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Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water

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ABSTRACT

Microplastics are anthropogenic contaminants which have been found in oceans, lakes and rivers. Investigations focusing on drinking water are rare and studies have mainly been using micro-Fourier Transform Infrared Spectroscopy (μ-FT-IR). A major limitation of this technique is its inability to detect particles smaller than 20 μm. However, micro-Raman spectroscopy is capable of detecting even smaller particle sizes. Therefore, we show that this technique, which was used in this study, is particularly useful in detecting microplastics in drinking water where particle sizes are in the low micrometer range. In our study, we compared the results from drinking water distributed in plastic bottles, glass bottles and beverage cartons.

We tested the microplastic content of water from 22 different returnable and single-use plastic bottles, 3 beverage cartons and 9 glass bottles obtained from grocery stores in Germany. Small (−50-500 μm) and very small (1−50 μm) microplastic fragments were found in every type of water. Interestingly, almost 80% of all microplastic particles found had a particle size between 5 and 20 μm and were therefore not detectable by the analytical techniques used in previous studies. The average microplastics content was 118 ± 88 particles/l in returnable, but only 14 ± 14 particles/l in single-use plastic bottles. The microplastics content in the beverage cartons was only 11 ± 8 particles/l. Contrary to our assumptions we found high amounts of plastic particles in some of the glass bottled waters (range 0−253 particles/l, mean 50 ± 52 particles/l). A statistically significant difference from the blank value (14 ± 13) to the investigated packaging types could only be shown comparing to the returnable bottles ($p < 0.05$).

Most of the particles in water from returnable plastic bottles were identified as consisting of polyester (primary polyethylene terephthalate PET, 84%) and polypropylene (PP; 7%). This is not surprising since the bottles are made of PET and the caps are made of PP. In water from single-use plastic bottles only a few micro-PET-particles have been found. In the water from beverage cartons and also from glass bottles, microplastic particles other than PET were found, for example polyethylene or polyolefins. This can be explained by the fact that beverage cartons are coated with polyethylene foils and caps are treated with lubricants. Therefore, these findings indicate that the packaging itself may release microparticles. The main fraction of the microplastic particles identified are of very small size with dimensions less than 20 μm, which is not detectable with the μ-FT-IR technique used in previous studies.

1. Introduction

Microplastic contamination is receiving increased attention and the impact on the environment but also on food has become evident. In the early seventies the tiny plastic pieces were reported for the first time being present in the Atlantic (Carpenter et al., 1972; Carpenter and Smith, 1972; Colton et al., 1974). Since then, a constant increase of microplastic pollution in marine waters has been observed (Thompson et al., 2004). In recent years, an increasing number of studies revealing the presence of microplastic particles in fresh water from sources, such as lakes and rivers (Ivleva et al., 2017; McCormick et al., 2016; Sruthy and Ramasamy,

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2017), as well as in the atmosphere (Cai et al., 2017; Dris et al., 2016) were reported.

In addition to the marine microplastics input into our diet by consuming fish and seafood (Cole et al., 2013; Davison and Asch, 2011) that may be contaminated by leaking pollutants and additives in microplastic (Bakir et al., 2014; Mato et al., 2001), the direct exposure of microplastics by humans via other sources, especially such as drinkable water, needs to be considered. Currently, there is almost no published data on the microplastics content of drinking- or mineral water available.

A publication about synthetic particles in German beers (Liebezeit and Liebezeit, 2014) has been discussed (Lachenmeier et al., 2015; Wiesheu et al., 2016). In this context one type of beer and one sample of bottled mineral water was analyzed by means of μ-Raman spectroscopy. The authors found fibers in the blank and the beverage samples, but the results did not show statistically significant differences (Wiesheu et al., 2016). In the study, by Wiesheu et al. (2016), only one single PET fiber was found in one of the water samples but no statements about the total amount of other fibers or particles were made. The study also didn't reveal anything about the type of water or the type of packaging. The authors recommended further analysis of fiber content in different beverages and investigations of the sources of contamination, pointing out the importance of working under extremely clean conditions in order to reduce contamination of the samples.

The Helmholtz Center for Polar and Marine Research (Alfred-Wegener-Institute, Bremerhaven, Germany) investigated a total of 40.000 L raw- and tap water in Lower Saxony by means of μ-FT-IR (Mintenig et al., 2014). In four blind samples fiber concentrations comparable to the other raw- and drinking water samples were detected, implying a contamination through the exposure of the water sample (fiber input through laboratory air). In 10 out of 24 samples 0.4–7 microplastic particles per m^3 drinking water were found which the authors affiliated to the abrasion of pipes and fittings used in the drinking water system. The particles found had sizes between 50 and 150 μm.

Using μ-FT-IR-spectroscopy, particles can be detected down to a lower size limit of 20 μm. Due to the higher resolution of up to 1 μm, μ-Raman spectroscopy is able to visualize very small microplastic particles that were most likely overlooked up to now (Ivleva et al., 2017). Especially for the analysis of food, the detection of very small microplastic particles is important, because of the possible implication on human health. It is expected that smaller particles could possibly be better absorbed by the digestive system than larger ones (Hussain et al., 2001). As a result, an increased accumulation in humans could be the consequence.

2. Material and methods

2.1. Materials

Particle counting and identification was done with the Single Particle Explorer (SPE, rapID, Berlin), a μ-Raman spectroscope with a 10x, 20x and 50x objective (NA = 0.55) and a 532 nm (≤20 mW; grating of 1040 lines/mm) and a 785 nm (≤50 mW; grating of 1450 lines/mm) Raman laser with adjustable laser power. A maximum of 5000 particles/scanning counts per measurement can be analyzed with the SPE. The smallest particle size that can be analyzed is 1 μm.

A specially manufactured filtering apparatus was needed for filtration (filtr.AID, rapID, Berlin) with a filter funnel that had a highly polished bottom, ensuring tight closure with the filter membrane. The inner diameter of the funnel was 4 mm, top diameter 10 cm and the filtration volume was 100 ml.

