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Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment.

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Abstract

Municipal effluent discharged from wastewater treatment works (WwTW) is suspected to be a significant contributor of microplastics (MP) to the environment as many personal care products contain plastic microbeads. A secondary WwTW (population equivalent 650,000) was sampled for microplastics at different stages of the treatment process to ascertain at what stage in the treatment process the MP are being removed. The influent contained on average 15.70 (± 5.23) MP. L⁻¹. This was reduced to 0.25 (± 0.04) MP. L⁻¹ in the final effluent, a decrease of 98.41%. Despite this large reduction we calculate that this WwTW is releasing 65 million microplastics into the receiving water every day. A significant proportion of the microplastic accumulated in

19 and was removed during the grease removal stage (19.67 (\pm 4.51) MP/2.5g), it was only in the
20 grease that the much publicised microbeads were found. This study shows that despite the
21 efficient removal rates of MP achieved by this modern treatment plant when dealing with such a
22 large volume of effluent even a modest amount of microplastics being released per litre of
23 effluent could result in significant amounts of microplastics entering the environment. This is the
24 first study to describe in detail the fate of microplastics during the wastewater treatment process.

25 **1 Introduction**

26 Plastic pollution in the aquatic environment is well studied and has been given considerable
27 attention for a number of decades.^{1,2,3} Due to the light weight nature of plastic it is easily
28 dispersed by wind and ocean currents across vast distances.⁴ In recent years the issue of small
29 plastic particles known as microplastics has been gathering increasing attention.⁵ Microplastic
30 are plastics that are <5mm in size⁶ and can be separated into two different types, primary
31 microplastics and secondary microplastics. Primary microplastics are plastics that are
32 manufactured to be of microscopic size these can be found in many cosmetic products where
33 they are used as facial scrubbers or as an air blasting media.⁷ Secondary microplastics are
34 formed from the breakdown of larger plastics debris,⁸ via exposure to sunlight, wind, water, and
35 other environmental stressors.⁹

36 Microplastics are ubiquitously found in aquatic water bodies^{10,11} and sediments^{12,13} and have
37 been ingested by various aquatic organisms.^{14,15,16} Trophic level transfer of microplastics has
38 also been shown to occur.¹⁷ Due to their small size microplastics may be more bio available to
39 lower trophic organisms,¹⁷ who tend to display limited selectivity and will often ingest anything
40 of appropriate size.¹⁸ While organisms of higher trophic levels may ingest microplastics

41 indirectly through trophic level transfer via their prey or by mistaking microplastics for a prey
42 item.¹⁹

43 However, despite this ubiquitous nature, the sources of microplastics in the environment are not
44 fully understood. Wastewater Treatment Works (WwTW) could potentially be a major source of
45 microplastics in the aquatic environment.²⁰ Microbeads used in facial scrubs, toothpaste and
46 other personal care products are transported in the raw effluent to WwTW,^{21,22} where due to their
47 small size they may bypass the waste treatment process. In recent years increased public pressure
48 has led companies and governments to regulate and ban the use of microbeads.²³ Synthetic
49 clothing such as polyester and nylon is also a concern as these fabrics can shed thousands of
50 fibres into the wastewater.²⁴

51 The growing issue of microplastics released from WwTW was recently reported by the
52 Norwegian Environmental Agency.²⁵ This report highlighted the knowledge gap regarding the
53 analysis of microplastics discharged from WwTW, particularly entering river systems and the
54 need for analysis of the fate and removal of microplastics during the treatment process. The
55 report also highlighted the need for more detailed analysis of microplastic particles in order to
56 classify them based on the polymer, size and type. Some research has been undertaken on
57 microplastics in WwTW final effluent^{24, 26, 27} but little work has been undertaken to determine
58 their removal efficiencies and at what stage in the process microplastics are extracted and
59 determining the composition of the polymers entering and exiting these treatment facilities.

60 There has been some research carried out on microplastics in WwTW^{24, 26, 27}. These studies have
61 mainly focused on the final effluent, with little work done on determining removal efficiencies
62 and where in the process microplastics are extracted. A Swedish study investigated the ability of
63 a WwTW to retain microplastics and found that 99% of the microplastic was removed from the

64 final effluent.²⁶ However, this was a relatively small WwTW serving 12,000 people and limited
65 identification was conducted on the specific polymers found. While Carr et al.,²⁷ looked at
66 microplastics from secondary as well as tertiary WwTW by filtering large volumes of effluent as
67 well as attempting to analyse different stages of the treatment process. Identification of MP was
68 primarily visual using MP extracted from personal care products as a visual reference with
69 limited FT-IR conducted. Browne et al.,²⁴ also examined effluent from a tertiary WwTW finding
70 1 MP.L⁻¹, however only small 750 ml samples were filtered. Concentrations of MP measured
71 previously in treated municipal effluents range from 0.0009 MP. L⁻¹²⁷ to 0.009 MP. L⁻¹²⁶ for
72 secondary treatment and 0.000002 MP.L⁻¹²⁷ to 1 MP.L⁻¹²⁴ for tertiary. However, comparison
73 between these concentrations is made difficult due to the variable sampling techniques and
74 identification methods employed.

