

1 Occurrence of microplastics in bottled water from Croatia: A 2 Raman spectroscopy approach

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10 **Abstract:**

11
12 The occurrence of microplastics (MPs) in bottled water is still largely unexplored in Croatia. This study fills this
13 gap by analysing six water brands available on the Croatian market, all bottled in either untreated or recycled
14 polyethylene terephthalate (PET). In order to analyse microplastics down to a size of 1 μm , the water from the
15 purchased bottles was filtered with silicon filters with a pore size of 1 μm and then micro-Raman spectroscopy
16 was performed. A significant reduction in analysis time was achieved by using a randomly selected filter area of
17 12.05 mm^2 instead of the conventional 100 mm^2 . The results showed that polyethylene (PE), PET, and polyamide
18 (PA) were found in all six Brands and the Blank. The highest detection frequency was found for PE (N=19),
19 followed by PET (N=17), and PA (N=14). Polyvinyl chloride (PVC) was found only in one Brand, but in all three
20 subsamples, packaged in recycled PET. By far the highest detection frequency of MPs occurred in the smallest
21 fraction at a length $<5 \mu\text{m}$. The highest number of MPs was for PET with 45% of the total amount, followed by
22 PE (37%). Although MPs were identified in the Blank water sample, the number was 12% of the total.
23 Interestingly, the bottled water in virgin PET containers had more MPs than that in recycled PET. It should be
24 noted that the purification system used to produce ultrapure water produced MPs, which poses an additional
25 analytical challenge. The results should be considered as a snapshot and further monitoring is necessary to assess
26 the extent of MP contamination of drinking water and to characterise the types of polymers and sources of the
27 MPs. The current findings raise concerns about the presence of MPs in bottled water and warrant a thorough risk
28 assessment to evaluate potential long-term health effects on humans.

29
30 **Keywords:** microplastics (MPs), mineral water, micro-Raman, polymers, contamination, MP size

33 1 Introduction

34 The global demand for plastics is increasing year on year due to its many beneficial properties, including
35 affordability, flexibility, strength, light weight, water resistance, ease of maintenance and sterilisation, and
36 excellent insulating properties (WHO 2022). In 2020, 367 million tonnes of plastic were produced worldwide,
37 generating 29.1 million tonnes of waste, of which only 9% is recycled (Lebreton and Andrady 2019, Patrício Silva
38 2021). It is worrying that a large proportion of these plastics are thrown away after they have fulfilled their purpose
39 (Geyer et al. 2017). As soon as plastics are released into the environment, they are broken down and decomposed,
40 producing smaller particles (Shi et al. 2023). The definition and properties of microplastics (MPs) have been
41 debated for several years. However, according to Gigault et al., MPs refer to plastic particles with a size of less
42 than 5 millimetres and a lower limit of 1 μm (Gigault et al. 2018). The term microplastic covers both intentionally
43 produced microplastics, known as primary microplastics, and secondary microplastics, which are created by the
44 degradation and fragmentation of macroplastics through external factors such as UV radiation, mechanical forces
45 and biological activity (Schymanski et al. 2021). Due to their widespread contamination and presence in food,
46 MPs have become a growing concern for both the scientific community and the public (Ziani et al. 2023). The
47 intentional use of nano- to micro-sized plastic particles and their unintentional formation and release into the
48 environment pose significant risks to ecosystems and are increasingly coming to the attention of the public (Dube
49 and Okuthe 2023, Ramsperger et al. 2023).

50 Numerous studies have reported on the presence of MP in drinking water, air, food and beverages,
51 highlighting the potential health risks resulting from exposure to these particles, as well as the chemical toxins
52 and biological pathogens they may contain (Vethaak and Leslie 2016, Ohlwein et al. 2019, Li et al. 2024,
53 Winiarska et al. 2024). MPs are found in various environments, including oceans (Andrady 2011, Reisser et al.
54 2013, ter Halle et al. 2016, Kwon et al. 2020), rivers (Horton et al. 2017), soils (Wang et al. 2019) and air (Dris et
55 al. 2015, Zhang et al. 2020). Due to their harmful effects on ecosystems and potential health risks for humans and
56 wildlife, MPs have become a global environmental problem. Along with inhalation, MPs ingestion is one of the
57 main routes by which MPs enter the human body (van Raamsdonk et al. 2020). As a result of environmental or
58 process contamination, they have been found in a variety of foods, including fish products (Bessa et al. 2018,
59 Smith et al. 2018), fruits and vegetables (Oliveri Conti et al. 2020), as well as milk, soft drinks, beer, honey and
60 packaged foods (Diaz-Basantés et al. 2020, Kedzierski et al. 2020). A recent report describes MPs as a new
61 pollutant that has been detected in the environment and in biological systems, although the risks to human health
62 are still unclear (Tang et al. 2024). The report focuses on exposure assessment, hazard identification and risk
63 characterisation and concludes that MP trigger oxidative stress, disrupt metabolism and impact gut, immune and
64 reproductive health. In addition, additives such as phthalates act as endocrine disruptors, while interactions with
65 other pollutants can exacerbate these risks.

66 According to the UN University Institute for Water, Environment and Health (Hamilton, ON, Canada),
67 the bottled water market has grown by 73% in the last ten years, with global sales of bottled water reaching 350
68 billion litres in 2021 (Brandt et al. 2020, Bouhleb et al. 2023). The consumption of bottled water is high in high-
69 income countries, even if they have a high-quality tap water supply. One of the reasons for the high dependence
70 on bottled water is the belief that bottled water is healthier than tap water. Numerous studies on the contamination
71 of bottled water have shown the presence of MPs in various brands available worldwide. The number of MPs in
72 bottled water is more variable than in tap water (Gambino et al. 2022). This number varies from 8.5 to 6292
73 particles/L of bottled water (Mason et al. 2018, Cox et al. 2019, Kankanige and Babel 2020, Makhdoumi et al.
74 2021, Schymanski et al. 2021). Nevertheless, the results of all studies were similar. The number of particles
75 increases with decreasing particle size (Oßmann et al. 2018, Schymanski et al. 2018, Winkler et al. 2019). The
76 results of the various studies vary with regard to the type of polymer. There is evidence that the largest proportion
77 of MP in bottled water consist of polypropylene (PP) (54%), nylon (16%), polystyrene (PS) (10%), polyethylene
78 (PE) (10%) and PEST (polyester + polyethylene terephthalate, 6%) (Mason et al. 2018). A study has shown that

79 the type of polymer used varies depending on the type of bottle (OBmann et al. 2018). Polyethylene terephthalate
80 (PET) is the predominant material in plastic bottles, while PE and styrene copolymers are more common in glass
81 bottle packaging. Other studies have shown that PET is the most commonly used polymer type, followed by PP
82 and PE (Schymanski et al. 2018, Kankanige and Babel 2020). Research from 2022 suggests that MP in bottled
83 water often originates from the packaging material itself, with reuse potentially increasing MP levels (2022).
84 Photodegradation can also lead to the embrittlement of plastics and is one of the main causes of the fragmentation
85 of plastics and the formation of secondary MP (Andrady 2011, Ha and Yeo 2018, Chamas et al. 2020).

86 Although it is not known whether MPs are dangerous to humans, a growing body of research suggests
87 that MPs can physically clog the gastrointestinal tract and subsequently deposit in the body (Li et al. 2024). MPs
88 less than 5 μm in size can enter the bloodstream and cause haemolysis of red blood cells, while MPs larger than
89 10 μm in diameter have shown no effect (Hwang et al. 2019). The many additives that are added to plastic
90 polymers during product processing and manufacturing can be potentially hazardous and cause oxidative stress,
91 endocrine disruption and impaired lipid metabolism (Hermabessiere et al. 2017, Pérez-Albaladejo et al. 2020).
92 The size of the MPs also influences the toxic effect, i.e. the smaller the particles, the stronger the effect. It has
93 been shown that MPs exhibit the highest toxicity in human cell lines, while large particles ($> 100 \mu\text{m}$) showed no
94 effect in experiments with fish (Jacob et al. 2019). The recent review emphasises that MPs are ubiquitous in the
95 environment and are frequently found in both environmental and human samples. It points to several routes of
96 exposure for humans, including ingestion, inhalation and dermal contact. Toxicity studies with cells, organoids
97 and animals show that MPs can lead to oxidative stress, DNA damage, organ dysfunction, metabolic problems,
98 immune responses, neurotoxicity and reproductive and developmental toxicity. Epidemiological studies also
99 indicate a possible link between chronic diseases and exposure to microplastics Yue Li (2023).

100 The aim of this study is to assess the presence, type of polymer and quantity of MP in commercial bottled
101 water brands on the Croatian market. As the presence of MPs in Croatian bottled water is still largely unexplored,
102 this study fills an important knowledge gap. When analysing MPs, Raman spectroscopy can identify particles
103 down to 1 μm in size by detecting and assigning specific vibrational modes, which appear as peaks in the Raman
104 spectrum.

105 2 Materials and methods

106 2.1 Identity of samples and designation

107 There were six brands of bottled water, later referred to as Brand W1 through W6; all with a volume of
108 0.5 L bought in grocery stores in Zagreb, Croatia. From each brand, three 0.5 L bottles, referred to as subsamples
109 'SS1', 'SS2', and 'SS3', were bought, assuming that they were of the same origin. All samples were stored in a
110 refrigerator before analysis.

111 In total, there were 18 bottles of commercially available mineral water; these are referred to as 'Sample'.
112 The mineral water from Brands W1, W2, and W6 were packaged in recycled PET bottles (referred to as 'recycl'
113 and coloured with reddish colours in visualization plots), the waters from Brands W3-W5 in virgin PET bottles
114 (coloured with greenish colours in visualization plots). For quality control, one blank sample, referred to as
115 'Blank' (coloured in blue) consisting of three subsamples of 0.5 L of freshly prepared ultrapure water was
116 analysed. Since there was no container, this sample is referred to as 'none'.

117 Further the samples were characterized by the length in μm of the MPs identified. The lengths were
118 grouped into increments of 5 μm or 10 μm and designated LengthCodes 'a' through 'e'. The SampleIDs contain
119 the Brand (W1-W6) followed by the subsample designation (SS1-SS3) and the LengthCode (a-e). The number of

120 final samples is 57 when grouped by Brand, subsample, and fractions according to length of the MPs; the summary
 121 is shown in Table 1.

122 Table 1: Summary of sample characteristics. ‘recycl’ refers to the samples packaged in recycled PET
 123 bottles, while ‘virgin’ refers to samples packaged in virgin PET bottles. SS1, SS2 and SS3 refer
 124 to subsamples.

