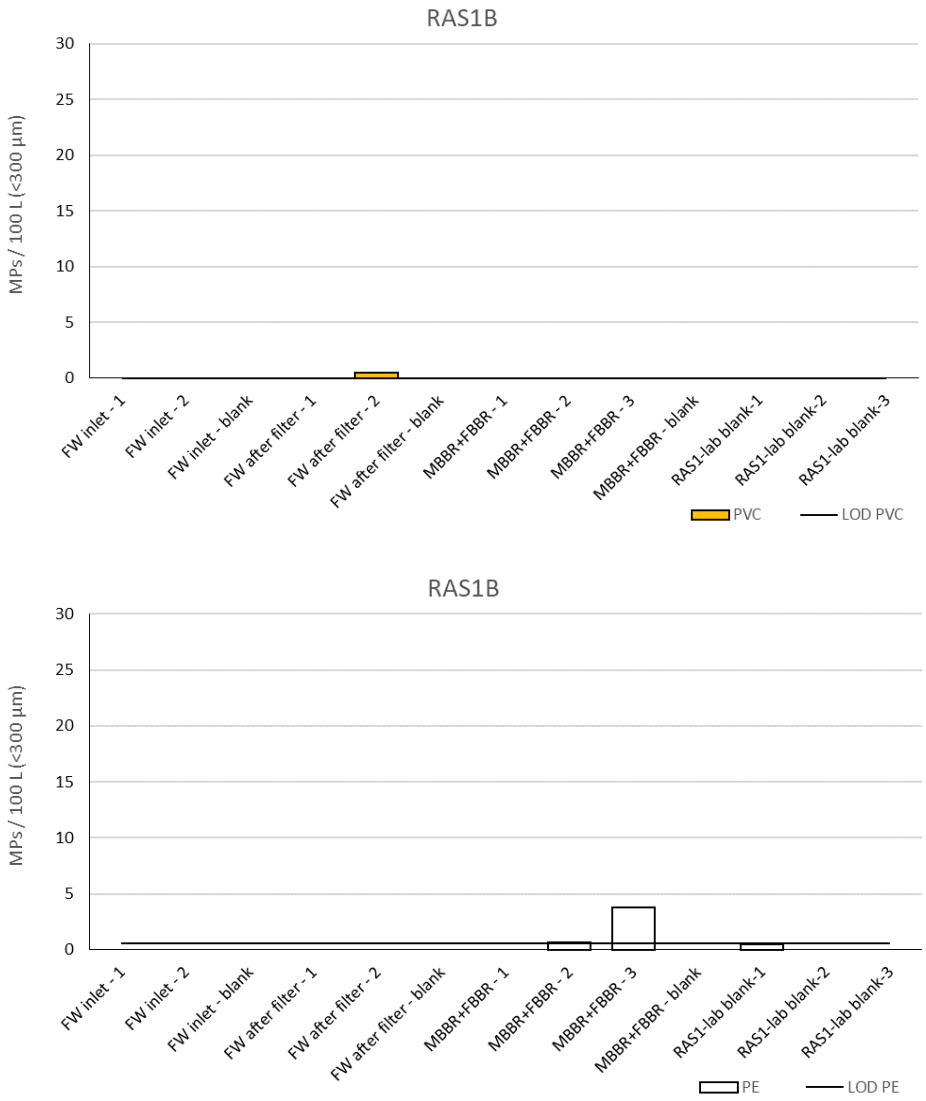
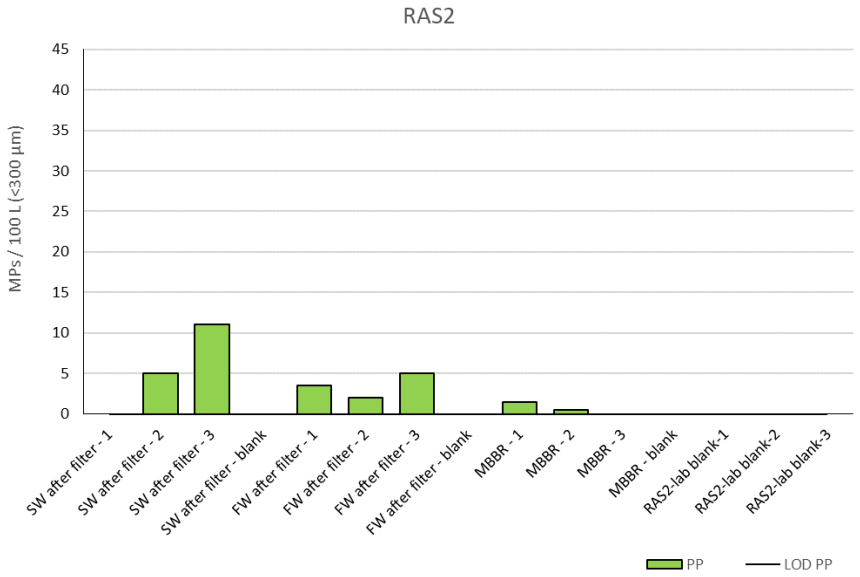
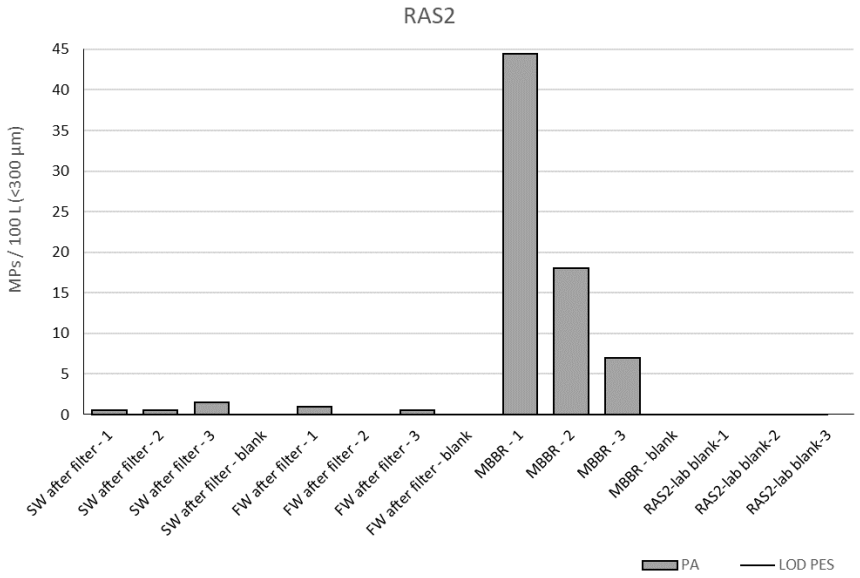
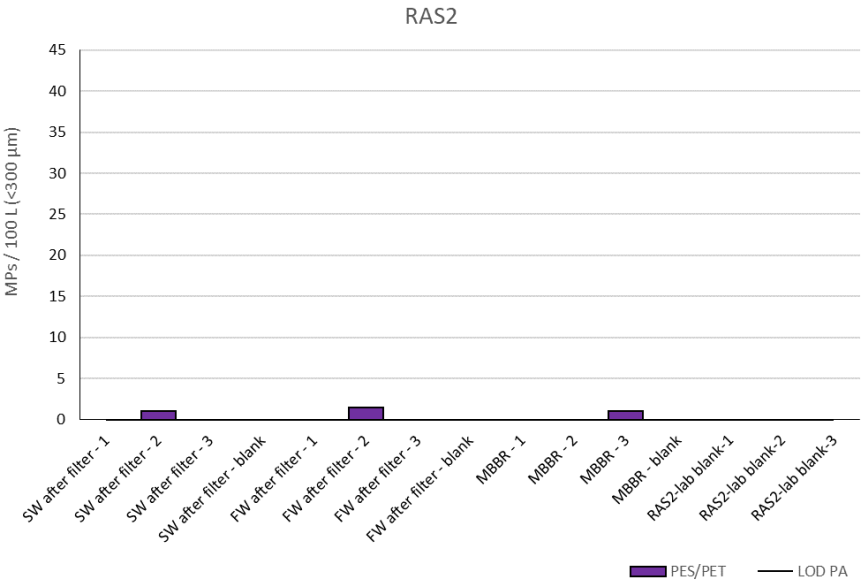