75 In this study we investigate the effectiveness of the WwTW process in the removal of
76 microplastic from municipal effluent at different stages during the treatment process of a large
77 secondary WwTW with a population equivalent of 650,000. We identify where in the treatment
78 process microplastics are being removed, identify the physical and chemical composition of the
79 microplastics found at each treatment stage. We provide the first systematic overview of the fate
80 of MP in municipal treatment plant, identifying and quantifying where MPs are removed at
81 various stage of the treatment process. There are three separate aspects to this study, the
82 examination of (i) Liquid fraction (ii) Solid fraction: comprising of grit, grease and sludge cake
83 (SC) (iii) 24 hr SC duplicate: SC sampled at two different time points on two consecutive days

84 **2 Materials & Methods**

85 **2.1 Sampling**

86 A large secondary WwTW located on the River Clyde, Glasgow was sampled for microplastics
87 at different stages of the treatment process. This site has the population equivalent of
88 approximately 650,000 and produces on average 260,954 m³ of treated wastewater every day
89 that is discharged into Glasgow's major waterway, the river Clyde. Samples were taken after
90 coarse screening in order to avoid larger debris clogging or damaging the equipment used to
91 filter the samples. Four stages of the treatment process were sampled (Fig. 1A): Influent after 19
92 mm coarse screening (S1), grit & grease effluent (S2), primary effluent (S3), and the final
93 effluent (S4) before it is released to the river Clyde (see Supporting Information (SI) Fig. S1 for
94 detailed description of the treatment process).

95 Steel buckets (10L) attached to steel wire were lowered into the turbulent effluent stream
96 (<50cm) for sample collection by an on-site technician. The sample was then passed through
97 steel sieves (65µm) to collect any debris present. Due to the large amount of debris it was only
98 possible to filter 30L (3 x 10L pooled sample) from sites 1 – 3 and 50L (5 x 10L pooled sample)
99 from site 4 before the sieves became clogged. This debris was then washed into clean glass
100 bottles using distilled H₂O and all equipment was cleaned using on-site hoses between samples.
101 All samples were taken in duplicate. The bottles were then sealed and brought to the laboratory,
102 where the samples were vacuum filtered through Whatman No. 1 qualitative circles, 90mm filter
103 paper, with a pore size of 11µm.

104 Samples of grit and grease were taken from the grit and grease removal stage (Fig.1), and SC
105 from the sludge centrifuge treatment for comparison of microplastics present in the solid effluent
106 fraction from the WwTW. On a separate day, SC samples were also collected at the centrifuge
107 treatment stage (Fig.1) at 09:30AM and again at 14:30PM on two consecutive days in order to
108 determine any variation in the amount of microplastic present.

109 **2.2 Contamination Mitigation**

110 A number of steps were taken to reduce the incidence of microplastic contamination. During
111 these steps clean white cotton lab coats were worn at all times, only natural fabric and no
112 clothing made from synthetic fibres was worn underneath the lab coats.

113 **2.2.1 Cleaning**

114 All equipment used was cleaned three times with distilled H₂O. All petri dishes, filter papers and
115 forceps were examined underneath a dissection microscope before use to ensure no
116 contamination was present. All work surfaces were wiped down with 70% ethanol three times
117 prior to work commencing.

118 **2.2.2 Taping**

119 The tape lifting techniques use in forensic science laboratories to check laboratory benches for
120 fibre and particle contamination was used in this study.²⁸ The tape consists of a plastic film with
121 one side covered with a layer of glue and is placed so that the glue makes contact with the area
122 being examined. The tape is then lifted and any trace particles present should adhere to the tape,
123 which is then placed on a clean sheet of acetate. To take a taping a piece of tape measuring 5cm x
124 5cm was randomly placed three times on the work surface after it was cleaned. After all lab work
125 was completed another taping was taken and was also placed on a sheet of acetate. The tapings
126 were then examined under a microscope for identification. Tapings were carried out before and
127 after all procedures. On average 3.3 fibres per taping taken were collected ranging from 0-14
128 fibres per taping.

129 **2.2.3 Atmospheric microplastic (MP)**

130 Clean filters in petri dishes were left out for the duration of the filtration in order to collect any
131 atmospheric MP that may be present. Before the liquid fraction filtering began 750ml of distilled
132 H₂O was vacuum filtered, and the filter examined for contamination. Clean filters in petri dishes
133 were also left out for the duration of lab work and were then checked for any contamination.

134 **2.3 Analysis**

135 Samples were vacuum filtered and all bottles containing the liquid fraction were rinsed three
136 times with distilled H₂O and filtered after each rinse. The filter was then observed under a
137 dissection microscope. Initially, all debris present was considered to be microplastic until proven
138 otherwise by FT-IR, as relying solely on visual identification is open to bias.^{29,30} Following the
139 FT-IR identification large amounts of material could be discounted (e.g. plant material) and the
140 microplastics were removed and characterised based on their colour, length and type (fibre, bead,
141 flake...etc.). Due to the large amount of debris on the filters from sites 1-3, it was neither
142 practical nor viable to identify all material present. It was therefore necessary to take sub samples
143 from these sites. It should also be noted that due to the complexity of the samples items of
144 similar colour to the background filter paper may have been overlooked.

145 To sub-section the samples from sites 1-3 the filters were divided into 24 pie sections, and
146 numbered 1-24. Using a random number generator, four sections were selected for each filter.
147 These sections were then excised using scissors and thoroughly analysed for microplastics. An
148 average of the four sections was used to get an estimate of the amount of microplastics present
149 for the whole filter. Using the equation below an estimated amount of microplastics released at
150 each site was made.

$$151 \quad MP \text{ Released} = \left(\frac{MP \text{ Present}}{\text{Litres Filtered}} \right) \times \text{Average Vol. of Effluent Released (Litres/Day)}$$

152
153 The solid fraction samples of grit, grease, SC and SC 24 hr duplicate were mixed thoroughly for
154 1 min before taking a 2.5g sub sample in triplicate. Initially a larger quantity was examined but
155 due to practical constraints and the time and effort needed to analyse these samples, a
156 homogenous representative sub-sample of 2.5g was chosen. This sample was left to dry at a low
157 heat <50°C for 2 hours, examined and analysed using a dissection microscope as above and the
158 amount of microplastics present per 2.5 gram was determined.