Brand of water	W1	W2	W3	W4	W5	W6	Blank	Overall
Number of samples	(N=10)	(N=8)	(N=8)	(N=10)	(N=10)	(N=7)	(N=4)	(N=57)
Number of SampleIDs according to Container								
recycl	10	8				7		25
virgin			8	10	10			28
none							4	4
Number SampleIDs in Subsample								
SS1	3	2	4	4	5	2	1	21
SS2	3	4	2	4	3	2	1	19
SS3	4	2	2	2	2	3	2	17
MPs identified at given Length (LengthCode in parenthesis)								
L<5 (a)	3	3	3	3	3	3	3	21
L_5-9.99 (b)	3	3	2	2	3	3	1	17
L_10-14.9 (c)	3	1	1	2	1	1	0	9
L_15-19.9 (d)	0	1	2	3	2	0	0	8
L_20-24.9 (e)	1	0	0	0	1	0	0	2

125

126 2.2 Preparation of the samples

127 To avoid the entry of airborne particles, sample preparation is carried out in a laminar flow box. The
 128 samples obtained, i.e. the filters, are stored in aluminium boxes previously washed with ethanol and ultrapure
 129 water (stakpure, Niederahr, Germany). A 100% cotton lab coat and particle-free nitrile gloves were used for all
 130 filtration and handling procedures. The use of plastic parts was avoided throughout sample handling, with the
 131 exception of Teflon supports and Teflon filter covers on the filtration system. If Teflon was found, it was excluded
 132 from the total number of polymers. The glassware was treated in an ultrasonic bath with ethanol for at least 5
 133 minutes and ultrapure water for a further 5 minutes, then placed in an oven at 450 °C for 24 h and stored in a
 134 laminar flow box. All chemicals used for the experiment were of analytical purity and obtained from Gram-mol,
 135 Zagreb, Croatia. High purity water (18 MΩ cm⁻¹) was used for all experiments.

136 The entire volume of each mineral water was mixed by inverting the bottle and then poured into a glass
 137 funnel. The water was filtered under vacuum through a Si filter. Macroporous silicon lift-off membranes (Si
 138 filters) with a pore diameter of 1 μm and a pore length of 200 μm were supplied by Smart Membranes (Halle,
 139 Germany). The vacuum was turned off and the Si filter was carefully removed to avoid scratching the filter
 140 surface. The samples were air-dried in the laminar flow. Once completely dry, they were stored in aluminium
 141 boxes for analysis.

142 2.3 Identification and quantification of microplastics with μ-Raman 143 spectroscopy

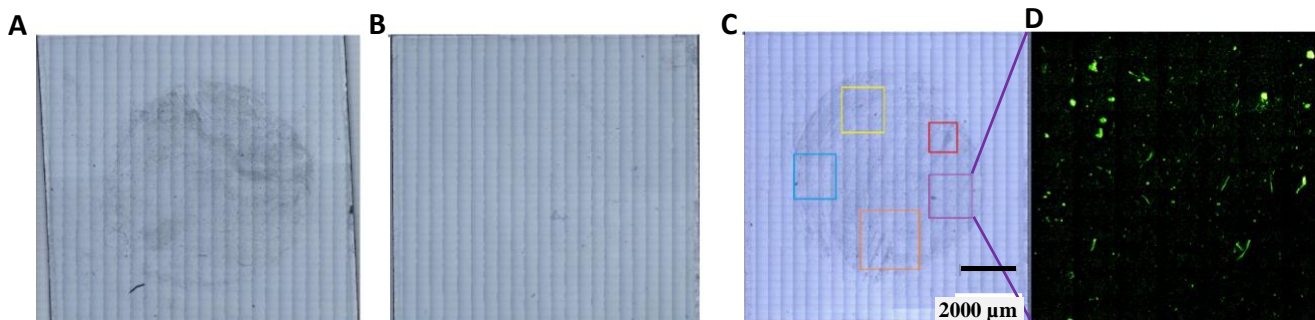
144 Microplastics were identified and quantified using a μ-Raman spectrometer (Renishaw Plc, Wotton-
 145 under-Edge, UK) by analysing their characteristic vibrational bands. Each polymer type had unique spectral
 146 properties that enabled accurate identification. Identification and quantification of the MPs followed the procedure
 147 described in the literature (Brandt et al. 2021, Schymanski et al. 2021, El Khatib et al. 2023). In brief, the dried
 148 particles were analysed directly on the filter surface using the Renishaw inVia system for automatic particle
 149 counting and identification, which is controlled by the Wire 5.5 software (Renishaw Plc, Wotton-under-Edge,

150 UK). A laser with a wavelength of 532 nm was used as the excitation laser, which offers a good balance between
151 sensitivity and resolution for the detection of MPs. The filters were first fully scanned in white light at 20×
152 magnification to visualise the collected particles on the Si filter as clearly as possible. Areas were then selected
153 for measurement and scanned in dark field (Fig. 1). When using the dark field mode, the background appears
154 black and the particles were illuminated to maximise the contrast between the substrate and the particles.
155 Identifying all particles on the 100 mm² filter area would be incredibly time consuming and tedious, so five areas
156 were selected for analysis.

157 According to the literature the most advisable subsampling strategy is the random model, therefore 5
158 randomly positioned areas were selected on the entire filter (Schymanski et al. 2021). The results obtained are
159 then extrapolated to express the data as the number of MPs in 1 L of bottled water. The spectroscopic parameters
160 were set as follows: a wavelength of 532 nm, a laser power of 20 mW, a grating of 2400 l/mm, an acquisition time
161 of 0.5 s, and a total of 3 accumulations. For image acquisition, the measurements were performed with a 20×
162 objective. The spectra were recorded in the range between 1050 cm⁻¹ and 3200 cm⁻¹ with a spectral resolution of
163 0.3 cm⁻¹. All particles > 1 µm on the analysed filter were analysed according to the criterion of equivalent circle
164 diameter > 1 µm. A polynomial baseline subtraction was applied to all spectra. The last step of the spectrum
165 processing is the direct comparison with a selected reference spectra library. A self-created spectra database with
166 over 75 spectra of polymers, materials used in laboratories and inorganic substances was used for the spectra
167 search. Spectral matches that had an HQI >70% were considered MP candidates and manually checked by a
168 trained interpreter to determine their validity (Woodall et al. 2014, Yang et al. 2015, Schymanski et al. 2021).
169 Attempts were made to identify and assign further polymers, but the quality of the hits was insufficient.

170 2.3.1 Extrapolation

171 To ensure comparability, an equal percentage of the area of each sample is analysed, i.e. a total of 12.05
172 mm² is analysed, which corresponds to 7.3% of the total filter area (Fig. 1). The decision to analyse a specific part
173 of the filter surface represents a practical balance between maintaining representativeness and managing the time-
174 consuming scanning process required to detect MPs. Imaging analysis of MPs is inherently time consuming,
175 especially when scanning at high magnification to detect small particles. Considering that typical MP analyses
176 often scan areas of less than 1 mm × 1 mm, increasing the size of the analysed area would significantly increase
177 the time and resources required, potentially limiting the practicality of routine testing. By selecting 12.05 mm² of
178 the total surface area, this study captures a representative subset while avoiding the resource-intensive
179 requirements of full-area scanning. To reduce potential bias due to the reduced area, a random subsampling model
180 recommended by Schymanski et al. was applied to ensure a representative selection of MP across the entire filter
181 area (Schymanski et al. 2021). This random selection reduces subjective errors and allows the analysed subset to
182 reflect the broader distribution of MP. In this context, five areas of the sample were analysed using a random
183 method (Figure 1c) and examined for the presence of microplastic particles. As only a portion of the filter surface
184 was analysed, extrapolation of the data was required to estimate the MP content for the entire sample, facilitating
185 comparison with other studies. Although there is an inherent trade-off between scan time and area analysed, this
186 approach achieves a balance between efficiency and accuracy that allows reliable detection of particles as small
187 as 1 µm in a representative area of each sample. In addition, descriptive statistical analyses, including the
188 calculation of mean and standard deviation, provide a reliable summary of the MP distribution in the sample.



189
 190 Figure 1: Microscopic images of filtered samples W5 (a) and W6 (b) with different particle distributions
 191 along their surfaces; Example of sampling area selection (squares in different colours) (c) and a dark-field
 192 image of the sampling area (d).
 193

194 2.4 Quality assurance and quality control

195 Precautions were taken to limit contamination of samples by airborne MP, with contamination control
 196 measures implemented at each step of sample processing. Plastic utensils were avoided and replaced wherever
 197 possible with metal and glass utensils which were rinsed with Milli-Q water prior to use. In all laboratory
 198 procedures, contact of samples, instruments and reagents with air and plastic surfaces was minimised by covering
 199 them with Milli-Q rinsed aluminium foil before and after use. After filtration in the laminar flow, filters were
 200 stored in metal boxes that had previously been rinsed with Milli-Q water. To validate the effectiveness of the
 201 contamination prevention, a blank sample was processed (filtered) and analysed in the same way as the other
 202 samples. A random subsampling model was used to ensure a representative selection of microplastic particles
 203 across the filter area, minimising potential bias and subjective error. Descriptive statistical analyses, including the
 204 calculation of mean and standard deviation, provided a reliable summary of the microplastic distribution in the
 205 sample.

206 Commercially available polystyrene spheres (PS) (Polysciences, Inc, Warrington, USA) with known
 207 concentrations were used to evaluate the recovery rates. The average particle size of these spheres was 10 or
 208 40 μm. A certain amount of particles of a specific size was added to the ultrapure water and subjected to the same
 209 procedure as for the samples. The MPs were collected using a Si filter. The number of these microplastic spheres
 210 was determined using Raman spectroscopy (Renishaw Plc, Wotton-under-Edge, UK). The recovery rate of
 211 microplastics of each size was expressed as the ratio between the number of microplastic spheres recovered and
 212 the number of microplastic spheres added. The protocol showed a recovery rate of 106% and 107% for PS beads
 213 of 40 and 10 μm respectively. These values can be explained by the fact that PS sphere size standards were used
 214 and their concentration is not an exact value. However, no corrections were made to the number of microplastics
 215 based on the recovery rates obtained. The lack of standards for different polymer sizes and types for analysing
 216 MP makes it difficult to accurately identify, quantify and compare MP contamination in different studies.

217 2.5 Statistical analysis

218 Statistical analyses and visualisations were performed using R version 4.4.1 (as of 2024-06-14) (R Core
 219 Team, 2024¹) with R-Studio (version 2024.12.0 Build 467; Posit Software, PBC). Following normality test using
 220 histogram and density tests, the samples did not show a normal distribution. Non-parametric tests were performed
 221 using the Kruskal-Wallis H-test to determine if there were statistically significant differences between the

¹ reference for R

R Core Team (2024). *R: A Language and Environment for Statistical Computing*. R Foundation for
 Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>>.