Figure 18 Microplastics (300 - 20 µm), broken down by polymer type, which gave positive results when compared against reference library created from the known plastics at RAS#1B. Limit of Detection (LOD) compared to the blanks is presented as a line.

8.2.3. RAS#2


RAS2
MPs / 100 L (<300 µm)
45
40
35
30
25
20
15
10
5
0
SW after filter - 1
SW after filter - 2
SW after filter - 3
SW after filter - blank
FW after filter - 1
FW after filter - 2
FW after filter - 3
FW after filter - blank
MBBR - 1
MBBR - 2
MBBR - 3
MBBR - blank
RAS2-lab blank-1
RAS2-lab blank-2
RAS2-lab blank-3
PP
LOD PP


RAS2
MPs / 100 L (<300 µm)
45
40
35
30
25
20
15
10
5
0
SW after filter - 1
SW after filter - 2
SW after filter - 3
SW after filter - blank
FW after filter - 1
FW after filter - 2
FW after filter - 3
FW after filter - blank
MBBR - 1
MBBR - 2
MBBR - 3
MBBR - blank
RAS2-lab blank-1
RAS2-lab blank-2
RAS2-lab blank-3
PA
LOD PES


RAS2
MPs / 100 L (<300 µm)
45
40
35
30
25
20
15
10
5
0
SW after filter - 1
SW after filter - 2
SW after filter - 3
SW after filter - blank
FW after filter - 1
FW after filter - 2
FW after filter - 3
FW after filter - blank
MBBR - 1
MBBR - 2
MBBR - 3
MBBR - blank
RAS2-lab blank-1
RAS2-lab blank-2
RAS2-lab blank-3
PES/PET
LOD PA