Since gold hardly emits any Raman signals gold coated polycarbonate filter (filtr.AID membranes, rapID, Berlin, 3.0 μm pore size and inner diameter of 24 mm) were used. In order to obtain a smooth surface of the filter membrane a specific gold filter sample holder was supplied with the device. This sample holder consisted of a base and a clamp, in between the filter was clamped (see Supporting Information S1). The gold filter sample holder was designed to fit exactly into the sample tray of the Single Particle Explorer. This guaranteed the center of the filter to be always in the middle and the filter membranes to stay plane. These settings were essential for the subsequent automatic image analysis, particle counting and Raman analysis.

2.2. Types of water

Water samples (volume range 700 ml–1500 ml) from 12 different returnable and 10 single-use plastic bottles, 3 beverage cartons and 9 glass bottles were obtained from grocery stores in Germany. The water samples were classified into “still mineral water”, “medium sparkling” and “sparkling”, corresponding to its carbonic acid content. Four plastic bottled waters (one single-use and three returnable bottled waters) of same brand/manufacturer but with different batch numbers were purchased six weeks later to check the influence of production dates on microplastic content.

All analyses were performed in triplicate.

2.3. Procedures performed to prevent particle contamination

2.3.1. Air-borne and water-borne contamination

In order to avoid a particle contamination from indoor air, all steps of the filtration process were conducted using a laminar flow workbench (cleanroom class ISO 3, Envair eco air H, Envair Deutschland GmbH, Emmendingen). The bench was periodically checked for proper operation with a particulate measuring device (Trotec, PC200, Heinsberg, Germany).

In all filtration and handling steps, a laboratory coat made of 100% cotton, particle free nitrile gloves and arm sleeves were worn in the laboratory. Before every work step, gloved hands were washed with detergent and thoroughly rinsed with Milli-Q water.

In order to create a blank value with very low microplastic content, different water sources were analyzed and a following cleaning process (see 2.3.2) developed. The overall lowest particle contamination was achieved using an ultra-pure water system (Milli-Q-, Advantage A10, Merck Millipore), fed with deionized water. As endfilter a 0.22 μm membrane filter for particulate-free and bacteria-free water (Millipak Express 40 Filter, Merck Millipore) was used.

Before filtration, each filter was analyzed with the μ-Raman spectroscope and tested for polymer particles present (“pre-counted”). The number of plastic particles detected in this “pre-count” was subtracted from the number of the detected polymer particles after filtration of the samples or the blanks. In general, these “pre-counted” values were very small (mostly between 0 and 4, highest value observed was 33 plastic particles).

2.3.2. Cleaning process

A complex cleaning process was necessary to assure that the filtration setup and the glass vessels did not contribute to particle input. Even the smallest irregularities in the glass vessels could be a source of particle accumulation. Therefore, all vessels were checked for any cracks or scratches.

In a first step, glass funnel and vessels were intensively cleaned with deionized water, detergent and cleanroom wipes. Then, they were sonicated in Milli-Q water for 30 min and subsequently extensively rinsed with Milli-Q water. All vessels were setup immediately on the clean bench. In order to ensure that no new particles accumulated an imaging microscope at the clean bench

was used. For this pre-check a picture of a clean gold filter ("flush filter") was taken with the imaging microscope. Then about 50 ml Milli-Q water were filter through this filter while washing over the surface area of the funnel. After this step, a new picture was taken. If the before/after-filtration picture altered and new fibers or particles accumulated, the rinsing steps were repeated. The "flush filter" was wiped off carefully with a cleanroom wipe so it could be used a few times. If finally the before/after-filtration picture does not alter anymore the funnel and used glass vessel were regarded as clean and the filtration of the sample was performed with a new, pre-counted filter (a flowchart of the procedure is illustrated in Supporting Information S2).

Before filtration the outside of water bottles or beverage cartons was also cleaned in order to reduce the risk of contamination from the outside. Whenever possible, the label was removed and the package was thoroughly rinsed with Milli-Q water and placed on the clean bench immediately, allowing to dry until filtration.

2.4. Filtration

The entire water volume of every mineral water bottle or beverage carton was filtered through a pre-counted filter under vacuum and then the container was rinsed with additional 50 ml of Milli-Q water. The funnel was then rinsed with 20 ml Milli-Q water. The vacuum was turned off and the funnel and the gold filter were taken off carefully to avoid scratches on the sensitive surface of the filter. The gold coated polycarbonate filter was stored in a capped thin petri dish until analysis. The petri-dish used was the original packaging the gold-coated polycarbonate filter was supplied/shipped. Although these petri-dishes were made out of polystyrol we could exclude contamination coming from these containers since no relevant amount of polystyrol particles were detected in our studies.

2.5. Counting and identification of microplastics with μ-Raman spectroscopy

In order to best visualize captured particles on the gold coated polycarbonate filter, the filters were first scanned completely in dark field mode with a 20-fold magnification. The scanned area was chosen to be 4.4 mm × 4.4 mm since this was the pre-set instrumental setting closest to the dimension of the entire filtration area (defined by dimension of 4 mm of the funnel used). In dark field mode, the background appeared black, while particles were illuminated to optimize contrast between substrate and particle, which ensured any white or transparent particles were not missed. With the help of binarization the computer determines quantity, size and morphology of the particles (image analysis). Five size ranges to be measured were set as: 5–10 μm, 10–20 μm, 20–50 μm, 50–100 μm and >100 μm. In a next step, all recorded particles starting with a size of 5 μm were analyzed for their chemical composition. The instrument settings were: 532 nm excitation laser, 5 s integration time, laser intensity of 12%, laser spot size was about 0.7 μm, spectral resolution of 5 cm^{-1} and spectral range was set to 200–3200 cm^{-1}. The spectrum of each particle was compared to a reference library (rapID, Berlin) for identification. The library contained all common polymers and materials of other groups of substances like cellulose, proteins or sugars. The library was extended with additional spectra from materials such as the blue plastic caps from the water bottles or the blue nitrile gloves which were used in the lab. Library matches with a ranking greater than 700 were accepted, in line with common practice in microspectroscopy established by Woodall (Woodall et al., 2014). Library matches with a ranking between ≥550 and ≤ 700 were analyzed and interpreted individually.