222 independent variables and the dependent variables. Post-hoc analysis was performed using the pairwise Wilcoxon
223 test. Adjustment of the p-value was made using the Benjamini-Hochberg method. The significance level was set
224 at $p=0.05$. The correlation between the variables was determined using the Pearson method.

225 Hierarchical cluster analysis (HCA) was used to assess similarities, correlations or differences between
226 datasets and metadata. It was used to find a natural grouping (clustering) of the dataset to achieve less variation
227 (greater similarity) within a group (cluster) and more variation (less similarity) between groups (clusters). The
228 clustering was performed using Euclidean distances and the Ward method. This method is used to form groups in
229 which the variance within the groups (clusters) is minimised. The data in a set are grouped into clusters with high
230 similarity to form a dendrogram. For statistical operations, the number of particles below the limit of quantification
231 (LOQ) was set to zero.

232 3 Results and discussion

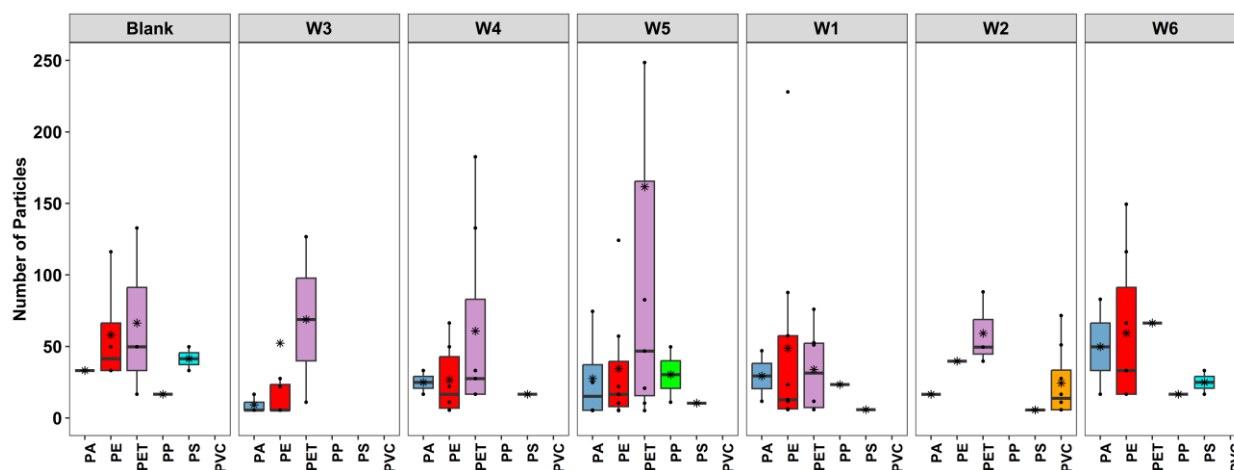
233 In total, 18 bottles of water from six Brands were analysed together with three subsamples of ultrapure
234 water. Therein, six MPs of the polymers PA, PE, PET, PP, PS, and PVC, could be identified by μ -Raman
235 spectroscopy. The MPs identified were grouped according to their lengths as described in section 2.1 and Table
236 1. The detailed SampleIDs and associated information as to subsample, length of particles identified as well as
237 the number and polymer type are shown in Table S1. From the Table S1a, it can be seen that there were 15
238 subsamples where no MP particle could be detected. All these waters were either from virgin PET packaging
239 ($N=9$, Brands W3, W4, and W5) or from the Blanks ($N=6$). Three more subsamples were excluded from further
240 consideration (Table S1b) whereby in the Sample W5_SS1 five PET particles were identified at a length of 60 μm -
241 69.9 μm , which was classified as an outlier due to low number of particles and the unusual length. In the two
242 samples WA_SS3 and W5_SS1, no particles could be identified automatically but manually.

243 3.1 Quantitative results

244 3.1.1 Brands and polymers

245 The summary of MPs by polymer in the six bottled waters and the Blank are shown in Figure 2. Each
246 boxplot contains the results from the three subsamples of the respective Brand (including the Blank). The samples
247 were either without a container (labelled as “none”, Blank), in virgin PET bottles (labelled as “virgin”; samples
248 W3, W4, and W5) or in recycled PET bottles (labelled as “recycl”, samples W1, W2, and W6). It can be seen
249 that three polymers, namely PE, PET, and PA were identified in all Brands (including the Blank). For easy
250 visualization, four pie charts displaying the average composition of the six polymers in the four groups of water
251 according to the container (none, virgin, recycle) and the overall composition across all samples is contained in
252 the supplementary information as Figure S1. It is further noticed that the values for PET in the water packaged in
253 virgin PET bottles, W3, W4, and W5, have large standard deviations around the mean value (for details, see Table
254 S 2).

255 The sum of particles per polymer and the sum of total MP polymers in the three subsamples of each
256 Brand are displayed in Figure S 2, while Figure S 3 shows stacked bar graphs of particles polymer in the
257 subsamples, coloured according to Brand.



258

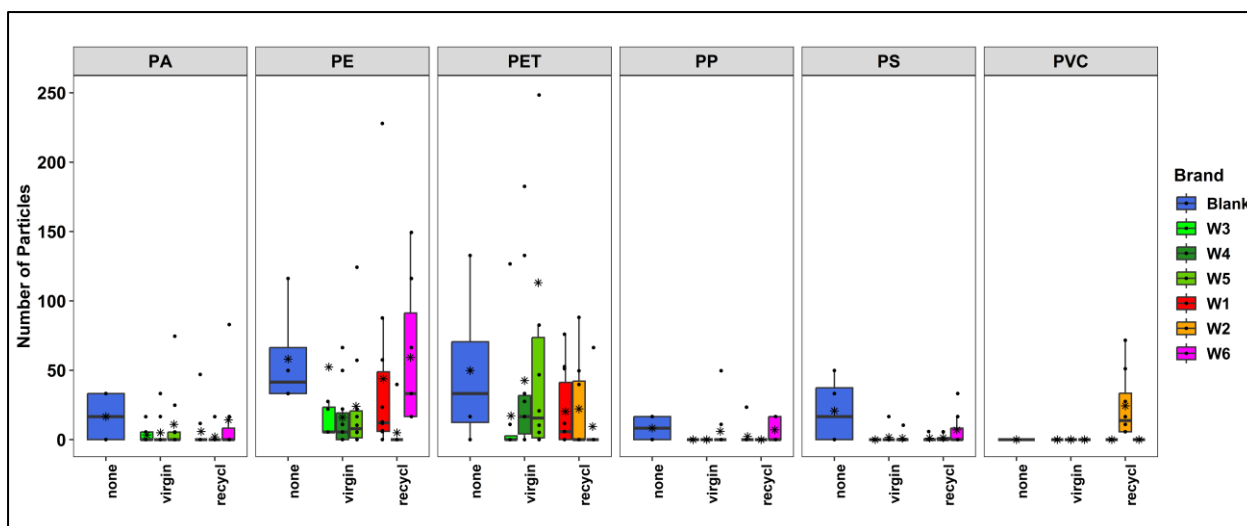
259 Figure 2: Unscaled box whisker plots displaying the number of MPs by polymer in the six Brands of
 260 bottled water and the Blank. Brands W3, W4, and W5 refer to water bottled in virgin PET,
 261 Brands W1, W2, and W6 to water bottled in recycled PET.
 262 The whiskers represent the minimum and maximum concentrations without the outliers. The
 263 lower border of the box represents the first quartile (25%) and the upper border the third quartile
 264 (75%). The line inside the box is the median value and the asterisk the mean value. The dots
 265 outside the whiskers are outliers, defined as all concentrations greater or smaller than the
 266 interquartile range multiplied by 1.5.

267

268 The statistical summary as box plots with respect to the container is shown in Figure 3. PVC was present
 269 in water bottled in recycled polymers; in W2 in all three subsamples, but in none of the subsamples of W1 and
 270 W6 (N=6).

271 Overall (across all 57 samples), a median value of zero was found for PA, PP, PS, and PVC. Grouped by
 272 container, the median value was zero for PA, PP, and PS in virgin and recycled, and for PET in recycled. For
 273 PVC, the median value was zero for all three containers. PE was the only polymer with a median value above
 274 zero in all three container and overall (11.0 particles/L). Also, PET had an overall median value above zero (5.2
 275 particles/L). With respect to the mean values, the overall mean value for PET was higher than for PE (41.1
 276 particles/L vs. 34.1 particles/L). It should be noted that only for PET, there was a large difference between the
 277 mean values for virgin and recycled containers (60.5 particles/L vs. 17.9 particles/L), whereas for all other
 278 polymers there was not much difference recorded.

279 The Blank samples often had higher mean and median values, especially for PE and PET, than the bottled
 280 waters, indicating that the water purification system may be the cause of MPs.



281

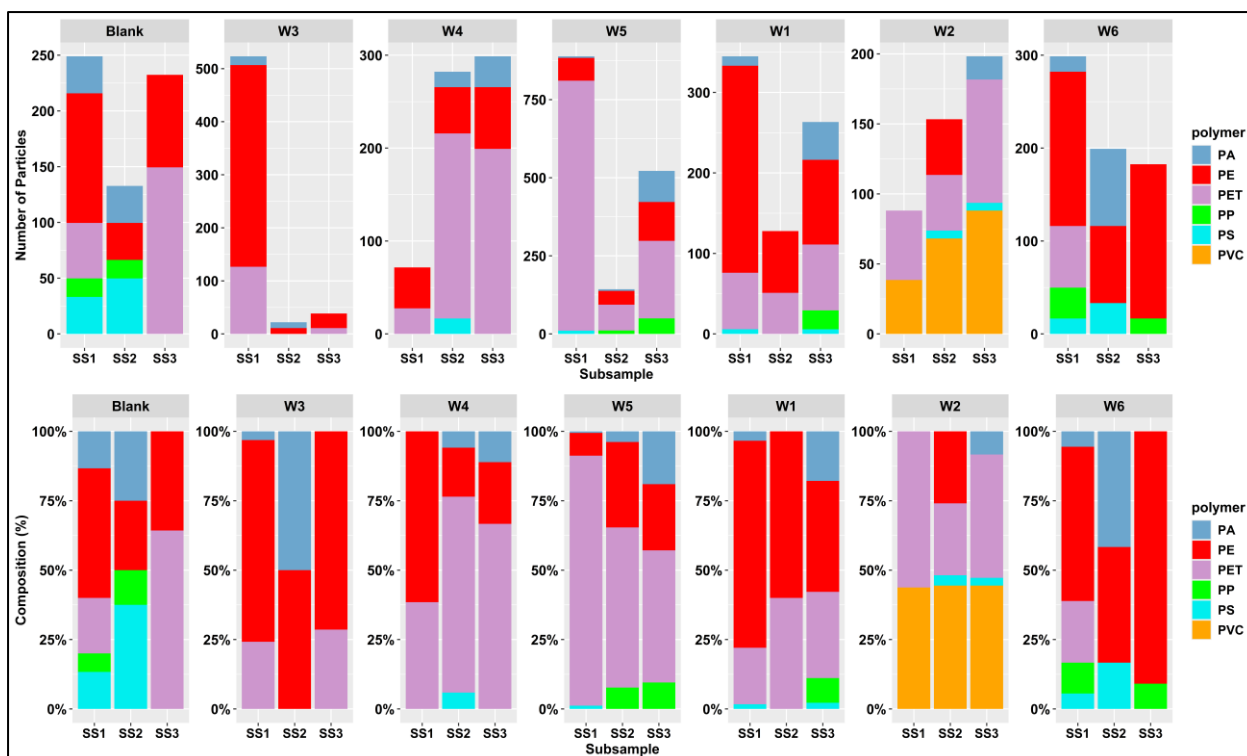
282 Figure 3: Unscaled boxplots for containers by polymer (coloured by Brand). Brands W3, W4, and W5 refer to
 283 water bottled in virgin PET (green colours), Brands W1, W2, and W6 to water bottled in
 284 recycled PET (red/orange colours).
 285 The whiskers represent the minimum and maximum concentrations without the outliers. The
 286 lower border of the box represents the first quartile (25%) and the upper border the third quartile
 287 (75%). The line inside the box is the median value and the asterisk is the mean value. The dots
 288 outside the whiskers are outliers, defined as all concentrations greater or smaller than the
 289 interquartile range multiplied by 1.5.