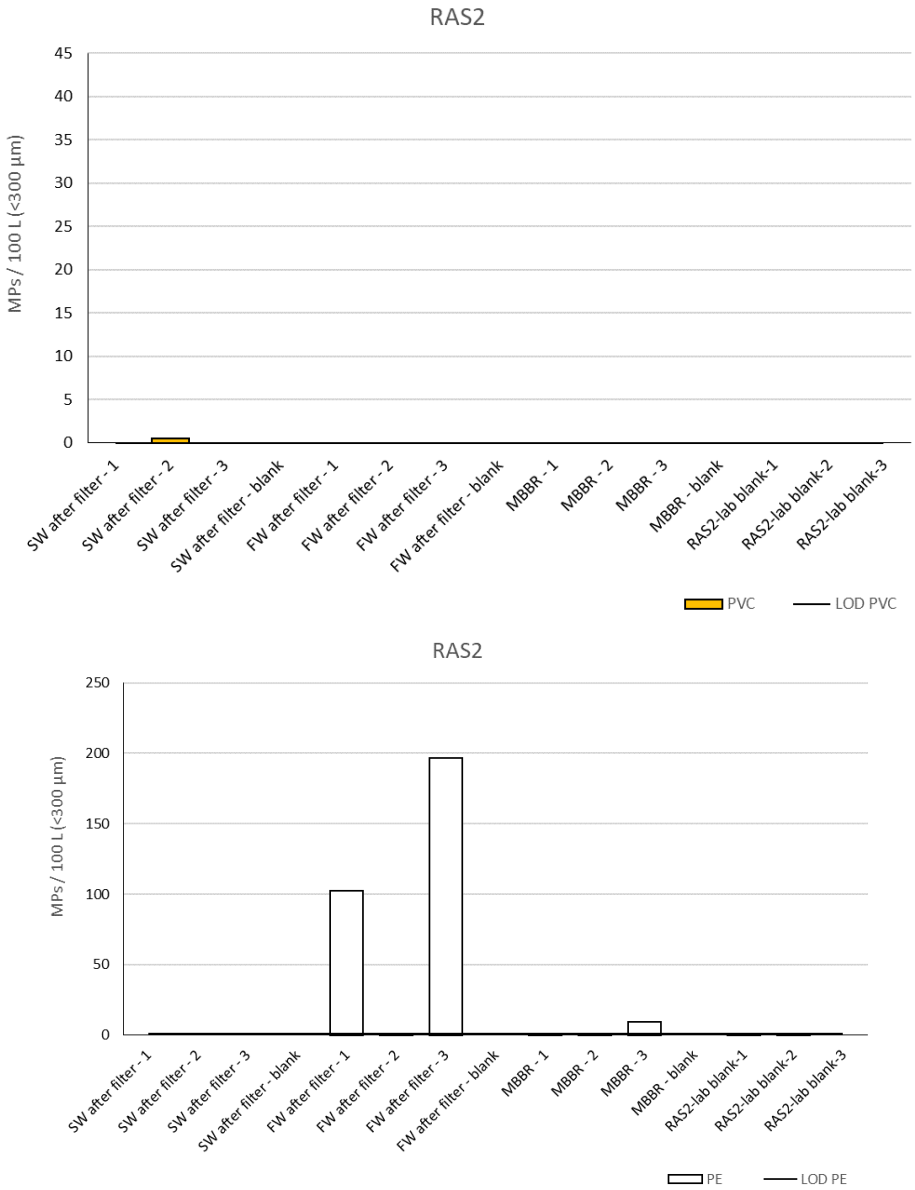
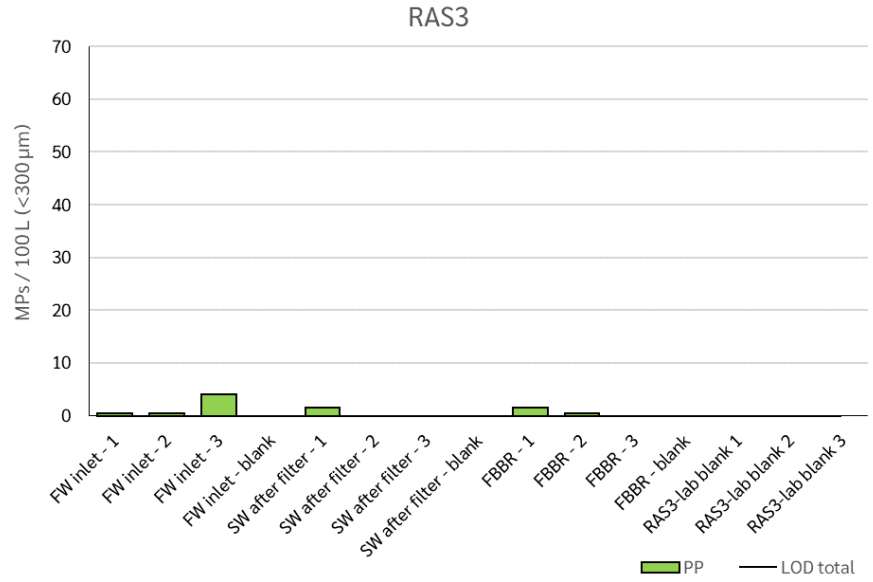