Microplastic particles made up only a few percent (min.: 0.03%, max.: 10.7%) of the total amount of counts per sample. In some cases it was not possible to analyze all scanning counts with Raman (if the total scanning counts were higher than the 5000 maximum analyzable counts) so the total microplastic counts had to be extrapolated. The function "select random particles" of the instrument allowed the randomized analysis of a predefined number of particles. Whenever possible, either all particles present or at least around 1000 particles of the smallest size range (5–10 μm) were analyzed. This was achieved using the "select random particles" function and then the overall microplastic count was extrapolated. In this case a multiplying factor of 10 was the highest value that has never been exceeded. The scanning counts in the other size ranges were all analyzed with Raman (for an example output list and calculation see Supporting Information S3). After the automated analysis was completed, it was possible to manually select every counted particle in the "verification mode" with "live view". Then the particles were analyzed again with longer integration time or a higher intensity at other spots to receive more significant spectra. This "verification mode" has been used for all particles that ranked lower than 700 but higher than 550. Because of this procedure, it took up to 18 h for counting and analyzing just only one filter.

2.6. Statistical analysis

Statistically analysis was performed using Microsoft Excel 2010 and a statistical software (STATISTICA, Statsoft). ANOVA (one way analysis of variance) and Tukey's HSD (honestly significant difference) post hoc test with $p < 0.05$ were applied.

3. Results

3.1. Blank samples, analyzed polymer types and sizes, correction for ambiguous particles

As blank sample, 1 L water from an ultra-pure water system (Milli-Q, Advantage A10, Merck Millipore, 0,45 μm endfilter "Millipak 40", fed with deionized water), was filtrated in a sequence (10 in a row) and also periodically between the measurements (after every fifth sample) to check for growing contamination trends. Within a total of 18 measurements 1–42 plastic particles have been found with a mean of 14 ± 13. The predominant polymer types were PET, but also PE, PS and PP.

In their critical assessment of visual identification of marine microplastic using Raman spectroscopy, Lenz et al. already pointed out that colored particles or polymers are difficult to differentiate (Lenz et al., 2015). In our samples we also found larger amounts of these particles and they had spectra similar to "blue nitrile gloves", followed in the ranking by "heliogen blue" and "polypropylene blue bottle cap". A differentiation is difficult since they all had very similar spectra (see Fig. 1). We suspect that this was caused due to a colored polymer or another substance that contained blue pigments. The blank controls did not show any of these particles, but we found them in large amounts in every water from glass bottles (rank 28–361, mean 115 ± 102 particles/l), from one type of beverage carton (39 particles/l), as well as from one type of returnable bottle (27 particles/l). Since blue nitrile gloves were worn during the experiments, they could have contributed to the observed contamination. However, these particles should then have also been observed in every sample or blank. Since this was not the case and we were unable to identify the source of these colored particles they were not included in the microplastic particle count. Furthermore, particles and films identified as "White Petroleum Jelly" and "Nylon" were also excluded, as they had always low rankings and similar spectra (some examples of the library

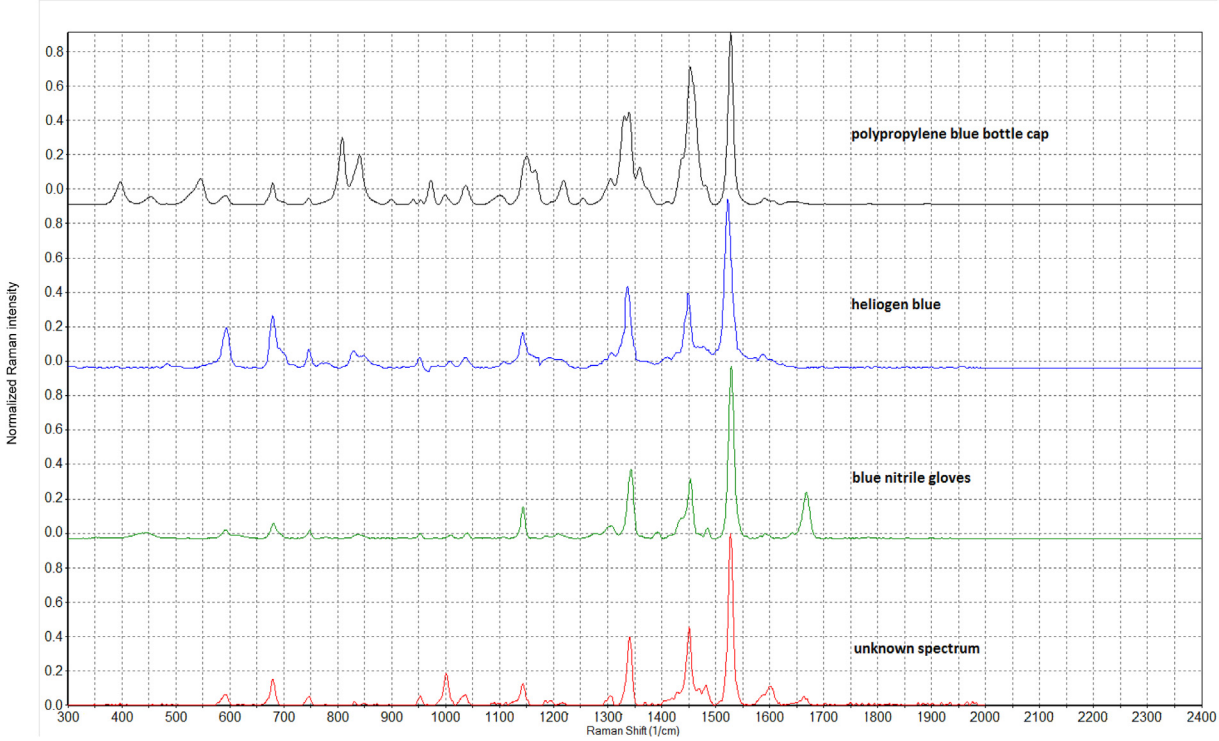


Fig. 1. Spectrum of an unknown particle and its closest database matches.

results are given in Supporting Information S4). In addition, some of these particles or films disappeared after a longer or higher intensity laser treatment (see Fig. 2). We suspect that the energy of the laser was too high and caused the particles to decompose. Since polymer gels are not classified as microplastics and additional verification was not possible, the findings were also not included in the microplastic particle count. The sum of "White Petroleum Jelly" and "Nylon" particles remained <10 particles/l in all four packaging types except for two samples of glass bottles (68 and 86 particles/l) and one sample of returnable bottles (21 particles). Calculated to the total microplastic amount of each packaging type this would have made up the following average percentages: glass bottles: 27%, single-use bottles: 10%, returnable bottles: 3%, beverage carton: 18%.