159 **2.4 Identification: Fourier Transform Infrared (FT-IR) spectrometry**

160 A dissection microscope was used to separate out and collect material for identification by FT-IR
161 analysis with any microplastic identified being photographed. A Perkin Elmer Spectrum One FT-
162 IR Microscope was used in the reflection mode using gold coated glass microscope slides.
163 Infrared radiation from 600 – 4000cm⁻¹ was used, with 16 scans taken to produce the spectra, a
164 variable aperture size was used and the spectral resolution was 4 cm⁻¹. FT-IR allows the
165 identification of chemical bonds present in the samples and gives a characteristic signal in the
166 “fingerprint” region. Samples are identified with the aid of reference spectra library (SI Fig. S2).
167 However, these reference spectra represent very clean and ideal samples, not typically found in
168 the environment. It was therefore deemed necessary to create a more representative library of
169 non-typical reference plastics taken from various sources such as beach debris, recycled waste
170 and microbeads from face washes amongst others. This allowed a comparison to much more
171 environmentally relevant samples to be made. As well as using reference spectra to make
172 identifications the presence of characteristic functional group signals at the correct wavenumber
173 values were checked to confirm the likely chemical structure of materials being examined.

174 **2.5 Statistical Analysis**

175 Statistical analysis was conducted using R statistical computing software. Differences in the
176 number of microplastics and their sizes between sites were determined using one-way
177 ANOVA's. Log10 transformation was used to transform data relating to the number of
178 microplastics present in order to meet the assumptions of normality and equal variance. Size data
179 did not need to be transformed as it already met the assumptions needed to carry out an ANOVA.
180 A Pearson correlation analysis was conducted on the stage of treatment and the amount of MP.
181 L⁻¹ present.

182 **3 Results**

183 In total 430 plastic items were identified across all the samples examined, the majority of which
184 came from the liquid fraction (n=303), followed by the solid fraction (n=79) and 24hr SC
185 duplicate (n=48) samples. Of the 430 items identified as plastic, 8 were >5mm.

186 **3.1 Liquid Fraction**

187 There was a significant difference in the amount of microplastic (MP) found between the four
188 sampling sites ($p = 0.0002$) (Fig. 1B). The influent sampled at S1 contained on average 15.70
189 (± 5.20) MP. L⁻¹ which was reduced by 98.4% in the final treated effluent sampled at S4 to 0.25
190 (± 0.04) MP. L⁻¹ (Table 1). Despite the highly efficient removal rate, using three years of flow
191 rate data from the WwTW an estimated 65,238,500 MP could be released from the WwTW
192 every day in the final effluent or 23 billion microplastics annually from this WwTW alone (Table
193 1). S2 (grit & grease removal) showed the biggest reduction in the amount of microplastic at
194 44.59%, this was further reduced by the primary settlement tanks by an additional 33.75%.
195 Aeration & clarification reduced the amount by 20.07% before the effluent was released into the
196 receiving water.

197 There was a significant negative correlation between the treatment stage and the number of MP.
198 L⁻¹ ($p = 0.014$). The most common polymers found in S1 were alkyds (28.7%), polystyrene-
199 acrylic (19.1%), polyester (10.8%), polyurethane (8.9%) and acrylic (8.3%) (Table 2). The most
200 common polymer found in the final effluent (S4) was polyester (28%), polyamide (20%),
201 polypropylene (12%), acrylic (12%), alkyd (8%), polyethylene (4%), polystyrene (4%) and PET
202 (4%) (Table 2).

203 The liquid fraction contained mainly flakes (67.3%), fibres (18.5%), film (9.9%), beads (3.0%)
204 and foam (1.3%) (Fig. 2A). The sampling process may have resulted in the number of flake
205 items being overrepresented as these flakes were very brittle and fragmented easily during
206 identification. There was no significant difference between the sizes of plastics found at each site
207 ($p = 0.913$). The MP found were predominantly red (26.7%), blue (25.4%) and green (19.1%)
208 but other colours were also present (Fig. 2B).

209 **3.2 Solid Fraction & 24hr SC duplicate**

210 There was a significant difference between the number of MP/2.5g in the three different solid
211 fractions investigated ($p = 0.002$) (Fig. 1C). The grease sample contained an average of 19.67 (\pm
212 4.51) MP/2.5g sample, which was significantly higher than both the grit sample ($p = 0.009$) and
213 the SC sample ($p = 0.002$). From the 24hr SC duplicate study, there was no significant difference
214 in the number of MP/2.5g SC found between the Day 1 and Day 2 or 09:30 and 14:30 ($p =$
215 0.383), or between the time of day the samples were taken (Fig. 1D). Polyester, acrylic,
216 polypropylene, alkyd, and polystyrene were the most commonly found polymers in the 24hr SC
217 duplicate study (Table 2).

218 **3.3 Size Comparison**

219 There was a significant difference in the size (mm) of MP found between the liquid fraction,
220 solid fraction, and 24hr SC duplicate study ($p = 0.002$) (Fig. 1E). MP taken from the liquid
221 fraction were on average 0.598mm (± 0.089) in size and were significantly smaller than both the
222 solid fraction 1.342mm (± 0.519) and the 24hr SC duplicate study 1.618mm (± 0.394) ($p =$
223 0.002). There was no significant difference between the solid fraction and the 24hr SC duplicate
224 study ($p = 0.4$). There was no significant difference in the sizes of the microplastics between the
225 times sampled in the 24hr SC duplicate study ($p = 0.782$).

226 **3.4 Contamination**

227 Throughout the course of the study 25 items were found to have accumulated on the filters that
228 were left out to test for atmospheric microplastic contamination. Following FT-IR examination
229 just one item was identified as a microplastic (polyester), with the rest identified as blue/black
230 cellulose/cotton fibres with a very distinctive ribbon like morphology when examined under light
231 microscope (SI Fig. S3). Similar fibres were also found on the tapings taken and following
232 observation under light microscope these were also identified as cellulose/cotton.

