Generation of microplastics from the opening and closing of disposable plastic water bottles

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ABSTRACT

There has recently been a significant increase in interest regarding the prevalence of microplastics in bottled water. Previous studies have shown that the composition of many of the microplastics in bottled water is consistent with the materials of the bottle and bottle cap. The focus of this study is to quantify microplastic particle generation from the cap and bottle interaction during open and close cycles. Nile Red dye was used for the detection of microplastics $>4.7\ \mu m$ in size. Microplastic contamination levels in the water were found to increase as the bottle cap is opened and closed repeatedly. The rate of generation of particles with bottle opening and closing cycles (553 ± 202 microplastics/L/cycle) is adequate to account for the total particle density in the water. This clearly demonstrates that the abrasion between the bottle cap and bottleneck is the dominant mechanism for the generation of microplastic contamination detected in bottled water. A large spread between the maximum and minimum levels of microplastic contamination for bottles from the same lot, regardless of the number of times the cap is opened and closed, suggests that mechanical tolerances in the manufacturing of bottles and caps might play an important role in microplastic generation.

Key words | abrasion, bottled water, drinking water, generation mechanism, microplastic, Nile Red

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HIGHLIGHTS

- The opening and closing of the cap generates the majority of the microplastics in bottled water.
- Improved repeatability of particle detection with Galaxy Count software.
- Improved protocol for detecting microplastics in bottled water.

INTRODUCTION

The rapid growth in the prevalence and production of plastics demands research into the risks posed by microplastics (MPs), to understand and avoid any health concerns. Microplastics are plastic pieces that are smaller than or equal to 5 mm in size (Bergmann *et al.* 2015). The existence of microplastics has been reported for decades, going back to the 1970s (Carpenter & Smith 1972). Microplastics are

often detected in the environment, but a lack of standardized methodology and the variability in composition and concentration of the particles make it difficult to determine the risks posed by the microplastics' existence (Koelmans *et al.* 2019).

With the ubiquity of microplastics comes the question of pollution in drinking water and food. Concerns about microplastics in our diet from consuming fish and seafood have been raised (Davison & Asch 2011). Recently, many studies (including Mason *et al.* 2018; Schymanski *et al.* 2018; Zuccarello *et al.* 2019) have pushed microplastics into the spotlight, when they detected microplastic particles in

bottled drinking water. In addition to drinking water, studies have found microplastic contamination in German beers (Liebezeit & Liebezeit 2014), honey and sugar (Liebezeit & Liebezeit 2013), and table salts (Karami et al. 2017).

The implications of microplastics on human health have been examined (Wright & Kelly 2017); however, the effect on humans is still unknown. There is limited data from animal studies, suggesting that the accumulation of microplastics in the body could induce an immune response, causing particle toxicity (Deng et al. 2017). The particles may also cause damage to the gastrointestinal system of animals (Wright & Kelly 2017), and small particles may transfer to tissues and penetrate organs (ESFA 2016). Chemical toxicity could also occur from the chemical additives and toxins in the plastic, which are used to alter the properties of the plastic polymers (Hammer et al. 2012). Some of these additives have been classified as hazardous to human health and the environment and can even be mutagenic and/or carcinogenic (Lithner et al. 2011). This makes research into the type and amount of microplastic consumed exceptionally important.

There have been various methods for separating and counting microplastics across the multiple fields microplastics research is conducted in. In studies about microplastics in sediment and other aqueous environments, density separation, filtration, and visual identification have been the most prevalent (Hidalgo-Ruz et al. 2012). In studies pertaining to food, particles are usually separated by filtration, and particle analysis is performed on the filter's surface. Particles can also be identified through micro-Fourier transform infrared spectroscopy (Erni-Cassola et al. 2017) or micro-Raman spectroscopy (Karami et al. 2017; Schymanski et al. 2018).

The quality of microplastic research has recently been discussed (Burton 2017). Using a microscope to visually identify microplastics is difficult for small, transparent, or fiber-like particles (Lenz et al. 2015). Fourier transform infrared and Raman spectroscopy do offer the precision to detect microplastics only tens of microns in size, but repeated trials and expensive equipment are often required to obtain reliable spectra (Lenz et al. 2015). There have been studies into automating infrared-microscopy procedures for a less labor-intensive approach, but this method is slow, costly, and has a poor spectral resolution (Maes et al. 2017).

Several recent studies have successfully used Nile Red (NR) dye as an accurate stain, to rapidly detect and count microplastics (Maes et al. 2017; Mason et al. 2018; Chen et al. 2021). NR selectively absorbs and fluoresces; both characteristics that make it an efficient technique to identify and quantify microplastics. NR's selective absorption has been tested for common organic and inorganic environmental contaminants (Maes et al. 2017), and NR's efficiency and reliability was further confirmed through analysis using Fourier transform infrared spectroscopy (Erni-Cassola et al. 2017).

An extensive study by Mason et al. (2018) demonstrated a large variation in MP levels between bottled water brands, and even between the bottles of the same brand, albeit sourced from different locations around the world. Although the average microplastic contamination level in Mason et al.'s (2018) study was 325 MPs/L, the highest case had an average contamination of 2,267 MPs/L, and the lowest lot's average was 3.72 MPs/L. Other studies have also noted a large difference in MP levels between brands (Schymanski et al. 2018; Winkler et al. 2019). A better understanding of variation within the same lot is necessary before comparing studies using similar and/or different detection methods. Therefore, this study is focused on better understanding variability between bottles of a single brand. A total of 31 bottles from two cases (purchased simultaneously) are analyzed for microparticles in this study.