Besides these blue pigments, white pigments identified as titanium dioxide were observed in a wide range of samples. Imhof et al. (2016) reported high numbers of microplastic particles in a subalpine lake. They were comprised of polymers with and without color pigments, paint particles with a high pigment content, as well as lower amounts of a polymeric binder. The most common pigments found were Pigment White 6, Pigment Blue 15 and Pigment Green 7. This correlates with the pigments found in this study. However, since we were unable to detect the underlying polymer type, this probably resulted in an underestimation of microplastic particles present in the samples.

Even though polyethylene terephthalate (PET) is a polyester (PES) there are different spectra available for PET and PES in the library used and the spectra differ only slightly. When the PES particles were analyzed with a longer duration or higher laser intensity mostly a clear PET spectra became apparent. Since not every

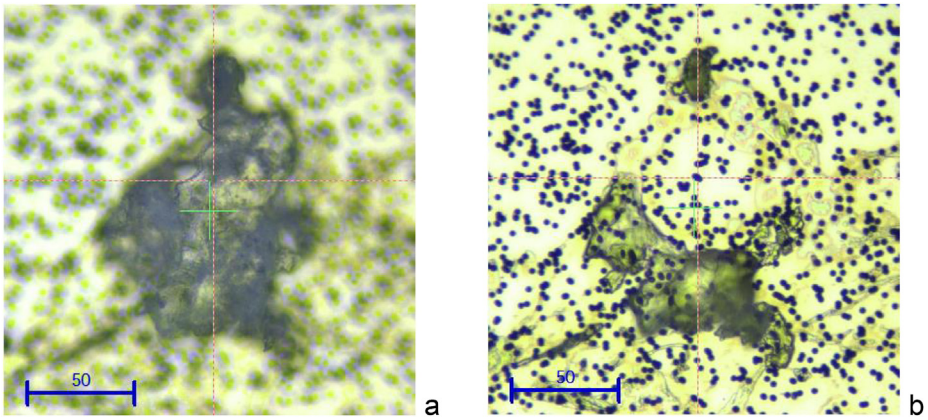


Fig. 2. Decomposing of particles after laser treatment (a: after 1 s exposure time with 12% laser intensity, b: same particle after 1 s/30% laser intensity); scale bars are equivalent to 50 μm.

Table 1
Analyzed polymers and their abbreviations.

PET	(Polyethylene terephthalate)	PEST	Poly(butyl methacrylate)
PES	(Polyester)		PPTA (Poly(p-phenylenterephthalamide), trade name Kevlar)
PE	(Polyethylene)		Paraformaldehyde
PA	(Polyamide)		PTFE (Polytetrafluoroethylene)
PS	(Polystyrene)		Poly(alpha-methylstyrene)
PP	(Polypropylene)		PVC (Polyvinyl chloride)
Parafilm			

polyester particle was analyzed individually for a second time we summarized them as PEST (polyester + polyethylene terephthalate).

Table 1 summarizes the polymers detected and that were included in the microplastics particle count in the respective size ranges 5–10 μm, 10–20 μm, 20–50 μm, 50–100 μm and >100 μm. All particles identified consisting of polycarbonate nature were excluded form the particle count since the filter material was made of this polymer. In general, particles consisting of polycarbonate were rare.

3.2. Microplastics in water

In total, 38 mineral waters were analyzed for their microplastic content. Thereof, 9 different brands of glass bottled waters, 3 different brands of beverage cartons and 26 different brands of plastic bottles (11 single-use and 15 returnable) were analyzed. We obtained a different batch of 4 plastic bottled waters at a later time. (one single-use and three returnable bottled waters) to determine whether the microplastics content varies between batches. All results were normalized to 1 L sample volume. For individual sample data see Supporting Information S5.

3.2.1. Plastic bottled waters

Water from the 11 single-use bottles contained a mean of 14 ± 14 particles/l (range 2–44 particles/l; see Fig. 3). The polymer identification revealed the following: 59% of the particles consisted of PEST, 9% of PE, 1% of PP and 1% of PA (see Fig. 4). The remainder of 30% is attributed to other polymers predominantly showing one type of spectrum (Fig. 5 red spectrum). A database search suggested "polysulfon" or beta-ionone and dimethoxybenzophenon. However, since polysulfone is a rare polymer and the rankings were not a perfect match and were only slightly above our acceptance score of 700, we suspect that the spectra could be derived from another substance not present in the database or derived from a mixture of polymers. A statistical difference of the microplastic particles from water of single-use bottles was only observed in the case of returnable bottles (see Fig. 3).

Only 2% of the microplastic particles were of the biggest size >100 μm and 5% were in the size range of 50–100 μm (see Fig. 6). 22% were sized between 20 and 50 μm, 30% between 10 and 20 μm and 41% between 5 and 10 μm.

The number of plastic particles in the water from 15 types of returnable plastic bottles resulted in a mean of 118 ± 88 particles/l (range 28–241 particles/l). This was on average 8 times higher than the number of plastic particles found in water from single-use plastic bottles (as well as in the average blank). The water from returnable plastic bottles had a 10 times higher number of plastic particles than the water from beverage cartons (see Fig. 3). These findings were statistically significant.

Identification of the composition of plastic particles found in water from returnable plastic bottles revealed that 84% consisted of PEST, 7% of PP, 5% of PE and 2% of PA (see Fig. 4). The rest of 2% consisted of other polymers.

The percentage polymer size distribution in returnable bottles were: >100 μm: 1%; 50–100 μm: 2%; 20–50 μm: 12%; 10–20 μm: 29% and 5–10 μm: 56% (see Fig. 6).