290

291 3.1.2 MPs in subsamples

292 The total number of MPs assigned and identified was 5 260, for details refer to Table S1. The results are
 293 visualized in Figure 4 for the three bottles (subsamples) for each Brand and the Blank sample in scale and pattern.
 294 In addition, Figure S 3 in the supplementary information assigns the Brands and subsamples to the polymers.
 295 From both figures, it must be noted that PE (W2_SS2) and PA (W2_SS3) were identified in only one of the
 296 subsamples whereas PET was found in all three subsamples. On the other hand, W2 is the only Brand where PVC
 297 was identified, consistently in all three subsamples. Visual inspection of the bar graphs shows that the scale of the
 298 MP particles varies highly between bottles. Also, the total number of MPs in the Blank subsamples varies highly
 299 (Blank_SS2 (133 particles/L) has only about half of the particles found in Blank_SS1 (249 particles/L) and
 300 Blank_SS3 (232 particles/L)).

301 The polymer pattern of the MPs in the lower row of Figure 4 shows the dominance of PET, especially in
 302 brands W4 (65% of all polymers) and W5 (73% of all polymers), both waters packaged in virgin PET, whereas
 303 PE is more abundant in the water bottled in recycled PET (W1 accounting for 60% and W6, accounting for 61%).
 304 The presence of PVC in the three subsamples of only Brand W2 is very constant. PVC accounts for 44% of the
 305 polymers in the sample and PET for 40% (across the three subsamples).



306

307

Figure 4: Stacked barplots of six MP polymers according to Brand and Subsample.

308

Brands W3, W4, and W5 refer to water bottled in virgin PET, Brands W1, W2, and W6 to water bottled in recycled PET.

309

310

311

Figure 5 shows the number of MPs in each subsample for each Brand together with the calculated average value for the Brand. The highest single value was found in W5 with 889 MP particles (subsample SS1). This Brand also had the highest average value with 518 particles/L water. The individual values in the three subsamples were 889 particles in SS1, 143 particles in SS2, and 522 particles in SS3. Notably, W5 was water bottled in virgin PET. Brand W2 had the lowest average particle count with 146 particles and was about 4 times smaller than W5. The detailed composition, broken down by particle length, can be found in Table S1.

312

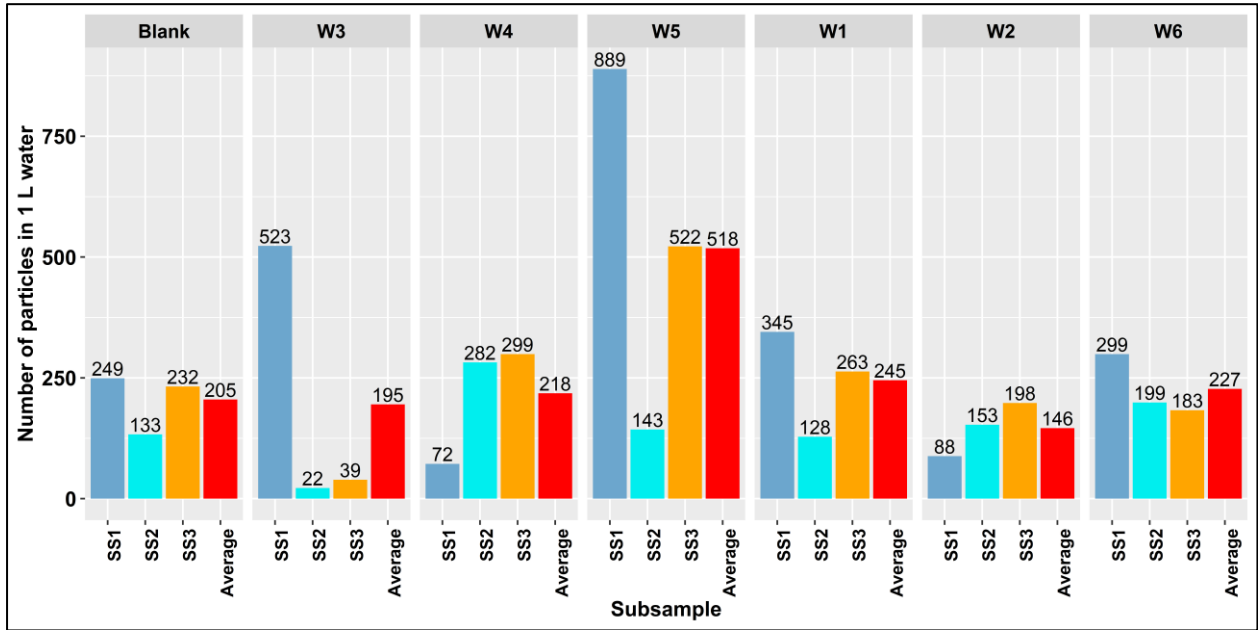
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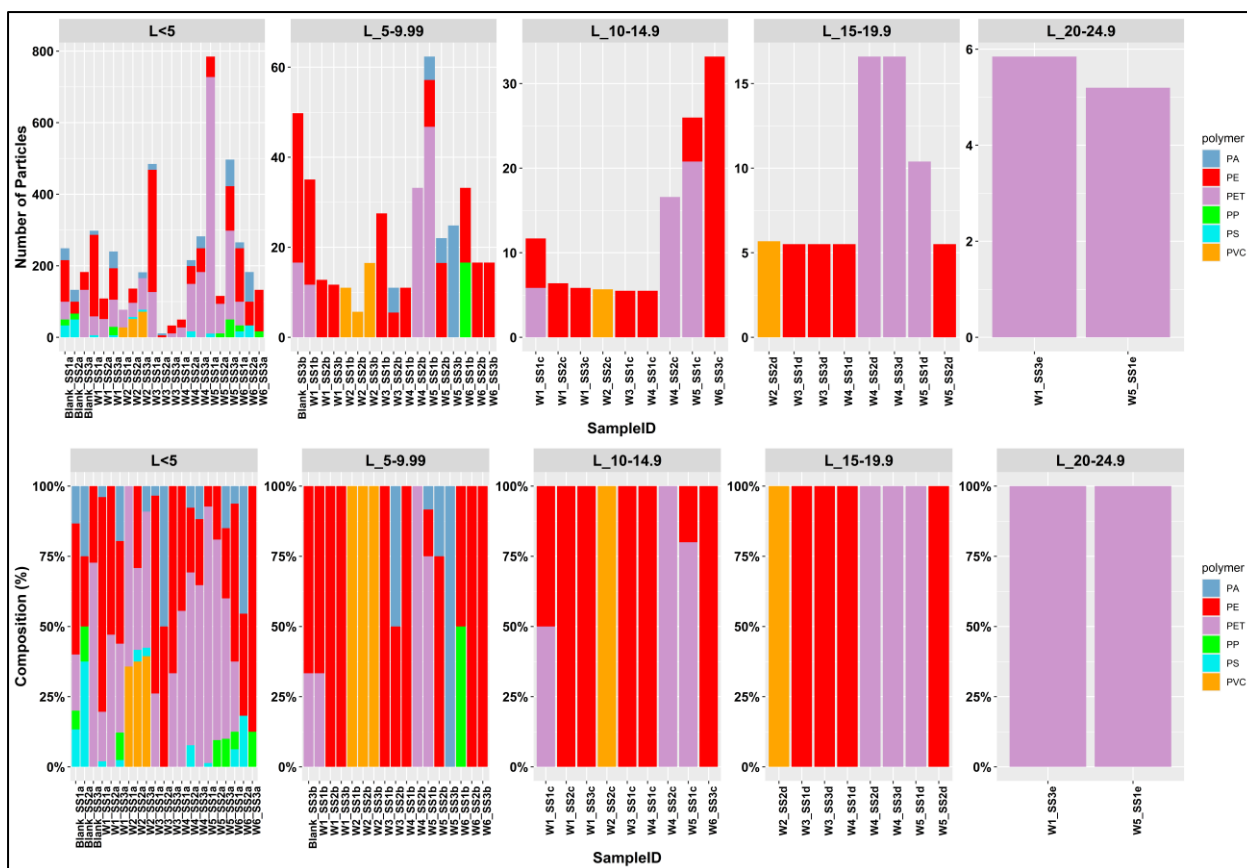
318

319 Figure 5: Sum of polymer particles per subsample and (calculated) average value (red colour) of each Brand (all
320 numbers refer to 1 L of water).
321 Brands W3, W4, and W5 refer to water bottled in virgin PET, Brands W1, W2, and W6 to water
322 bottled in recycled PET.

323

324 3.1.3 Size distribution

325 The total number of 5 260 identified particles in the 57 samples and their distribution are shown in Figure
326 6. The upper panel shows the number of microplastic particles by size class in different water samples, with the
327 smallest size fraction ($L < 5 \mu\text{m}$) having the highest number of particles in most samples. The bottom panel shows
328 the composition of microplastic particles in each size range. A consistent trend can be observed in all samples:
329 More than 80% of the microplastic particles are in the smallest size range, with the proportion decreasing as the
330 particle size increases.