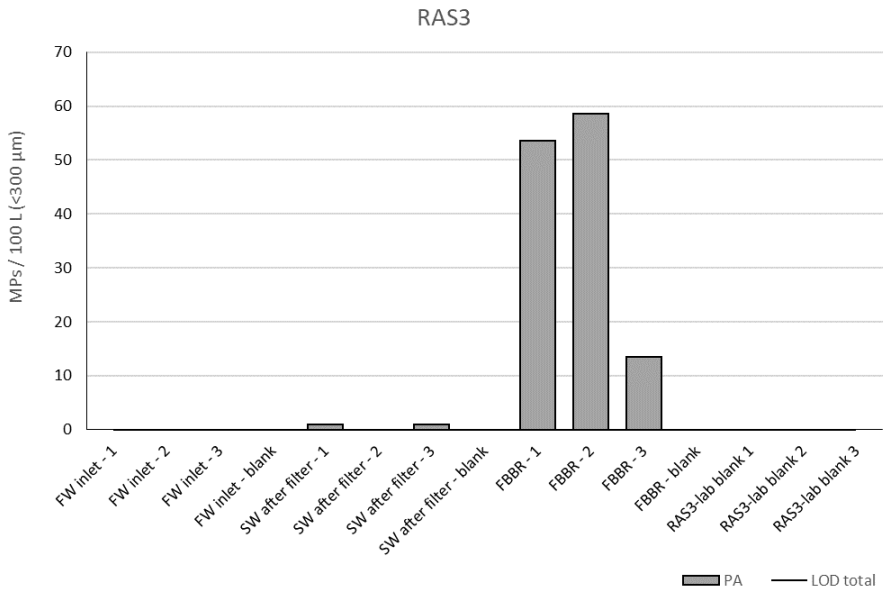
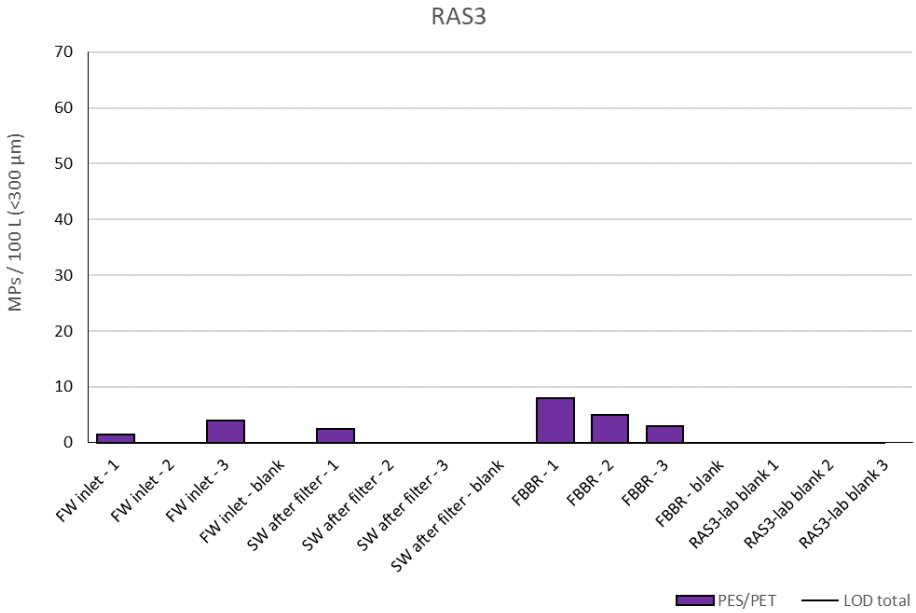