3.2.2. Microplastics in glass bottles

Very different microplastic concentrations were found in the nine analyzed waters from glass bottles. The number of particles varied between 4 and 156 particles/l and the samples had an average of 50 ± 52 particles/l. About 41% of the plastic consisted of PEST, 35% of PE and 8% of PP. In comparison to the other packaging materials the particles found in water from glass bottles showed the highest concentration of PA (12%) (see Fig. 4).

However, the number of plastic particles found in water from glass bottles (50 ± 52) were statistically not different compared to returnable bottles (118 ± 88), single use bottles (14 ± 14), beverage cartons (11 ± 8) or the blank value (14 ± 13).

Comparing the microplastic particle numbers with the five size ranges in glass bottles the following results were obtained: >100 μm: 3%; 50–100 μm: 7%; 20–50 μm: 14%; 10–20 μm: 32%; 5–10 μm: 45% (see Fig. 6).

3.2.3. Microplastics in beverage cartons

Three different brands of water from beverage cartons were analyzed. The water from beverage cartons showed the lowest microplastic counts of all examined packaging types. We found between 5 and 20 particles/l with an average of 11 ± 8 particles/l.

As mentioned before in Section 3.1, one of the samples contained a comparatively high amount of particles classified as „nitrile gloves blue" (46, 11 and 29 particles/l), which were not added to the total microplastic particle count.

Aside from this, the remaining plastic particles consisted of PE (38%), PEST (32%) and PP (26%) (Fig. 4). In addition to microplastic particles, many larger particles in the size range 50–100 μm and >100 μm (largest particle was 1359 μm, see Supporting Information S6) could be seen on the filter. These larger particles were only detected in water from beverage cartons but not in water from plastic or glass bottles. Some of the larger particles were identified as cellulose fibers, most likely derived from the carton itself. A few other larger particles were identified as PE.

7% of the microplastic particles were in the biggest size range of >100 μm, whereas the biggest particle was 1349 μm. The remaining size ranges had the following amounts: 50–100 μm: 10%;

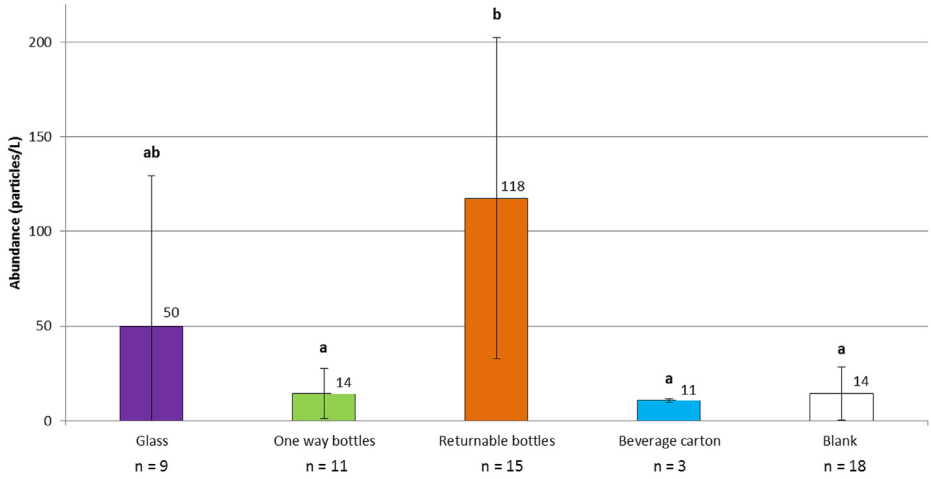
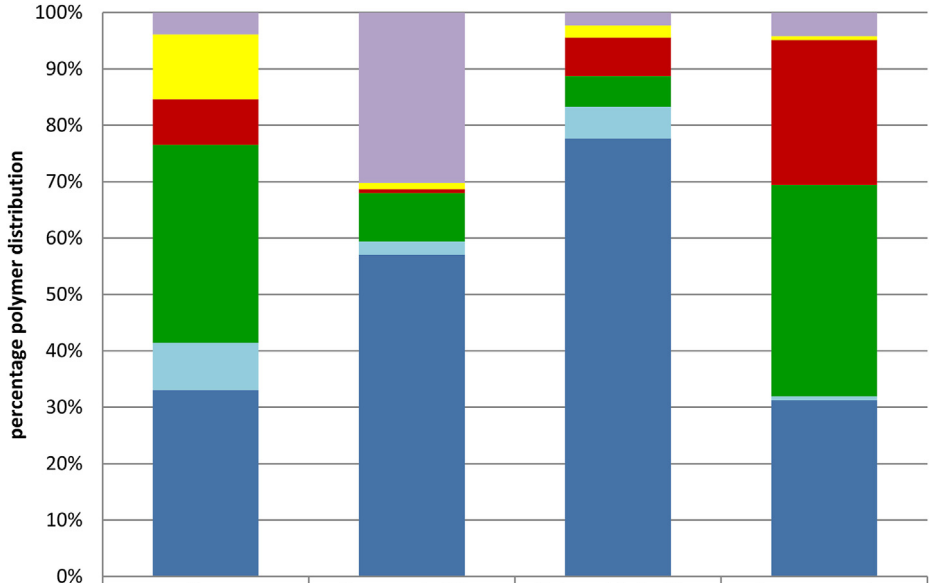


Fig. 3. Mean microplastic content of water from different packaging. Different letters above the error bars indicate significant difference; same letters indicate no significant difference ($p < 0.05$). Error bars represent ± 1 standard deviation.



	Glass	One way bottles	Returnable bottles	Beverage carton
others	4%	30%	2%	4%
PA	12%	1%	2%	1%
PP	8%	1%	7%	26%
PE	35%	9%	5%	38%
PES	8% (41% PEST)	2% (59% PEST)	6% (84% PEST)	1% (32% PEST)
PET	33%	57%	78%	31%

Fig. 4. Polymer distribution of the microparticles found in water from different packaging types (for abbreviations of polymer type see Table 1).

20–50 μm: 16%; 10–20 μm: 28%; 5–10 μm: 39% (see Fig. 6).