233 **4 Discussion**

234 **4.1 Liquid Fraction**

235 Preliminary and primary treatment effectively removed 78.34% of the microplastics from the
236 liquid fraction. Preliminary treatment involves the removal of large items such as rags and sticks
237 as well as the removal of floatables, grit and grease that may damage or interfere with the
238 equipment used in the treatment process.³¹ Primary treatment involves the removal of a portion
239 of the suspended solids and organic matter, achieved through the use of chemical additives
240 (flocculation agents) and sedimentation.³² The secondary treatment stage managed to remove a

241 further 20.1%. Secondary treatment involves the removal of biodegradable organic matter as
242 well as suspended solids during the aeration and clarification treatment (Fig. 1A).³¹

243 Chemicals such as ferric sulphate are used in the treatment process in order to cause suspended
244 particulate matter to aggregate together forming a “floc”.³² It is likely that the amount of ferric
245 sulphate or other flocculating agents will have an effect on the particulate matter present in the
246 wastewater. For the WwTW in the current study the amount added is flow dependent but on
247 average a total of 7-9 g/m³ are used, with primary treatment receiving 2 g/m³ and secondary
248 treatment getting 5-7 g/m³ during aeration (Fig. 1A). Polyacrylamide is also used as a
249 flocculation agent (Fig. 1A), this is a white water soluble powder. Samples of this were taken
250 and examined in order to exclude it from the final results. Bacterial jelly-like balls are also
251 formed at the aeration stage, likely aiding in the accumulation of particulate debris.

252 A study conducted by the Swedish Environmental Research Institute found high removal rates of
253 99% in a smaller WwTW serving a population equivalent load of 12,000.²⁶ Although
254 microplastic polymer composition was not fully described as the identification of items was
255 mainly visual, several items were identified using FT-IR and included polyester, polyethylene,
256 and polypropylene. This study estimated that 2,000 microplastics were released in the effluent on
257 one particular day, equivalent to 0.16 MP/Person/Day or 0.009 MP. L⁻¹. This microplastic
258 concentration is considerably lower than the 100 MP/Person/Day or 0.25 MP. L⁻¹ of final
259 effluent found in the current study. While a concentration of 1 MP. L⁻¹ was found in the effluent
260 from two tertiary treatment plants in New South Wales, Australia.²⁴ This comparatively high
261 concentration was surprising considering the additional treatment process involved in tertiary
262 treatment. However direct comparison is difficult as the specific treatment processes, the
263 volumes of effluent treated and the population equivalent was not described in this study. A

264 recently published study carried out in Southern California also examined effluent from several
265 tertiary and a secondary WwTW.²⁷ 423,000 L of effluent were filtered at the secondary WwTW
266 and 373 MP were counted or 0.0009 MP. L⁻¹. However this study conducted limited FT-IR,
267 identification was mainly visual and relied upon comparison of MP derived from personal care
268 products. This may have resulted in MP being underestimated, while the number of MP found in
269 the tertiary effluent was even lower with the highest count being 0.000002 MP. L⁻¹ at one site.
270 This study also attempted to investigate the transport of microplastics at each stage of the tertiary
271 treatment process. But owing to practical issues with clogging of filtration equipment (sieves)
272 and the use of density separation, only 5 L of the raw influent was examined with no MP
273 identified making determining reduction rates difficult. Therefore the current study is the first to
274 provide detailed data on the removal rates of MP at various stages of municipal effluent
275 treatment as well as the detailed characterisation of MP found. The concentrations reported in
276 these four studies are all considerably lower than what was reported in a Dutch survey of
277 microplastics in the environment.³³ The concentrations reported ranged from 9 MP. L⁻¹ to 91 MP.
278 L⁻¹, averaging 52 MP. L⁻¹ however identification of microplastic was visual so the concentrations
279 are likely to have been overestimated.

280 The three most common polymers found in the final effluent in the Australian study were
281 polyester (30.4%), polyamide (21%), and acrylic (13%) the same as in the current study. Browne
282 et al.,²⁴ reported the presence of only fibres in municipal effluent which is in contrast to the
283 various different types of microplastics found in the current study. This is most probably due to
284 the large difference in the sizes of the two facilities but may also be influenced by the difference
285 in influent composition from the surrounding catchment, the degree of urbanisation and the time
286 of day that sampling was conducted as well as the specific treatment process used at that facility.

287 4.2 Solid Fraction

288 Analysis of the solid fraction (grit, grease and SC) samples showed high amounts of microplastic
289 accumulating in these three stages. This was most evident in the grease stage, which showed a
290 significantly higher amount of microplastic present. It was only from the grease samples that the
291 much publicised microbeads from face washes were found²² (Fig. 3). It has been suggested that
292 due to their small size, microbeads are capable of passing through the coarse and fine screens
293 (designed to remove large debris to prevent damage to the equipment) and through the
294 wastewater treatment process without removal. The microbeads found in the majority of face
295 washes consist of polyethylene,³⁴ which is positively buoyant in water and is likely to sit on the
296 surface of the wastewater where it can be easily skimmed off the surface layer during grease
297 removal. In this study no microbeads were found in the final effluent, indicating that microbeads
298 from face washes may not be a major issue for the receiving environment if appropriate
299 treatment processes are implemented. However, owing to the small sample size, this study may
300 not be entirely representative. Microbeads from face wash products have been previously found
301 in the Great Lakes in North America,¹¹ but due to the size of the area studied it is difficult to
302 determine their source.

303 Certain aspects of the grit and grease removal stage implemented in this WwTW are site specific
304 and may not be normal practice in other locations. Typically, at this stage of the treatment
305 process skimmers are placed on the surface of the effluent to skim off any floating grease, while
306 the grit portion settles at the bottom. The WwTW sampled in the current study uses aeration to
307 cause frothing of the grease, making it more likely to be collected and removed from the
308 effluent. On average 12-15m³ of grease is removed from the effluent each day. This is then

309 incorporated along with the grit to the sludge (Fig. 1A) where it is then sent for incineration in a
310 waste to energy scheme.