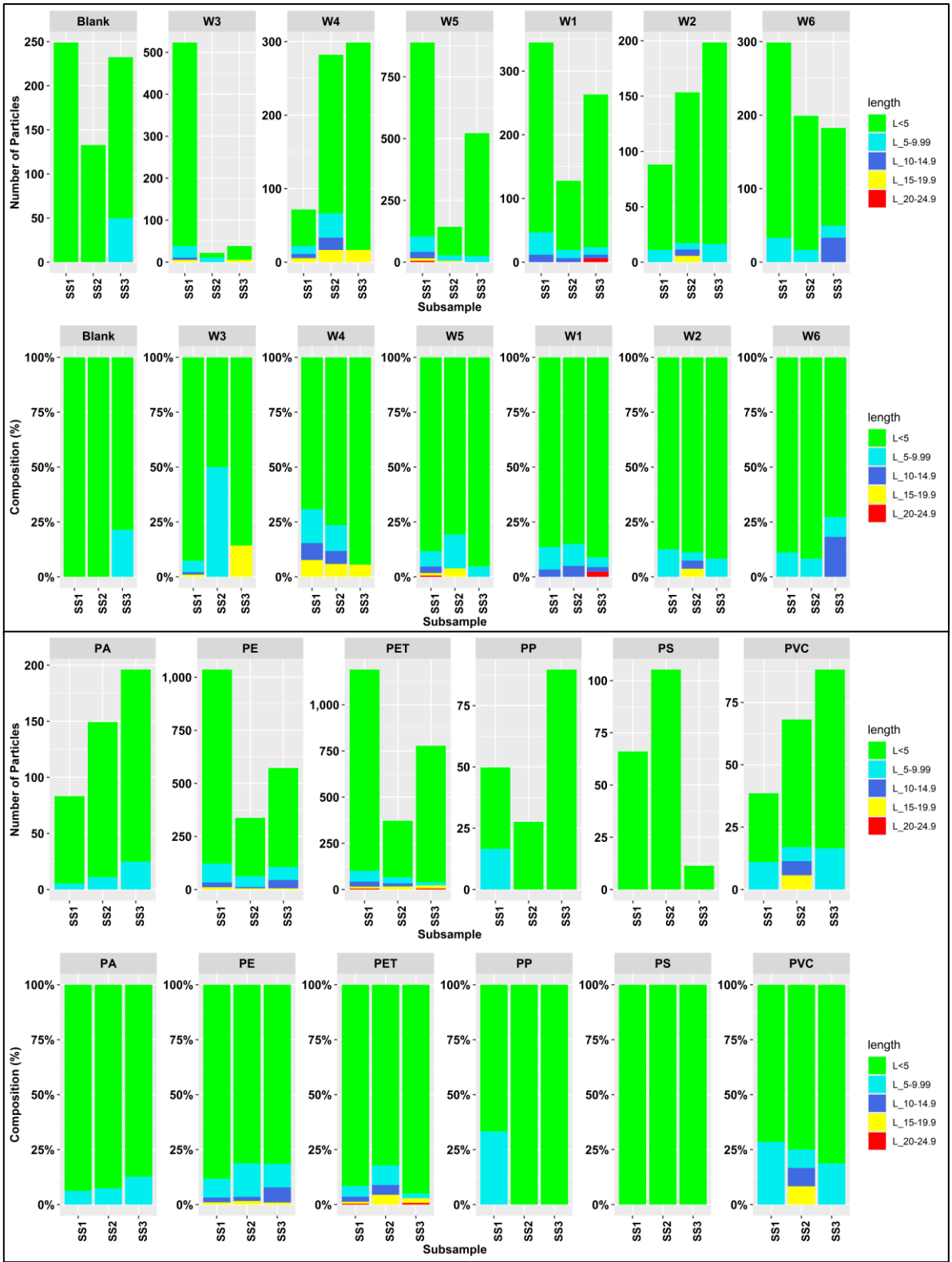
331

332 Figure 6: Scale and pattern of polymers in the 7 samples shown for length of particles (n=57).

333

334

335 Figure 7 lower row shows the length of the MPs in the subsamples. Statistically, 89% or 4 660 of the
 336 MPs were found in the smallest length fraction (L<5 μm , green colour), 7.6% (or 401 particles) were in the second
 337 length (between 5 μm and 9.99 μm). There were only 2.2% and 1.4% MPs at longer sizes (L 10-14.9 μm , L = 15-
 338 19.9 μm). At the largest length (red colour), all 11 particles found in 1 L of water were PET, the only polymer
 339 identified in all sizes; no other polymer was found at L_{20-24.9} μm .



340

341

342 Figure 7: Stacked bars displaying length distribution in subsamples according to Brand or polymer. The
 343 numbers for the length refer to μm .

344 3.1.4 Significance tests

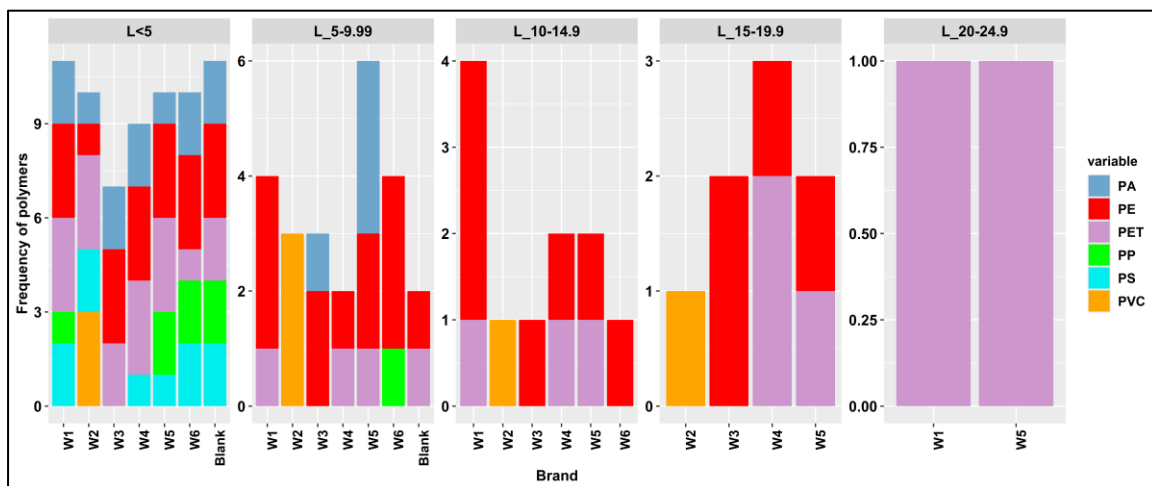
345 The Kruskal-Wallis significance test (p-values <0.05) showed that there were no statistically significant
 346 differences between the brands in terms of number of MP, length of MP particles and containers (p-value=0.1199),
 347 including the Blank sample (the smallest p-value was 0.077 for W3). The polymers differed significantly (p-value
 348 2.087×10^{-15}); the pairwise test showed statistically significant differences for most pairs, but not for: PA vs. PP
 349 (p=0.1075), PS (p=0.2283) and PVC (p=0.1075); PE vs. PET p=0.2603) and PS vs. PVC (p=0.791).

350 The Pearson correlation coefficients together with the significance level are contained in Figure S 4.
 351 Although the p-values indicate significance (except for PA vs. PE), only the number of PET particles is strongly
 352 correlated (R=0.87) to the sum of all MPs (across all samples, N=57). Thus, the number of PET particles in the
 353 water represents a strong indicator for the sum of the particles.

354 No significant difference was found between the brands in terms of length of MPs and polymers (p-
 355 value=0.11), and there was also no significant difference in containers (p-value=0.1282). The shortest length
 356 (L<5) showed significant differences from the other lengths (p-value=0.00014, p-value=0.00039, p-
 357 value=0.00053), except for the longest (L_20-24.9; p-value=0.072).

358 3.2 Frequency distribution

359 As can be seen from the quantitative data, PET and PE were the most abundant polymers, and in general
 360 the MP with the shortest length (<5 μm) were the most abundant. Figure 8 shows the polymers in the 57 samples
 361 by brand. As there are three subsamples for each Brand, the maximum frequency of detection per Brand is three.
 362 It can be seen that at a length of <5 MPs were found 11-times in the Blank and the (virgin) W1 Brand; both had
 363 five polymers present. At the same length, W2 had three occurrences of PVC, meaning that PVC was found in
 364 each of the three subsamples. Brand W3 had only PE in all three subsamples, PVC, PP, and PS were not found at
 365 all. See also Table S 3. The highest detection frequency was for PE (42) followed by PET (29). PP and PVC were
 366 identified only in eight samples, each (Table S 3). Applying multivariate analysis, such as HCA, and visualize as
 367 dendrogram as shown in Figure S 5, the first two clusters at right contain almost all MPs with a length <5 μm,
 368 which indicates a stratification of particles in the bottles and the Blank.
 369



370
 371 Figure 8: Stacked bars displaying the occurrence of polymers in each Branch at a given length according
 372 to Brand or polymer.

373 3.3 Comparison with literature

374 Table 2 shows the global research on bottled water over the last five years. It should be noted that the
 375 current studies are not directly comparable as there are no harmonised methodologies, including differences in

376 sample preparation, analytical techniques, size thresholds and extrapolation methods. However, given the limited
 377 data available, they represent the best available comparison of the current state of the art. To summarise, PET, PE
 378 and PP are the most common MPs found in bottled water worldwide.

379 Table 2: Comparison of global studies from the last five years.

Country	Bottle Type	Average MP/L	Size	Polymer	Reference
Germany	Single use PET	2649 ± 2857	>5 µm	PET	(Oßmann et al. 2018)
	Reusable PET	4889 ± 5432	>5 µm	PET	
	Glass	6292 ± 10,521	>5 µm	PE, styrene butadiene copolymer	
Germany	Single use	14 ± 14	5, 10, < 20 µm	PET, PP, PE	(Schymanski et al. 2018)
	Returnable	118 ± 88			
	Glass	50 ± 52			
	Beverage	11 ± 8			
Global	Single use PET	315	NR	PP, nylon	(Mason et al. 2018)
Italy	Single use PET	148 ± 253	0.5-40 µm	PET, HDPE	(Winkler et al. 2019)
Italy	Single use PET 10 brands	$5.42 \times 10^7 \pm 1.95 \times 10^7$	not reported	not reported	(Zuccarello et al. 2019)
Thailand	Single use PET	140 ± 19	6.5-50 µm	PET, PP, PE, PA, PVC	(Kankanige and Babel 2020)
	Returnable glass	52 ± 4			
Iran	PET bottles	8.5 ± 10.2	1280–4.200 µm	PET, PS, PE	(Makhdoumi et al. 2021)
Turkey	PET bottles and Tetra pak	8.9 ± 2.95	10-1000 µm	PA, PE, PET	(Altunışık 2023)

380

381 Our MP sum as the mean value for all samples is 92 particles/L (including the Blank samples). This value
 382 is in the same order of magnitude as the results for Germany, Italy, Thailand but higher than for Iran and Turkey.
 383 The two studies by (Oßmann et al. 2018) and (Zuccarello et al. 2019) report orders of magnitude higher values
 384 for Italy and Germany.