3.2.4. Results from difference batches and influence of the carbon dioxide content

Different batches of a single-use and three returnable bottled waters were analyzed some weeks later to check for influences of variations during manufacturing. The single-use bottled water "O6 still"/"O6 still (2)" showed similar low microplastic contents, even though the mean variation of the blank value was larger (see Fig. 7). Also, the microplastic contents of the three returnable bottled waters "R12 medium", "R12 classic" and "R4 classic" were found to be within the same range.

The highest carbon dioxide content was present in classic water, a lower content was present in medium water and almost none was present in still water types. Comparing the microplastic counts in water with different carbon dioxide contents a statistically significant difference between classic (mean 99 ± 82 particles/l) and still water (mean 12 ± 9 particles/l) was observed (see Fig. 8).

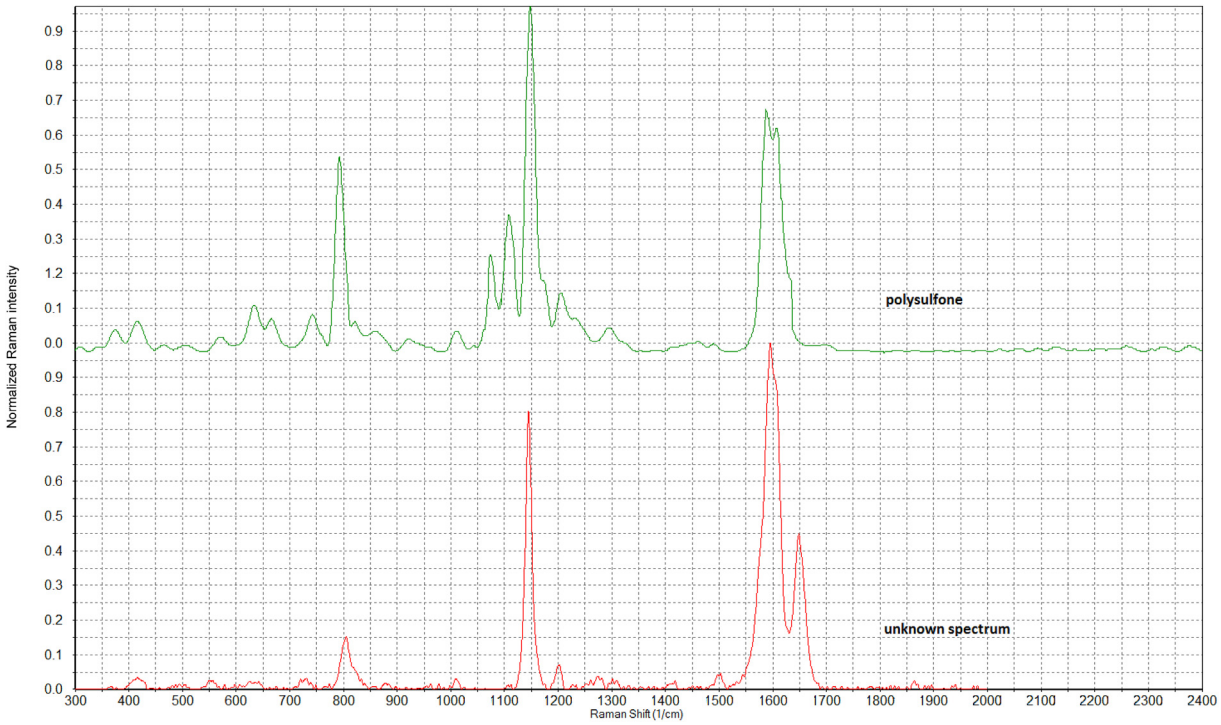


Fig. 5. Spectrum of an unknown particle in single-use plastic bottles (red) and its closest database match (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

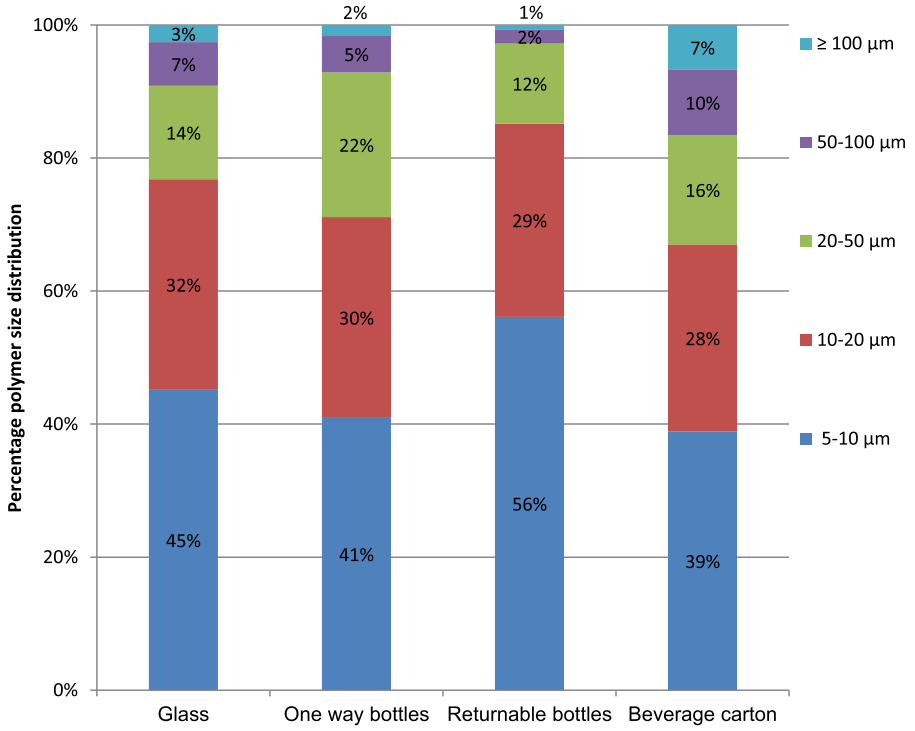


Fig. 6. Polymer size distribution of the microparticles found in water from different packaging types. Particles were classified into 5 size ranges: 5–10 µm, 10–20 µm, 20–50 µm, 50–100 µm and ≥100 µm.