Figure 19 Microplastics (300 - 20 µm), broken down by polymer type, which gave positive results when compared against reference library created from the known plastics at RAS#2. Limit of Detection (LOD) compared to the blanks is presented as a line.

8.2.4. RAS#3


RAS3
MPs / 100 L (<300 µm)
70
60
50
40
30
20
10
0
FW inlet - 1
FW inlet - 2
FW inlet - 3
FW inlet - blank
SW after filter - 1
SW after filter - 2
SW after filter - 3
SW after filter - blank
FBBR - 1
FBBR - 2
FBBR - 3
FBBR - blank
RAS3-lab blank 1
RAS3-lab blank 2
RAS3-lab blank 3
PP
LOD total


RAS3
MPs / 100 L (<300 µm)
70
60
50
40
30
20
10
0
FW inlet - 1
FW inlet - 2
FW inlet - 3
FW inlet - blank
SW after filter - 1
SW after filter - 2
SW after filter - 3
SW after filter - blank
FBBR - 1
FBBR - 2
FBBR - 3
FBBR - blank
RAS3-lab blank 1
RAS3-lab blank 2
RAS3-lab blank 3
PA
LOD total


RAS3
MPs / 100 L (<300 µm)
70
60
50
40
30
20
10
0
FW inlet - 1
FW inlet - 2
FW inlet - 3
FW inlet - blank
SW after filter - 1
SW after filter - 2
SW after filter - 3
SW after filter - blank
FBBR - 1
FBBR - 2
FBBR - 3
FBBR - blank
RAS3-lab blank 1
RAS3-lab blank 2
RAS3-lab blank 3
PES/PET
LOD total