311 Synthetic material has previously been found in sludge samples,^{35,36} as well as effluent
312 samples.^{24,37} A study examining the presence of synthetic fibres in WwTW sludge found 4 fibres
313 per gram of sludge sampled.³⁶ However this study only examined synthetic fibres as an indicator
314 of soil pollution and may have resulted in other non-fibrous polymers being overlooked.

315 **4.3 24hr SC duplicate**

316 The 24hr duplicate study showed no difference in the amount of microplastic present in the
317 sludge over this time period investigated. However, this study may have used too short a time
318 between sampling periods to provide a solid conclusion and it would be more appropriate to look
319 at longer time frames such as a monthly comparison and more frequent sampling in future
320 studies.

321 **4.4 Destination**

322 Microplastic size was considerably smaller in the liquid fraction than in the solid fraction and
323 24hr SC duplicate study samples. This may be due to the smaller items remaining suspended
324 within the liquid fraction, while the larger items are more likely to settle at the bottom of
325 settlement tanks or be captured in the grit & grease stage. It could also be due to only the
326 smallest items being capable of passing through the treatment process. The final destination of
327 these microplastics released in the treated effluent remains unknown but evidence suggests they
328 may be accumulating in the river banks of the Clyde or carried out into the estuary and
329 eventually the Clyde Sea. A preliminary study conducted by Habib et al.,³⁵ examined sediments
330 collected from a bay downstream of a sewage treatment plant. It was found that the sediment

331 contained numerous synthetic fibres and as distance increased from the sewage treatment plant
332 the size and number of fibres decreased. The difference in concentrations of microplastics up and
333 downstream from a WwTW has also been examined³⁸ finding a higher downstream
334 concentration of MP (17.93 m³) compared to the upstream concentration (1.91 m³), with
335 primarily fibres and fragments being found. The river Clyde receives the effluent from a number
336 of WwTW which could all be contributing to the microplastic load. Microplastics have been
337 previously identified in the Clyde Sea, with 83% of *Nephrops* examined found to contain
338 microplastics,¹⁴ although it was thought that these were primarily sourced from discarded fishing
339 line and rope.

340 **4.5 Contamination**

341 An important aspect of this study was the implementation of various contamination controls to
342 ensure the validity of the findings. Contamination has been put forward as a topic of concern in
343 microplastic research.^{24,30} Similar methods to reduce and determine the incidence of
344 contamination such as avoiding wearing synthetic clothing, thorough cleaning, the use of filters
345 to collect atmospheric microplastics, as well as forensic taping techniques were also developed
346 for microplastic sediment analysis.³⁹ Through the use of these contamination controls it was
347 determined that the incidence of microplastic contamination from clothing or atmospheric
348 particulate matter is very small provided appropriate controls are put in place. The methods used
349 in the current study are simple, cheap and require little technical training to carry out but do
350 require care to be taken to prevent contamination. Implementation of these contamination
351 controls in future microplastic research should be included to provide additional validity to the
352 results obtained.

353 **4.6 Limitations of this study**

354 Sampling in wastewater treatment systems presents a number of challenges as reviewed by Ort et
355 al.,⁴⁰ This review examined the study of pharmaceuticals and personal care products but should
356 also be applicable to sampling for microplastics. The review highlights practical limitations in
357 sampling such as environmental and the daily variability of flow rates as well as variability in
358 pollutant concentration. In future studies the time of day, year and weather patterns should all be
359 considered when sampling. Due to the great variation of flow rates it may be more appropriate to
360 take frequent samples throughout the day rather than taking a snapshot as was done in this study.

361 This current study did not take into account storm water runoff, where untreated effluent is
362 released directly into the river when the volume of incoming water exceeds the treatable volume.
363 According to flow rate data taken from the WwTW, when averaged out over the year 39,000 m³
364 of effluent with limited treatment (settlement in storm tanks) is released every day or potentially
365 an additionally 620 million microplastics/day using the figure of 15.70 MP. L⁻¹ taken from S1.
366 However, this normally occurs in large volumes across short periods of time during spells of bad
367 weather, for example on one particular day over 700,000m³ was recorded to have been released
368 as storm water. This untreated wastewater may potentially heavily increase the amount of
369 microplastic entering the receiving environment. However, it's important to take into account the
370 dilution factor that would occur, although the volume of wastewater increases it is unlikely to
371 increase the amount of microplastics present. Although large storm tanks used to hold excess
372 untreated wastewater, allow some settlement to occur reducing the amount of particulate matter
373 and the amount of denser microplastic present before being released/treated, the issue of storm
374 water overflow has yet to be investigated in relation to microplastic contamination.