385 4 Conclusion

386 This study represents the first investigation of bottled water contamination with microplastics on the
 387 Croatian market and makes a valuable contribution to the broader understanding of MP in bottled water in Europe
 388 and worldwide. Using Raman spectroscopy, the study successfully identified and quantified MP down to 1 µm in
 389 size. With this method, we were able to identify 40.7% of all microplastics whereby polyethylene terephthalate
 390 (PET) had an overall contribution of 44.5% across all samples (including the Blanks and all replicates). This was
 391 the highest percentage for the MP_{identified}, followed by polyethylene (PE) with 37.0%. All other plastics were below
 392 5%, namely, polyvinylchloride (PVC) had 3.7%, polystyrene (PS) 3.5%, and polypropylene (PP) 3.2%. Nylon
 393 could not be identified. The percentages of the identified individual plastics as share of the MP_{total} correspond to
 394 18.1% for PET, 15.1% for PE, 1.5% for PVC, 1.4% for PS, and 1.3% for PP.

395 A subsampling approach significantly reduced the analysis time while ensuring robust and comparable
 396 results for all samples.

397 Polyethylene, polyethylene terephthalate, polyamide and occasionally polyvinyl chloride were among
 398 the MP identified, with over 80% of the particles in all samples being smaller than 5 µm. Remarkably, bottled

399 water in virgin PET containers contained more MP than in recycled PET, raising questions about possible
400 differences in manufacturing processes or material properties.

401 The study provided detailed insights by focussing on specific particle sizes and polymer compositions,
402 allowing a clear understanding of the types of microplastics present. In addition, the detection of microplastics in
403 the ultrapure water used for the analysis emphasises the difficulties associated with assessing the level of pollution.

404 This research highlights the need for standardised methods for the testing and monitoring of MP in
405 bottled water to ensure consumer safety and environmental health. The results could inform regulatory policy and
406 quality control standards, particularly with regard to the use of recycled plastic. Future research should investigate
407 the long-term health effects of MP ingestion through bottled water and seek to develop effective strategies to
408 minimise human exposure.

409

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557

558 **Statements and Declarations**

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567 **Authors Contributions**

568 Ana Medić: Methodology, Investigation, Formal analysis, Data curation, Writing – original draft; Heidelore
569 Fiedler: Data curation, Visualization, Writing – Review & editing; TamilSelvi Selvam: Formal analysis, Data
570 curation; Vlasta Mohaček Grošev: Formal analysis, Data curation, Visualization; Sandy Lovković: Resources,
571 Supervision; Lara Mikac: Methodology, Investigation, Visualization, Writing – Review & editing; Mile Ivanda:
572 Conceptualization, Resources, Supervision.

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579 **Competing Interests**

580 The authors have no relevant financial or non-financial interests to disclose.

581 **Data availability statements**

582 The data cannot be made available for reasons of confidentiality.

583

Supplementary Information

Occurrence of microplastics in bottled water from Croatia: A Raman spectroscopy approach

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Table S1: Summary of data and assignment of metadata.

Colour codes as follows: green highlight=number of particles quantified; pink=0 no particles quantified
Length: highlight green=<5 µm, yellow=5 µm-9.99 µm, blue=10 µm-14.9 µm, orange=15 µm-19.9 µm, grey=20 µm-24.9 µm. Subsamples: green=SS1, yellow=SS2, pink=SS3.

SampleID	Sample	Length Code	Length	Brand	Container	Sub sample	PA	PE	PET	PP	PS	PVC	MP sum
W1_SS1a	W1_SS1	a	L<5	W1	recycl	SS1	12	228	53	0	6	0	298
W1_SS1b	W1_SS1	b	L_5-9.99	W1	recycl	SS1	0	23	12	0	0	0	35
W1_SS1c	W1_SS1	c	L_10-14.9	W1	recycl	SS1	0	6	6	0	0	0	12
W1_SS2a	W1_SS2	a	L<5	W1	recycl	SS2	0	57	51	0	0	0	109
W1_SS2b	W1_SS2	b	L_5-9.99	W1	recycl	SS2	0	13	0	0	0	0	13
W1_SS2c	W1_SS2	c	L_10-14.9	W1	recycl	SS2	0	6	0	0	0	0	6
W1_SS3a	W1_SS3	a	L<5	W1	recycl	SS3	47	88	76	23	6	0	240
W1_SS3b	W1_SS3	b	L_5-9.99	W1	recycl	SS3	0	12	0	0	0	0	12
W1_SS3c	W1_SS3	c	L_10-14.9	W1	recycl	SS3	0	6	0	0	0	0	6
W1_SS3e	W1_SS3	e	L_20-24.9	W1	recycl	SS3	0	0	6	0	0	0	6
W2_SS1a	W2_SS1	a	L<5	W2	recycl	SS1	0	0	50	0	0	28	77
W2_SS1b	W2_SS1	b	L_5-9.99	W2	recycl	SS1	0	0	0	0	0	11	11
W2_SS2a	W2_SS2	a	L<5	W2	recycl	SS2	0	40	40	0	6	51	136
W2_SS2b	W2_SS2	b	L_5-9.99	W2	recycl	SS2	0	0	0	0	0	6	6
W2_SS2c	W2_SS2	c	L_10-14.9	W2	recycl	SS2	0	0	0	0	0	6	6
W2_SS2d	W2_SS2	d	L_15-19.9	W2	recycl	SS2	0	0	0	0	0	6	6
W2_SS3a	W2_SS3	a	L<5	W2	recycl	SS3	17	0	88	0	6	72	182
W2_SS3b	W2_SS3	b	L_5-9.99	W2	recycl	SS3	0	0	0	0	0	17	17
W3_SS1a	W3_SS1	a	L<5	W3	virgin	SS1	17	341	127	0	0	0	485
W3_SS1b	W3_SS1	b	L_5-9.99	W3	virgin	SS1	0	28	0	0	0	0	28
W3_SS1c	W3_SS1	c	L_10-14.9	W3	virgin	SS1	0	6	0	0	0	0	6
W3_SS1d	W3_SS1	d	L_15-19.9	W3	virgin	SS1	0	6	0	0	0	0	6
W3_SS2a	W3_SS2	a	L<5	W3	virgin	SS2	6	6	0	0	0	0	11
W3_SS2b	W3_SS2	b	L_5-9.99	W3	virgin	SS2	6	6	0	0	0	0	11
W3_SS3a	W3_SS3	a	L<5	W3	virgin	SS3	0	22	11	0	0	0	33
W3_SS3d	W3_SS3	d	L_15-19.9	W3	virgin	SS3	0	6	0	0	0	0	6
W4_SS1a	W4_SS1	a	L<5	W4	virgin	SS1	0	22	28	0	0	0	50
W4_SS1b	W4_SS1	b	L_5-9.99	W4	virgin	SS1	0	11	0	0	0	0	11
W4_SS1c	W4_SS1	c	L_10-14.9	W4	virgin	SS1	0	6	0	0	0	0	6
W4_SS1d	W4_SS1	d	L_15-19.9	W4	virgin	SS1	0	6	0	0	0	0	6
W4_SS2a	W4_SS2	a	L<5	W4	virgin	SS2	17	50	133	0	17	0	216
W4_SS2b	W4_SS2	b	L_5-9.99	W4	virgin	SS2	0	0	33	0	0	0	33
W4_SS2c	W4_SS2	c	L_10-14.9	W4	virgin	SS2	0	0	17	0	0	0	17
W4_SS2d	W4_SS2	d	L_15-19.9	W4	virgin	SS2	0	0	17	0	0	0	17
W4_SS3a	W4_SS3	a	L<5	W4	virgin	SS3	33	66	183	0	0	0	282

SampleID	Sample	Length Code	Length	Brand	Container	Sub sample	PA	PE	PET	PP	PS	PVC	MP sum
W4_SS3d	W4_SS3	d	L_15-19.9	W4	virgin	SS3	0	0	17	0	0	0	17
W5_SS1a	W5_SS1	a	L<5	W5	virgin	SS1	0	57	717	0	10	0	785
W5_SS1b	W5_SS1	b	L_5-9.99	W5	virgin	SS1	5	10	47	0	0	0	62
W5_SS1c	W5_SS1	c	L_10-14.9	W5	virgin	SS1	0	5	21	0	0	0	26
W5_SS1d	W5_SS1	d	L_15-19.9	W5	virgin	SS1	0	0	10	0	0	0	10
W5_SS1e	W5_SS1	e	L_20-24.9	W5	virgin	SS1	0	0	5	0	0	0	5
W5_SS2a	W5_SS2	a	L<5	W5	virgin	SS2	0	22	83	11	0	0	116
W5_SS2b	W5_SS2	b	L_5-9.99	W5	virgin	SS2	6	17	0	0	0	0	22
W5_SS2d	W5_SS2	d	L_15-19.9	W5	virgin	SS2	0	6	0	0	0	0	6
W5_SS3a	W5_SS3	a	L<5	W5	virgin	SS3	75	124	248	50	0	0	497
W5_SS3b	W5_SS3	b	L_5-9.99	W5	virgin	SS3	25	0	0	0	0	0	25
W6_SS1a	W6_SS1	a	L<5	W6	recycl	SS1	17	149	66	17	17	0	266
W6_SS1b	W6_SS1	b	L_5-9.99	W6	recycl	SS1	0	17	0	17	0	0	33
W6_SS2a	W6_SS2	a	L<5	W6	recycl	SS2	83	66	0	0	33	0	183
W6_SS2b	W6_SS2	b	L_5-9.99	W6	recycl	SS2	0	17	0	0	0	0	17
W6_SS3a	W6_SS3	a	L<5	W6	recycl	SS3	0	116	0	17	0	0	133
W6_SS3b	W6_SS3	b	L_5-9.99	W6	recycl	SS3	0	17	0	0	0	0	17
W6_SS3c	W6_SS3	c	L_10-14.9	W6	recycl	SS3	0	33	0	0	0	0	33
Blank_SS1a	Blank_SS1	a	L<5	Blank	none	SS1	33	116	50	17	33	0	249
Blank_SS2a	Blank_SS2	a	L<5	Blank	none	SS2	33	33	0	17	50	0	133
Blank_SS3a	Blank_SS3	a	L<5	Blank	none	SS3	0	50	133	0	0	0	183
Blank_SS3b	Blank_SS3	b	L_5-9.99	Blank	none	SS3	0	33	17	0	0	0	50

603 Table S1a: Subsamples where no MPs could be automatically assigned
604 No LengthCode was assigned to MPs with a length >35 µm.