4. Discussion

In this study a total of 38 mineral waters of different types and with different type of packaging materials were analyzed for their microplastic content by means of µ-Raman spectroscopy.

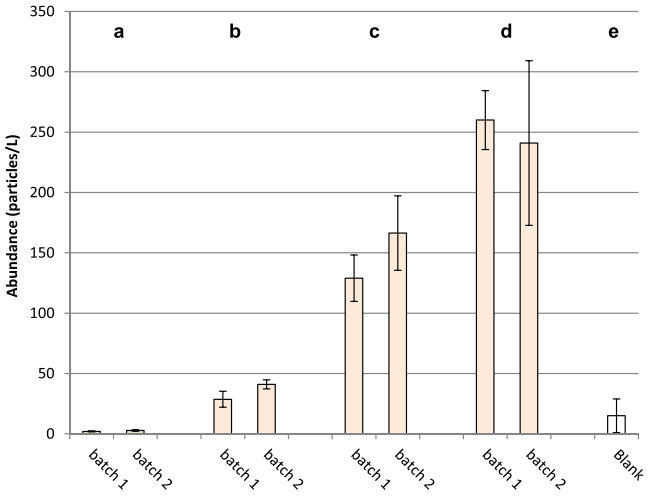


Fig. 7. Number of particles per liter of water found in different batches of **a** O6 still water (single-use bottle), **b** R12 medium water (returnable bottle), **c** R12 classic water (returnable bottle), **d** R4 classic (returnable bottle), and **e** blank value. Error bars represent ± 1 standard deviation.

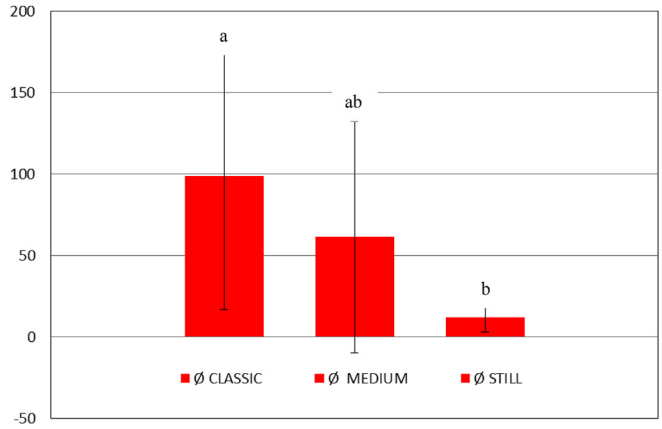


Fig. 8. Mean microplastic particle counts of waters with different carbon dioxide content. Different letters above the error bar indicates significant difference; same letters indicate no significant difference ($p < 0.05$). Error bars represent ± 1 standard deviation.

4.1. Blank values

As blank samples, 1 L of Milli-Q water was filtered in a sequence and also periodically between measurements to check for increasing or decreasing (trend) contamination. The number of particles found in the blank samples were referred to as "blank values". In all of the 18 blank samples no trend in the contamination levels was observed. Despite our efforts to avoid particle contamination it was not possible to obtain a particle count ("blank value") of zero in the blank samples. By using another ultra-filtration filter (ultra-filtration cartridge „Biopak C", Merk Millipore) we were able to further decrease the blank-values. In the present study however, all data were generated using the blanks with the filters mentioned above (see Section 2.3.1). "Biopak C filter" is the recommended filter for future studies.

4.2. Microplastic contents, polymer types and size distribution

A statistically significant difference between the number of microplastic particles in the blank values and water from returnable plastic bottles was observed. The number of plastic particles in water from returnable bottles was 8 times higher than from water in single-use plastic bottles and nearly 10 times higher than that from beverage cartons (see Fig. 3).

Since the majority of microplastic particles in PET-bottled mineral waters consist of polyester, we conclude that microplastics were derived from the polymer bottle itself. This is also supported by the fact that more PEST particles have been found in water from returnable bottles. These type of bottles are under more "stress" due to their reutilization than single-use bottles or beverage cartons. Therefore, microparticles can be a cause of increased abrasion from the packaging material.

A relation of carbon dioxide contents of the waters and number of microplastic particles was observed in this study. Still water had very a low amount of particles whereas sparkling water appeared to have high amount. We suspect that the higher pressure in the bottles with sparkling water compared to the lower pressure of still water distributed in beverage cartons and bottles lead to increased stress on the plastic material resulting in the release of more particles.

The predominant amount of PE plastic particles in water from beverage cartons, as well as the larger cellulose particles found on the filters, were another indication that the contamination was derived from the packaging itself: The beverage carton were coated with PE from the in- and outside and also consisted of a layer of cellulosic material.

We didn't expect the larger amounts of microplastic found in water from glass bottles. It is plausible that the hard glass material caused extra wear on the soft plastic material from the bottle cap and sealing, and thus particles are released due to abrasion. On average, the microplastic content (including the mean variation) was higher in water from reusable glass and PET bottles than from disposable bottles/cartons. Therefore, we recommend to take a closer look at their production, cleaning and refilling processes since this might reveal possible input sources of microplastic contamination. Further examination is required to confirm the source of these findings.

The analysis of different batches of the same water (one single-use bottle and three pairs of returnable bottles) showed similar microplastic contents. Although the number of the repetition samples were small, the similar plastic contents may indicate, that the production date has no influence on the microplastic contents. More data are needed, to support this statement.

Our study revealed that almost 80% of all microparticles were smaller than 20 μm. Therefore, it is not surprising that previous studies did not find (or only small amounts) of microplastics in drinking water or beverages. The main reason is attributed to the fact that these studies used μ-FT-IR not capable of detecting particle sizes smaller than 20 μm.

In addition, we used an automated process of scanning and counting the gold filter in the dark field mode which ensured we did not miss the smallest particles which are often white or transparent. These particles might not be visible when only the bright field mode or a white filter (often used in spectroscopic methods) is applied. Furthermore, our method analyzed the entire filter area which reduced the probability of missing particles. However, this procedure is time- and cost-intensive. For a higher sample throughput it is conceivable to analyze only a sector of the filter or a smaller number of particles if reproducibility is provided.