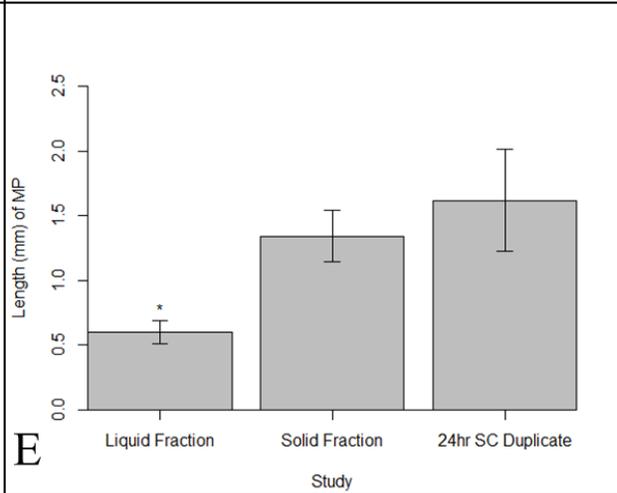
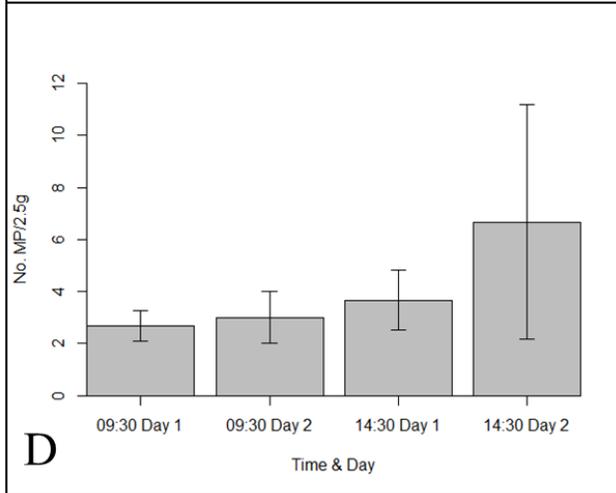
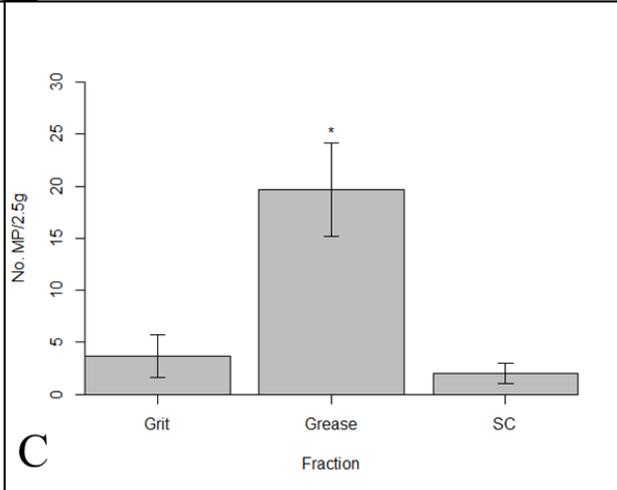
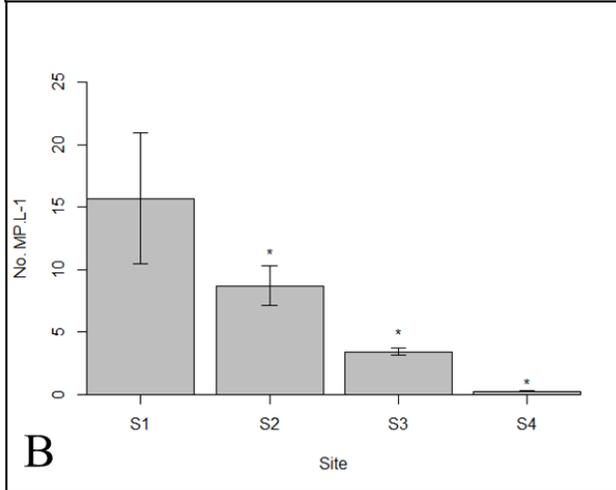
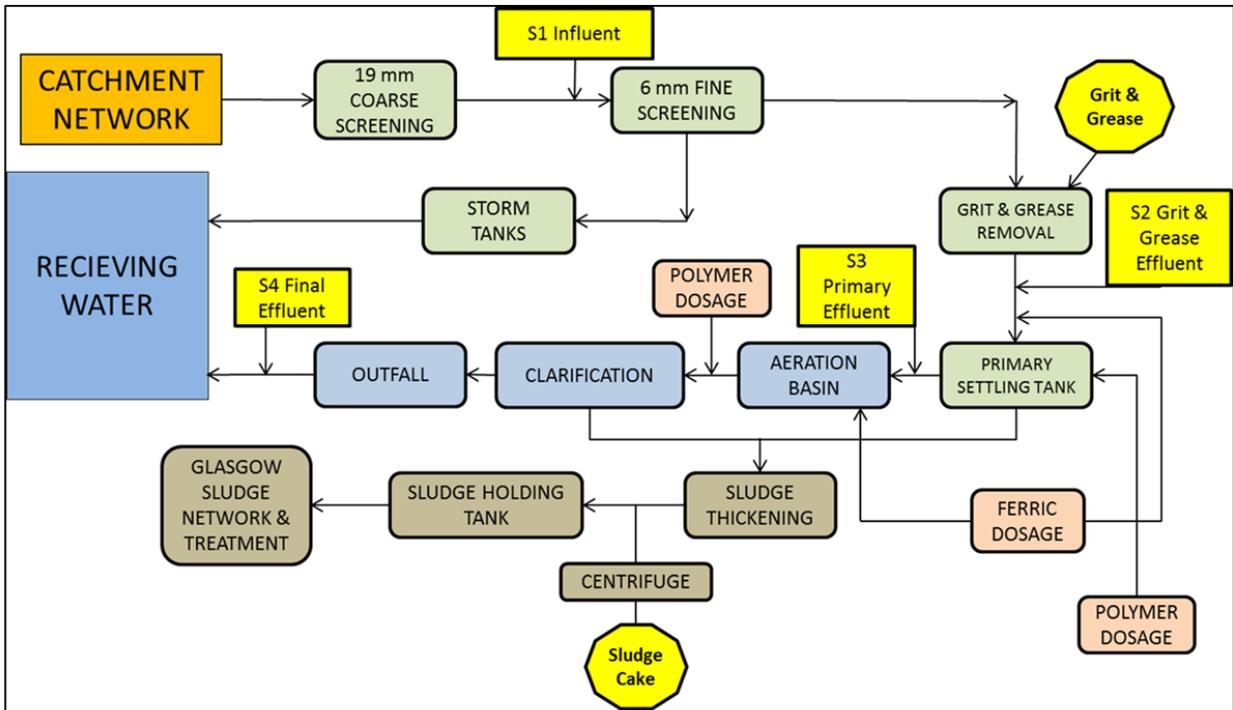
375 **5 Conclusion**