SampleID	Sample	Length Code	Length	Brand	Container	Sub sample	PA	PE	PET	PP	PS	PVC	MPsum
W3_SS1e	W3_SS1	e	L_20-24.9	W3	virgin	SS1	0	0	0	0	0	0	0
	W3_SS2		L_35-39.9	W3	virgin	SS2	0	0	0	0	0	0	0
	W3_SS2		L>100	W3	virgin	SS2	0	0	0	0	0	0	0
W3_SS3b	W3_SS3	b	L_5-9.99	W3	virgin	SS3	0	0	0	0	0	0	0
W3_SS3c	W3_SS3	c	L_10-14.9	W3	virgin	SS3	0	0	0	0	0	0	0
	W3_SS3		L_50-59.9	W3	virgin	SS3	0	0	0	0	0	0	0
W4_SS3b	W4_SS3	b	L_5-9.99	W4	virgin	SS3	0	0	0	0	0	0	0
W4_SS3c	W4_SS3	c	L_10-14.9	W4	virgin	SS3	0	0	0	0	0	0	0
W5_SS2c	W5_SS2	c	L_10-14.9	W5	virgin	SS2	0	0	0	0	0	0	0
Blank_SS1b	Blank_SS1	b	L_5-9.99	Blank	none	SS1	0	0	0	0	0	0	0
Blank_SS2b	Blank_SS2	b	L_5-9.99	Blank	none	SS2	0	0	0	0	0	0	0
Blank_SS2d	Blank_SS2	d	L_15-19.9	Blank	none	SS2	0	0	0	0	0	0	0
Blank_SS3c	Blank_SS3	c	L_10-14.9	Blank	none	SS3	0	0	0	0	0	0	0
Blank_SS3d	Blank_SS3	d	L_15-19.9	Blank	none	SS3	0	0	0	0	0	0	0
Blank_SS3e	Blank_SS3	e	L_20-24.9	Blank	none	SS3	0	0	0	0	0	0	0

605 Table S1b: Subsamples excluded for QA/QR reasons.

Sample	Length	Brand	Container	Subsample	PA	PE	PET	PP	PS	PVC	MPsum
W5_SS1	L_60-69.9	W5	virgin	SS1	0	0	5	0	0	0	5
W4_SS3		W4	virgin	SS3	33	66	199	0	0	0	299
W5_SS1		W5	virgin	SS1	5	73	800	1 803	10	0	2 692

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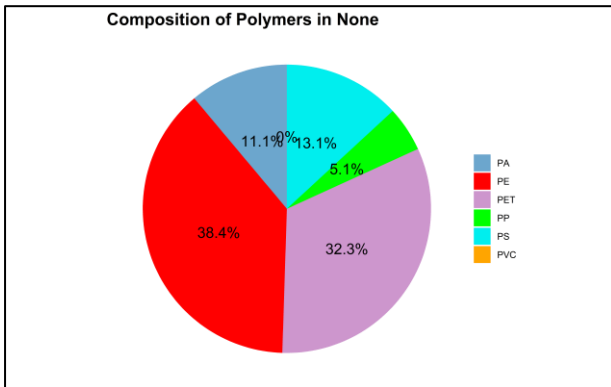
608

609 Table S 2: Descriptive statistics for number of polymer MPs by Brand (six Brands and one Blank; three
610 subsamples each).
611 Cells characterizing the Blank are coloured in light blue, for virgin PET in light green, for
612 recycled PET in light red).

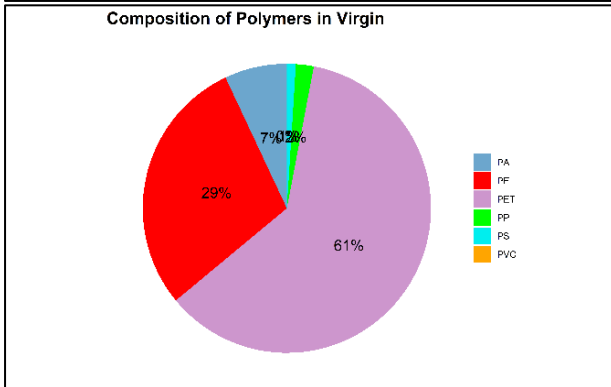
	Blank	W3	W4	W5	W1	W2	W6	Overall
PA								
Mean (SD)	16.6 (19.2)	3.44 (5.84)	4.98 (11.2)	11.0 (23.6)	5.87 (14.9)	2.07 (5.84)	14.2 (30.9)	7.52 (17.3)
Median	16.6	0	0	0	0	0	0	0
[Min, Max]	[0, 33.2]	[0, 16.5]	[0, 33.2]	[0, 74.5]	[0, 47.0]	[0, 16.5]	[0, 83.0]	[0, 83.0]
PE								

Mean (SD)	58.1 (39.5)	52.3 (117)	16.0 (23.5)	24.1 (39.2)	43.9 (70.4)	4.97 (14.1)	59.3 (54.1)	34.1 (60.4)
Median	41.5	5.51	5.51	7.95	12.2	0	33.2	11.0
[Min, Max]	[33.2, 116]	[5.51, 341]	[0, 66.4]	[0, 124]	[0, 228]	[0, 39.7]	[16.6, 149]	[0, 341]
PET								
Mean (SD)	49.8 (59.1)	17.2 (44.4)	42.6 (62.8)	113 (225)	20.3 (28.4)	22.2 (33.5)	9.48 (25.1)	41.1 (104)
Median	33.2	0	16.6	15.6	5.85	0	0	5.20
[Min, Max]	[0, 133]	[0, 127]	[0, 183]	[0, 717]	[0, 76.0]	[0, 88.1]	[0, 66.4]	[0, 717]
PP								
Mean (SD)	8.30 (9.58)	0 (0)	0 (0)	6.07 (15.7)	2.34 (7.39)	0 (0)	7.11 (8.87)	2.93 (8.48)
Median	8.30	0	0	0	0	0	0	0
[Min, Max]	[0, 16.6]	[0, 0]	[0, 0]	[0, 49.7]	[0, 23.4]	[0, 0]	[0, 16.6]	[0, 49.7]
PS								
Mean (SD)	20.7 (24.9)	0 (0)	1.66 (5.25)	1.04 (3.29)	1.17 (2.46)	1.40 (2.59)	7.11 (13.1)	3.20 (9.34)
Median	16.6	0	0	0	0	0	0	0
[Min, Max]	[0, 49.8]	[0, 0]	[0, 16.6]	[0, 10.4]	[0, 5.85]	[0, 5.68]	[0, 33.2]	[0, 49.8]
PVC								
Mean (SD)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	24.4 (24.6)	0 (0)	3.42 (12.2)
Median	0	0	0	0	0	13.8	0	0
[Min, Max]	[0, 0]	[0, 0]	[0, 0]	[0, 0]	[0, 0]	[5.68, 71.6]	[0, 0]	[0, 71.6]
MPsum								
Mean (SD)	154 (84.0)	73.0 (167)	65.3 (99.0)	155 (267)	73.6 (108)	55.0 (69.6)	97.2 (98.4)	92.3 (148)
Median	158	11.0	16.6	25.4	12.2	13.8	33.2	24.8
[Min, Max]	[49.8, 249]	[5.51, 485]	[5.51, 282]	[5.20, 785]	[5.85, 298]	[5.68, 182]	[16.6, 266]	[5.20, 785]

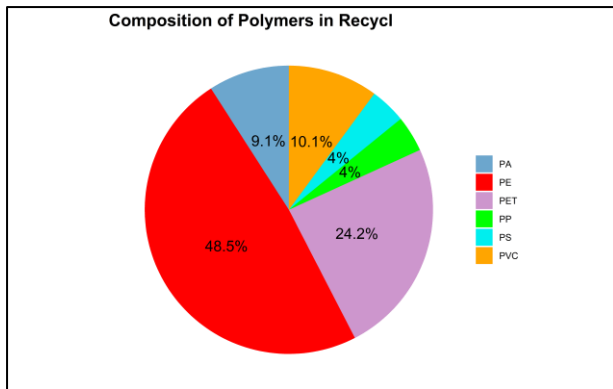
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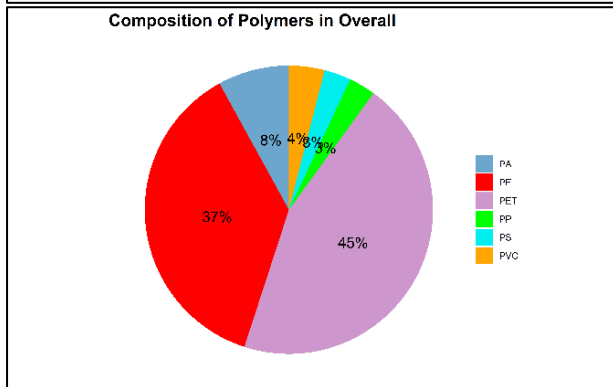
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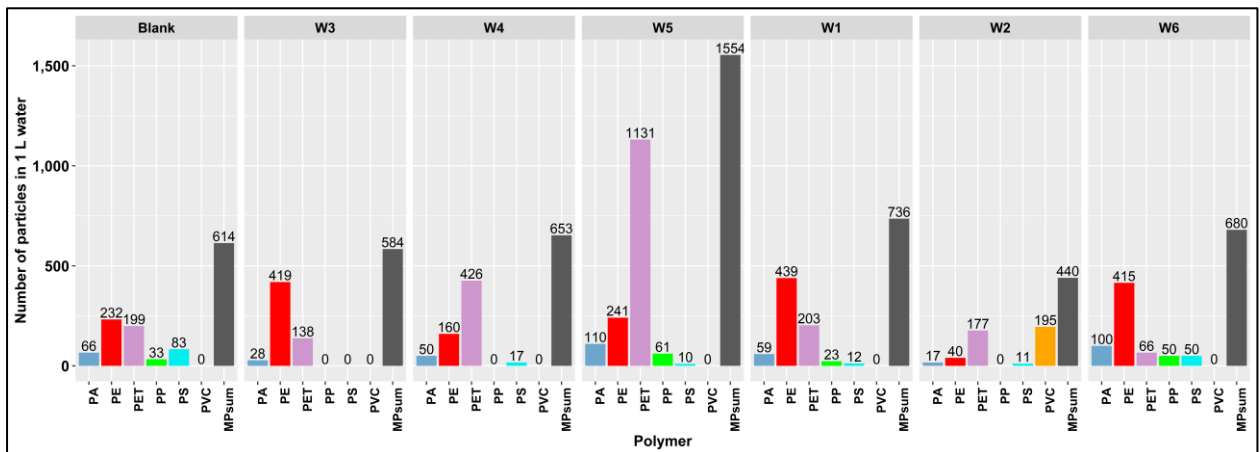


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618 Figure S 1: Piecharts displaying overall polymer composition according to the container of the waters.