4.3. Limitations of the method and results

We only had 3 water samples from beverage cartons because we were unable to obtain other samples from super markets in our area. A larger number of samples for this packaging type would have led to more representative results. We are also aware that there is a possibility that the larger cellulose particles could have overlaid some smaller particles which then could have not been analyzed. An underestimation of small particles would have been the result.

Another problem is that a maximum of 5000 particles in total could be identified with Raman. The highest total particle counts were obtained within the smallest size range (5–10 μm) but not all particles could be analyzed by Raman. Therefore, the microplastic content had to be extrapolated based on the total particle counts. Regardless of a multiplying factor of a maximum of 10, an over- or underestimation of plastic particles especially within this size range has to be taken into account.

The removal of "ambiguous" particle matches like "Blue nitrile gloves", "White Petroleum Jelly" or "Nylon" can be argued. It is unclear what the origin substance of these particles is. In general, any occurring colored pigment, fluorescence, additive or carbon bond cause a problem because the underlying polymer spectra may be overlapped (compare (Lenz et al., 2015)). Therefore, the removed "ambiguous" particles might indeed be of plastic nature and as a consequence the present microplastic particle numbers in this study might have been underestimated.

5. Conclusions

We have successfully applied μ-Raman spectroscopy to detect and analyze very small microplastic particles (<50 μm) in mineral waters. The high resolution of μ-Raman spectroscopy was necessary to detect the entire particle population mainly (>80%) consisting of particles with sizes smaller than 20 μm. Previous studies using μ-FT-IR spectroscopy have underestimated the microparticle numbers due to the inability of μ-FT-IR to detect sizes <20 μm.

Water from reusable plastic bottles had the highest averaged polymer particle counts and the particles consisted primarily of polyester and polypropylene. Since the bottles are made of these materials, it can be seen as an indication of a potential wear with the result of packaging material leaching into the water. Additional investigations should be conducted to further corroborate this theory. Abrasion or brittleness of the reusable bottles over time are concerning and could impact their reusability.

The fact that even with greatest care a zero blank value was not feasible demonstrates how ubiquitous microplastic particles are already distributed in the environment and that every processing and filtration step needs to be investigated for contamination sources.

Sources of microplastics have been known for a while, such as microplastic particle generation as a result of plastic littering or microbeads from cosmetic products. However, our results indicate that plastic packaging can also emit microparticles. This is especially alarming since a wide range of food is packed or wrapped in plastic. Released microplastic particles could then be directly ingested by consumers. Further research and analysis is needed with a special focus on particles in the size range below 50 μm.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2017.11.011.

References

Bakir, A., Rowland, S.J., Thompson, R.C., 2014. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environ. Pollut. 185, 16–23.

Cai, L., Wang, J., Peng, J., Tan, Z., Zhan, Z., Tan, X., Chen, Q., 2017. Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. Environ. Sci. Pollut. Res. Int. https://doi.org/10.1007/s11356-017-0116-x (Epub ahead of print).

Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P., Peck, B.B., 1972. Polystyrene spherules in coastal waters. Science 178 (4062), 749–750.

Carpenter, E.J., Smith, K.L., 1972. Plastics on the sargasso sea surface. Science 175 (4027), 1240–1241.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47 (12), 6646–6655.

Colton, J.B., Burns, B.R., Knapp, F.D., 1974. Plastic particles in surface waters of the northwestern atlantic. Science 185 (4150), 491–497.

Davison, P., Asch, R.G., 2011. Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre. Mar. Ecol. Prog. Ser. 432, 173–180.

Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? Mar. Pollut. Bull. 104 (1–2), 290–293.

Hussain, N., Jaitley, V., Florence, A.T., 2001. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. Adv. Drug Deliv. Rev. 50 (1), 107–142.

Imhof, H.K., Laforsch, C., Wiesheu, A.C., Schmid, J., Anger, P.M., Niessner, R., Ivleva, N.P., 2016. Pigments and plastic in limnetic ecosystems: a qualitative and quantitative study on microparticles of different size classes. Water Res. 98, 64–74.

Ivleva, N.P., Wiesheu, A.C., Niessner, R., 2017. Microplastic in aquatic ecosystems. Angew. Chem. Int. Ed. 56 (7), 1720–1739. https://doi.org/10.1002/anie.201606957.

Lachenmeier, D.W., Kocareva, J., Noack, D., Kuballa, T., 2015. Microplastic identification in German beer – an artefact of laboratory contamination? Dtsch. Lebensm. 111, 437–440.

Lenz, R., Enders, K., Stedmon, C.A., Mackenzie, D.M.A., Nielsen, T.G., 2015. A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement. Mar. Pollut. Bull. 100 (1), 82–91.

Liebezeit, G., Liebezeit, E., 2014. Synthetic particles as contaminants in German beers. Food Addit. Contam. Part A 31 (9), 1574–1578.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ. Sci. Technol. 35 (2), 318–324.

McCormick, A.R., Hoellein, T.J., London, M.G., Hittie, J., Scott, J.W., Kelly, J.J., 2016. Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages. Ecosphere 7 (11), e01556.

Mintenig, S., Löder, M.G.J., Gerdts, G., 2014. Mikroplastik in Trinkwasser: Untersuchung im Trinkwasserversorgungsgebiet des Oldenburgisch-Ostfriesischen Wasserverbandes (OOWV)in Niedersachsen. Probenanalyse mittels Mikro-FTIR Spektroskopie. Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung (AWI), Helgoland, p. 50. . (Accessed 20 April 2016).

Sruthy, S., Ramasamy, E.V., 2017. Microplastic pollution in Vembanad Lake, Kerala, India: the first report of microplastics in lake and estuarine sediments in India. Environ. Pollut. 222, 315–322 (Barking, Essex : 1987).

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304 (5672), 838.

Wiesheu, A.C., Anger, P.M., Baumann, T., Niessner, R., Ivleva, N.P., 2016. Raman microspectroscopic analysis of fibers in beverages. Anal. Methods 8 (28), 5722–5725.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1 (4), 140317.