376 The results of this study show that WwTW can be effective in the removal of microplastic from
377 the municipal effluent. However even a small amount of microplastic being released per L⁻¹ can
378 result in significant amounts of microplastics entering the environment due to the large volumes
379 being treated. These treatment processes are standard wastewater treatment practices and are
380 implemented worldwide. The study goes some way to determine what the most important steps in
381 the treatment process are in the removal of microplastics i.e. grit & grease removal and primary
382 settlement and to address the knowledge gaps highlighted by the Norwegian Environmental
383 Report.²⁵ Treatment facilities where these particular processes are less efficient may be making a
384 greater contribution to microplastic pollution in the environment. This will provide important
385 information in the reduction of microplastic pollution in guiding waste management processes. It
386 is also important that future research on microplastics in WwTW takes a site specific approach
387 by detailing any unique practices carried out by the WwTW studied. More research is needed to
388 determine the difference between the ability of primary, secondary and tertiary WwTW to
389 remove microplastics as well as the potential temporal differences in the release of microplastics
390 from this source into the environment.

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394



396 **Figure 1.** (A) Diagram of WwTW showing the location of the liquid fraction sampling sites (S1-
397 4) where: S1 = Influent, S2 = Grit & grease effluent, S3 = Primary effluent, S4 = Final effluent.
398 Sludge cake samples were taken from the same area for both the 24 hr SC duplicate comparison
399 and the comparison between grit and grease. (B) Barplot of the number of microplastic (MP). L⁻¹
400 at each liquid fraction site sampled (S1-4), (error bars = standard deviation, * = significance
401 <0.05). (C) Barplot of the number of MP/2.5g from solid fraction comparison (error bars =
402 standard deviation, * = significance <0.05). (D) Barplot of the number of MP/2.5g sample of
403 24hr SC duplicate (error bars = standard deviation). (E) Barplot of mean length of microplastic
404 (mm) from each study (Liquid Fraction, Solid Fraction & 24hr SC duplicate) conducted (error
405 bars = standard deviation, * = significance <0.05).

406 **Table 1.** Average number of MP Released at each sampling site per day and per year with
407 percentage removal rates based on average outflow of 260,954 m³/Day. S1 = Influent, S2 = Grit
408 & grease effluent, S3 = Primary effluent, S4 = Final effluent.

Site	MP. L ⁻¹	Million MP/Day	Million MP/Year	% Removal
S1	15.70 (±5.23)	4,097 (±1,365)	1,495,397 (±498,395)	0.00
S2	8.70 (±1.56)	2,270 (±406)	828,659 (±148,171)	44.59
S3	3.40 (±0.28)	887 (±74)	323,844 (±26,940)	78.34
S4	0.25 (±0.04)	65 (±11)	23,812 (±4,041)	98.41

409 **Table 2.** The microplastics found in the liquid fraction (S1 = Influent, S2 = Grit & grease effluent, S3 = Primary effluent, S4 = Final
 410 effluent.), solid fraction and 24hr SC duplicate as a percentage of the total plastic found (PET = polyethylenterephthalat, PS acrylic =
 411 polystyrene acrylic, PV Acrylate = polyvinyl acrylate, PVA = polyvinyl acetate, PVC = polyvinyl chloride, PVE = polyvinyl
 412 ethelene).

Polymer	Liquid Fraction (303 MP)				Solid Fraction (79 MP)			24hr SC duplicate (48 MP)			
	S1	S2	S3	S4	Grit	Grease	SC	Day 1		Day 2	
								09:30:00	14:30:00	09:30:00	14:30:00
Acrylic	8.3	12.6	5.9	12.0	0.0	6.8	16.7	12.5	18.2	33.3	15.0
Alkyd	28.7	17.2	20.6	8.0	54.6	13.6	16.7	0.0	27.3	33.3	5.0
PET	3.8	12.6	2.9	4.0	0.0	13.6	0.0	0.0	0.0	0.0	0.0
Polyamide	4.5	2.3	14.7	20.0	9.1	0.0	0.0	0.0	0.0	0.0	10.0
Polyaryl ether	0.0	1.2	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0
Polyester	10.8	13.8	29.4	28.0	27.3	23.7	16.7	25.0	36.4	11.1	30.0
Polyethylene	4.5	1.2	14.7	4.0	0.0	32.2	33.3	0.0	9.1	0.0	5.0
Polypropylene	2.6	1.2	5.9	12.0	0.0	5.1	0.0	12.5	0.0	22.2	20.0
Polystyrene	2.6	17.2	5.9	4.0	9.1	1.7	16.7	37.5	0.0	0.0	10.0
Polyurethane	8.9	8.1	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0
Polyvinylfluride	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PS Acrylic	19.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PV Acrylate	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PVA	3.2	10.3	0.0	4.0	0.0	1.7	0.0	12.5	9.1	0.0	0.0
PVC	1.3	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PVE	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