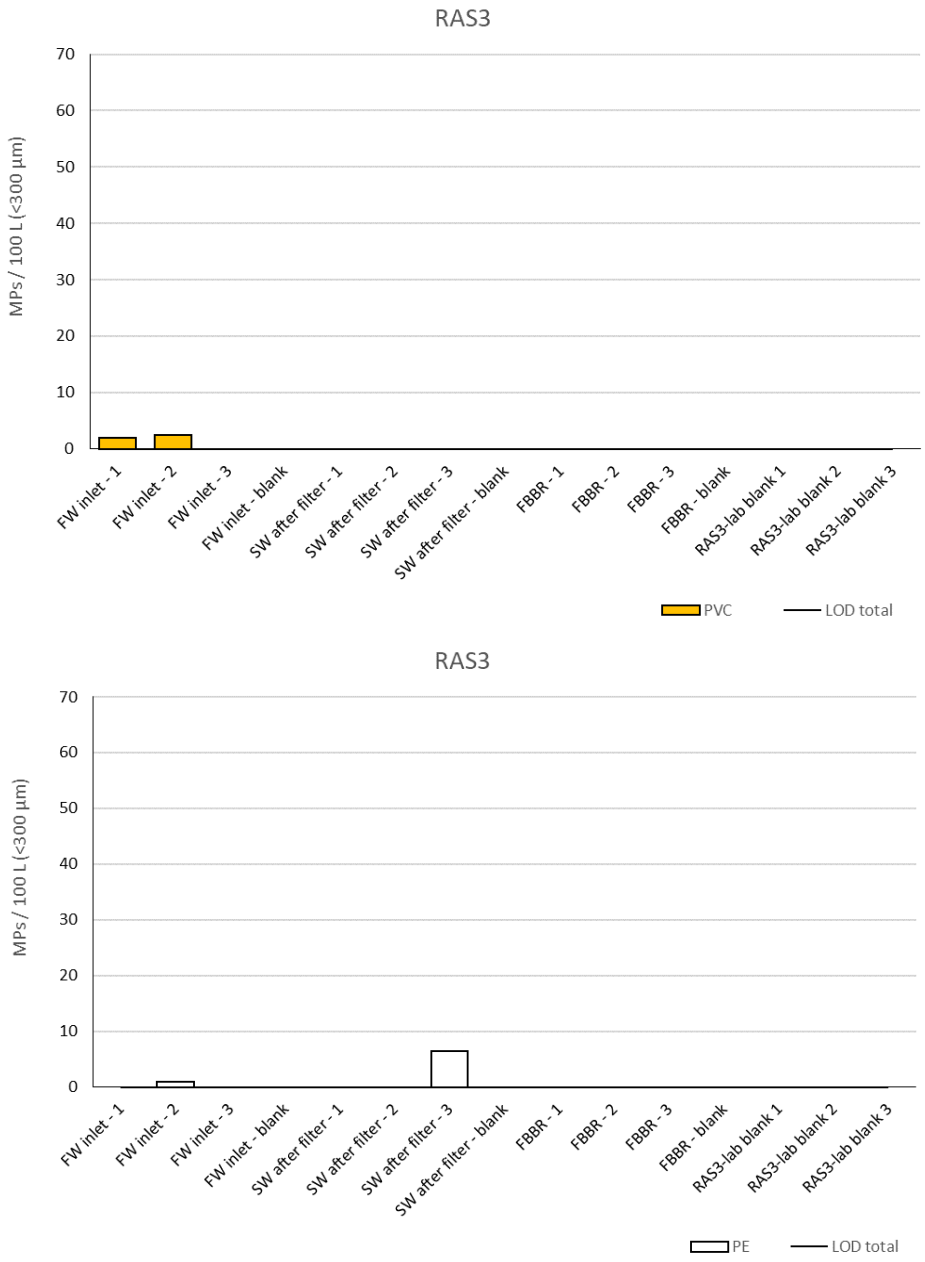


Figure 20 Microplastics (300 - 20 µm), broken down by polymer type, which gave positive results when compared against reference library created from the known plastics at RAS#3. Limit of Detection (LOD) compared to the blanks is presented as a line.

The Norwegian Institute for Water Research

We are Norway's premier research institute in the fields of water and the environment. We are experts on ecosystems in both freshwater and marine environments, from mountains, lakes and rivers, to fjords, coasts and oceans. We develop science-based knowledge and solutions to challenges related to the interaction between water and climate, the environment, nature, people, resources and society.