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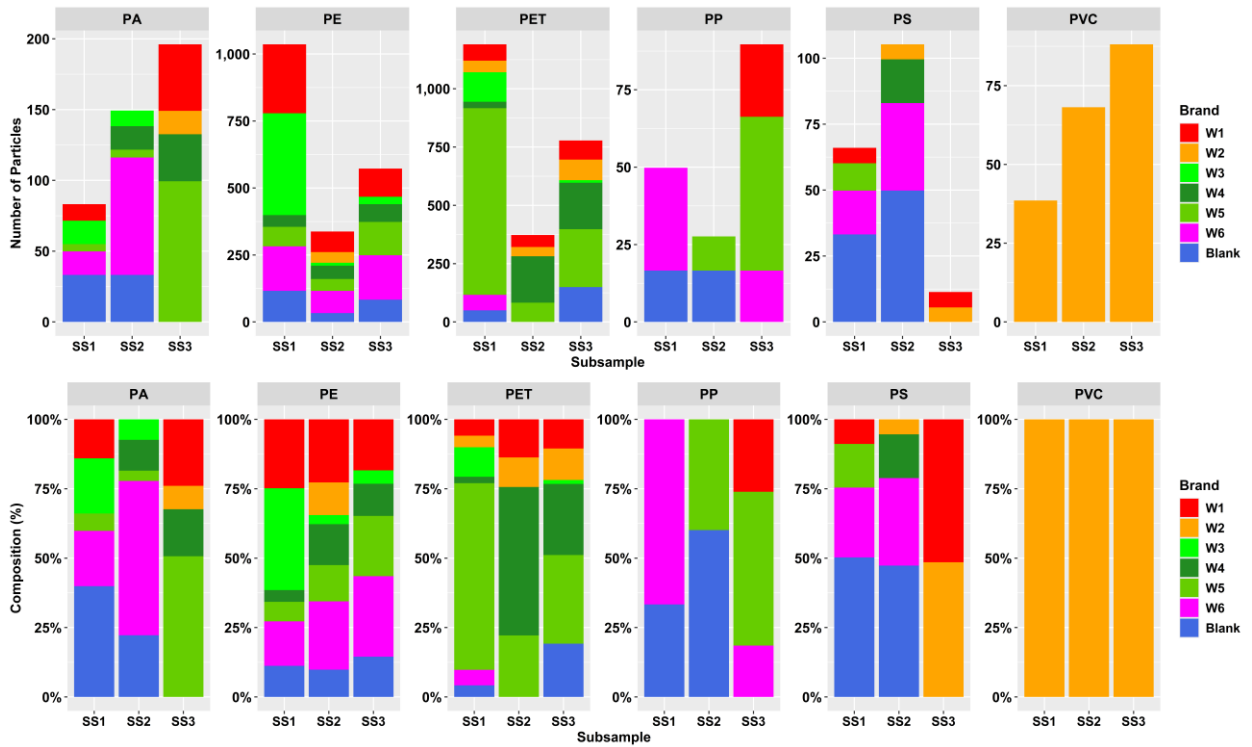
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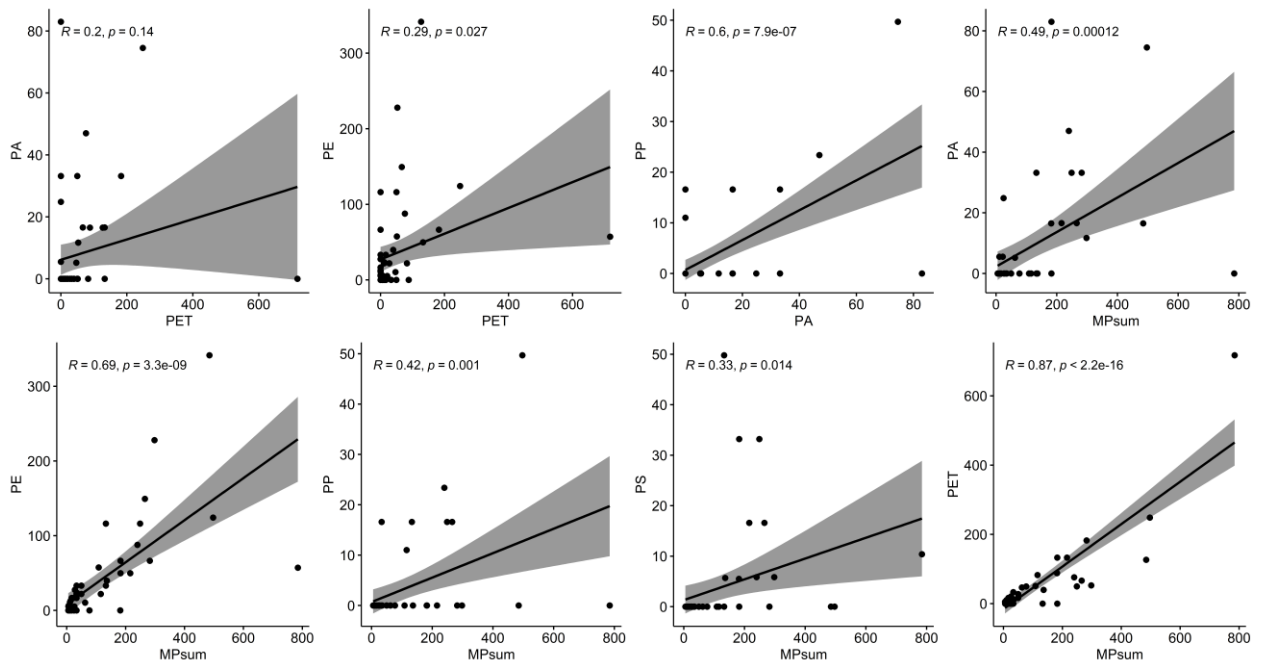
622 Figure S 2: Overview of sum polymers and sum of total MP polymers in the three subsamples of each
623 Brand.

624 Brands W3, W4, and W5 refer to water bottled in virgin PET, Brands W1, W2, and W6 to water
625 bottled in recycled PET.



627

628 Figure S 3: Stacked barplots on particles per polymer in subsamples coloured according to Brand.



629

630 Figure S 4: Pearson correlation coefficients and significance for MP polymers (quantitative as to the
631 number of particles in 1 L).

632 Table S 3: Detection frequency of polymers at various lengths and in Brands.

Brand	Count L<5	Count L_5-9.99	Count L_10-14.9	Count L_15-19.9	Count L_20-24.9	Subtotal
Blank	11	2				13
W3	7	3	1	2		13

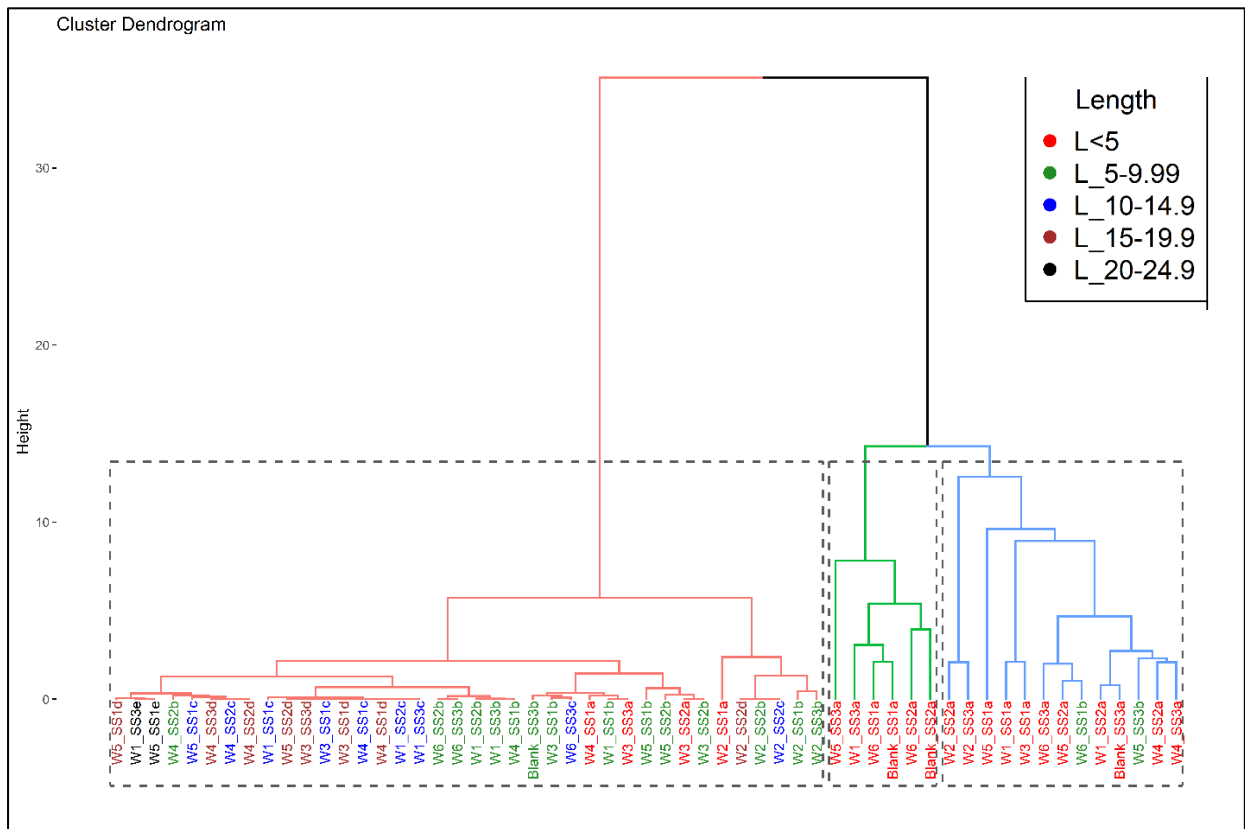
W4	9	2	2	3		16
W5	10	6	2	2	1	21
W1	11	4	4		1	20
W2	10	3	1	1		15
W6	10	4	1			15
Total	68	24	11	8	2	113

633

Polymer	Count L<5	Count L_5-9.99	Count L_10-14.9	Count L_15-19.9	Count L_20-24.9	Subtotal
PA	12	4				16
PE	19	12	7	4		42
PET	17	4	3	3	2	29
PP	7	1				8
PS	10					10
PVC	3	3	1	1		8
Total	68	24	11	8	2	113

634

635



636

637 Figure S 5: HCA dendrogram (using subsamples, Euclidean distances, Ward method) coloured by length (μm).
 638 The first two clusters at right contain only small particles (red and green colour).