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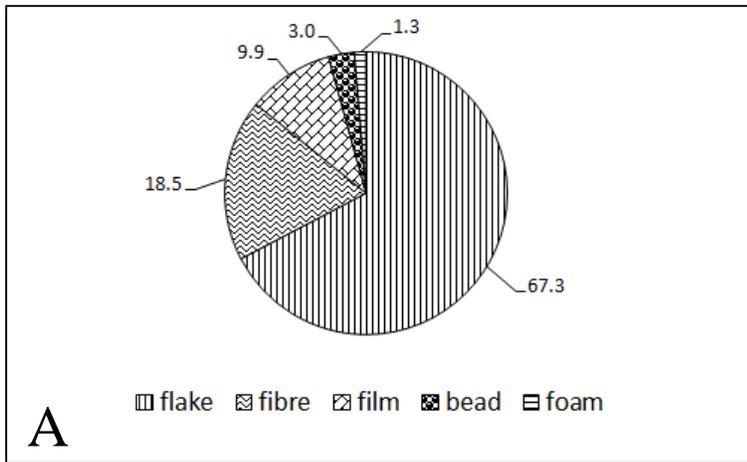
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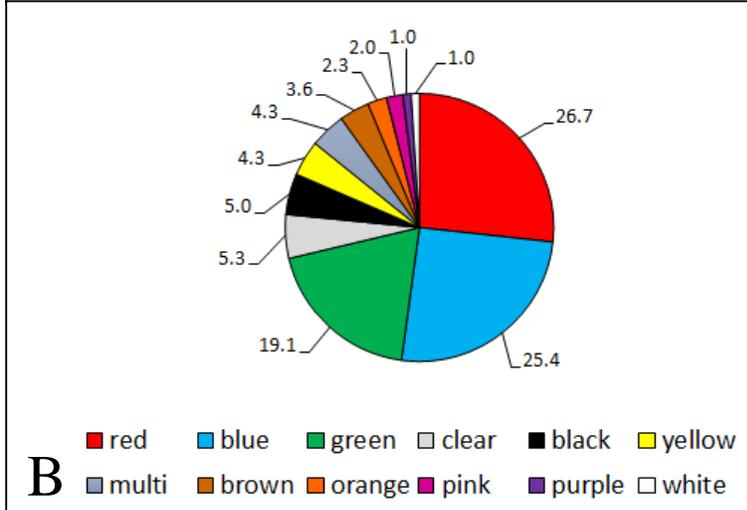
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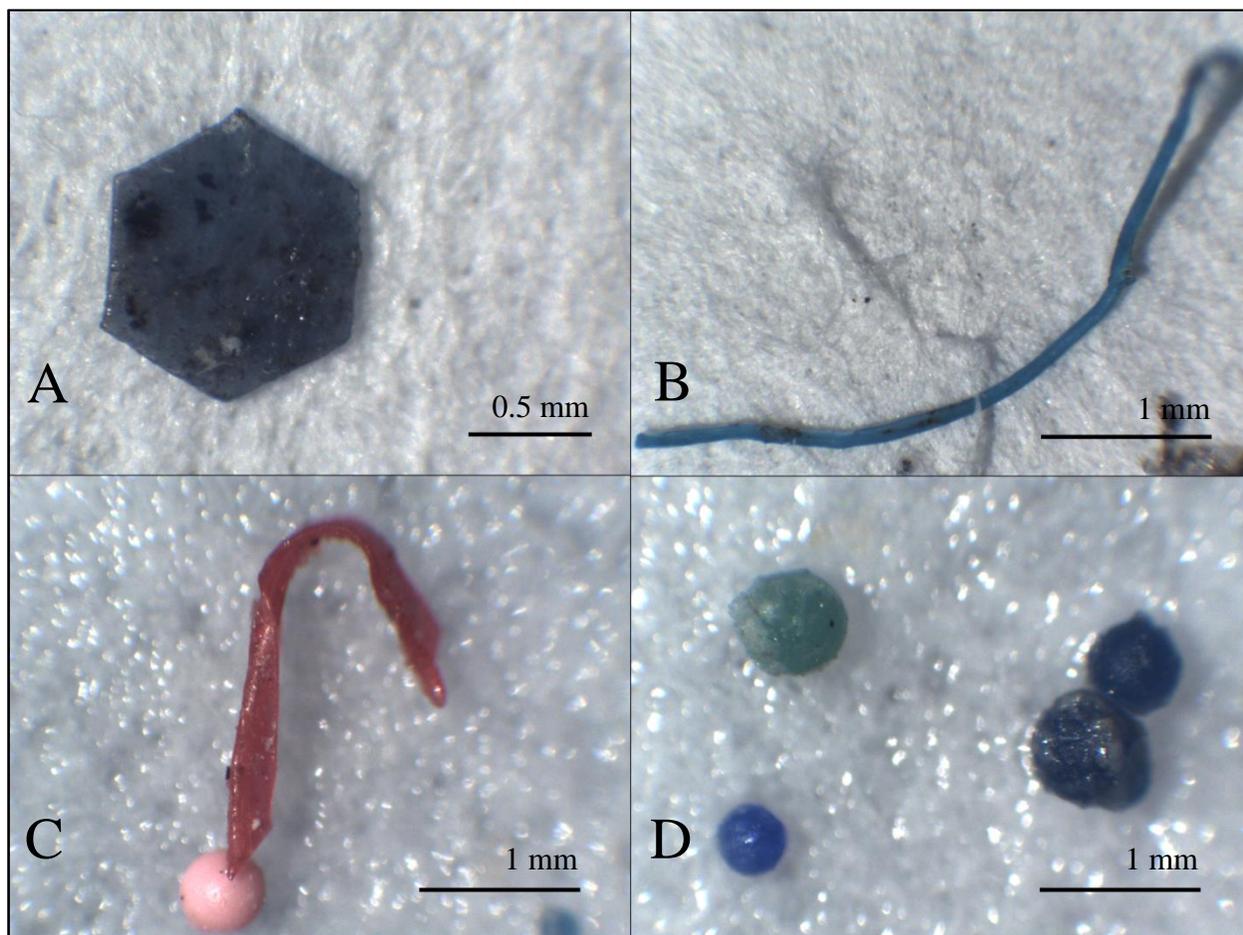
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425 **Figure 2.** Pie charts of the different types (A) and colour (B) of microplastic found in all of the
426 liquid fraction samples examined from a secondary WwTW. Results shown as a percentage.

427



428

429 **Figure 3.** Photos of microplastics found in the solid fraction taken from a secondary WwTW.
430 (A) Alkyd fragment taken from the sludge cake (SC) fraction. (B) Polypropylene fibre taken
431 from the grit fraction. (C) A single polyethylene microbead and red PET fragment taken from the
432 grease fraction. (D) 4 polyethylene microbeads extracted from the grease fraction.

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532 **TOC Art**



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