MATERIALS AND METHODS

Sample collection and processing

Two cases of 0.5 L, single use (disposable) plastic water bottles of a major bottled water brand were purchased simultaneously in California, USA, for this study. Each case contained 24 single use polyethylene terephthalate (PET) plastic water bottles. Bottles from the two cases were used randomly in this study to ensure that case-to-case variation does not impact the results of this study.

The bottles' caps were opened and closed 1, 5, 10, and 15 times before analyzing the number of particles generated per open-close cycle. For the cases when the bottle cap was opened and closed more than one time, the bottle cap was

fully loosened, but not removed, from the bottle before retightening it. This was done to ensure that no ambient air could enter the bottle during the open-close cycles. Hereafter, an open-close cycle is simply defined as a combination of the bottle cap being completely loosened and retightened, regardless of whether the cap is removed in between the open and close events. Each open-close cycle was performed in approximately 5 s, and all cycles were performed in quick succession such that 15 cycles were completed in just under 2 min.

After completing the requisite open and close cycles, each bottle was dosed with 5 mL of 1 mg/mL stock NR dye solution to yield a working concentration of 10 μg/mL (Maes et al. 2017). NR dye stock solution was prepared in Acetone (Pharmco HPLC-UV grade) and stored in a glass bottle at 4 °C between experiments. The water bottles were then re-capped and placed on their side during the 30 min dye incubation time period, to ensure that any microplastic particles trapped at the area where the cap meets the bottle are also tagged. The water was then vacuum-filtered through a Whatman glass microfiber filter (934-AH GE Healthcare Life Sciences, 1.5 μm pore). The water bottle was rotated about its axis four times, while the water was being poured for filtration to ensure that all particles on the inside surface of the bottle mouth are flushed out.

All glassware used in the experiments were thoroughly washed after each experiment. Furthermore, glassware was rinsed twice with high-performance liquid chromatography (HPLC) grade 0.22 μm filtered deionized water (Pharmco) just prior to experiments. Processed filters were stored in covered glass Petri dishes to minimize contamination. Furthermore, only cotton clothing was worn by the researcher while performing experiments, as recommended by Koelmans et al. (2019). The order of various repeat experiments was randomized to ensure that the impact of any systemic effects (e.g. buildup of contamination on glassware) is minimized.

Imaging and excitation-emission wavelengths

The filters were inspected with a trinocular optical microscope (AmScope SM-1TNZ, 3.5-90X) fitted with a longpass (LP) filter (Omega Optical Rapidedge), and imaged with a 10M pixel integrated camera (AmScope MU1000). Six images were taken for each filter. Each 10 M pixel image has a field of view of 17 mm × 13 mm, making 1 pixel equal to 4.7 μm. Therefore, this study can detect >4.7 μm particles. There is no size upper bound limitation on particles detected by this method (though particles >1 mm were not observed in this study). Filters were placed on a manual X–Y stage mounted on the microscope base, allowing for easy and precise positioning of the filters for imaging.

Shim et al. (2016) have shown that many plastics can be detected by NR dye tagging using 450–490 nm excitation with 515–565 nm emission, and 534–558 nm excitation with >590 nm emission. For this study, three light sources (Chanzon 100 W High Power LED: Royal Blue 440–450 nm, Cyan 495–500 nm, and Light Green 520–525 nm) were tested in combination with three emission wavelengths using LP filters (Omega Optical Rapidedge LP: 560, 580, and 600 nm). The light sources were operated at 15 W power using a DC power supply (Tekpower TP3005T).

Particle counting

Fluorescent particles in all the images were counted using 'Galaxy Count' software (Faltas 2018) following the approach used by Mason et al. (2018). Particle numbers reported by Galaxy Count are quite sensitive to the threshold value setting used for an image. Human judgment for setting the threshold value can be a major source of variability in the particle counts reported. Whereas Mason et al. (2018) used two researchers to independently set the threshold value in Galaxy Count software and averaged the resulting particle counts, a new procedure for setting an appropriate threshold value for the Galaxy Count software was developed in this work. The slope of reported particle counts vs. threshold value in the software was found to show a significant change when background noise stops getting reported as particles. The threshold value associated with this slope change gives optimal MP counts.

Blanks and positive control

A blank sample was processed with every set of experiments (typically 4–6 bottles processed per set) to measure the impact of ambient air particles and other sources of

contamination on the results of this study. The blank samples were processed using the same procedure as the single use plastic water bottles. 500 mL HPLC grade 0.22 μm filtered deionized water (Pharmco) was poured in a glass beaker and tagged with 5 mL NR stock solution for the blank samples. The beaker was covered for 30 min and then the water was filtered. The filter paper was imaged in the same manner as described previously.

The positive control for this study was established following the methodology used by Mason *et al.* (2018). Three solutions containing 81, 193, and 352 particles (Cospheric, PE microspheres. 75–90 μm diameter, density = 1.25 g/mL) in 500 mL HPLC grade DI water were processed in a manner identical to all other samples. Furthermore, establishing a positive control for this study using 20–27 μm diameter particles (Cospheric, PE microspheres, density =

1.25 g/mL) in HPLC grade DI water was not feasible due to the severe agglomeration of the microspheres (see Supplementary material for details).

RESULTS

Excitation and emission wavelength selection

The results from evaluating the combinations of three excitation and emission wavelengths on a single filter paper sample are shown in Figure 1. NR dyed fluorescent particles are most visible with an excitation wavelength of 520–525 nm (Figure 1, top row) for the emission wavelengths used in this study. A higher level of noise is visible in the image for >560 nm emission wavelengths (Figure 1,
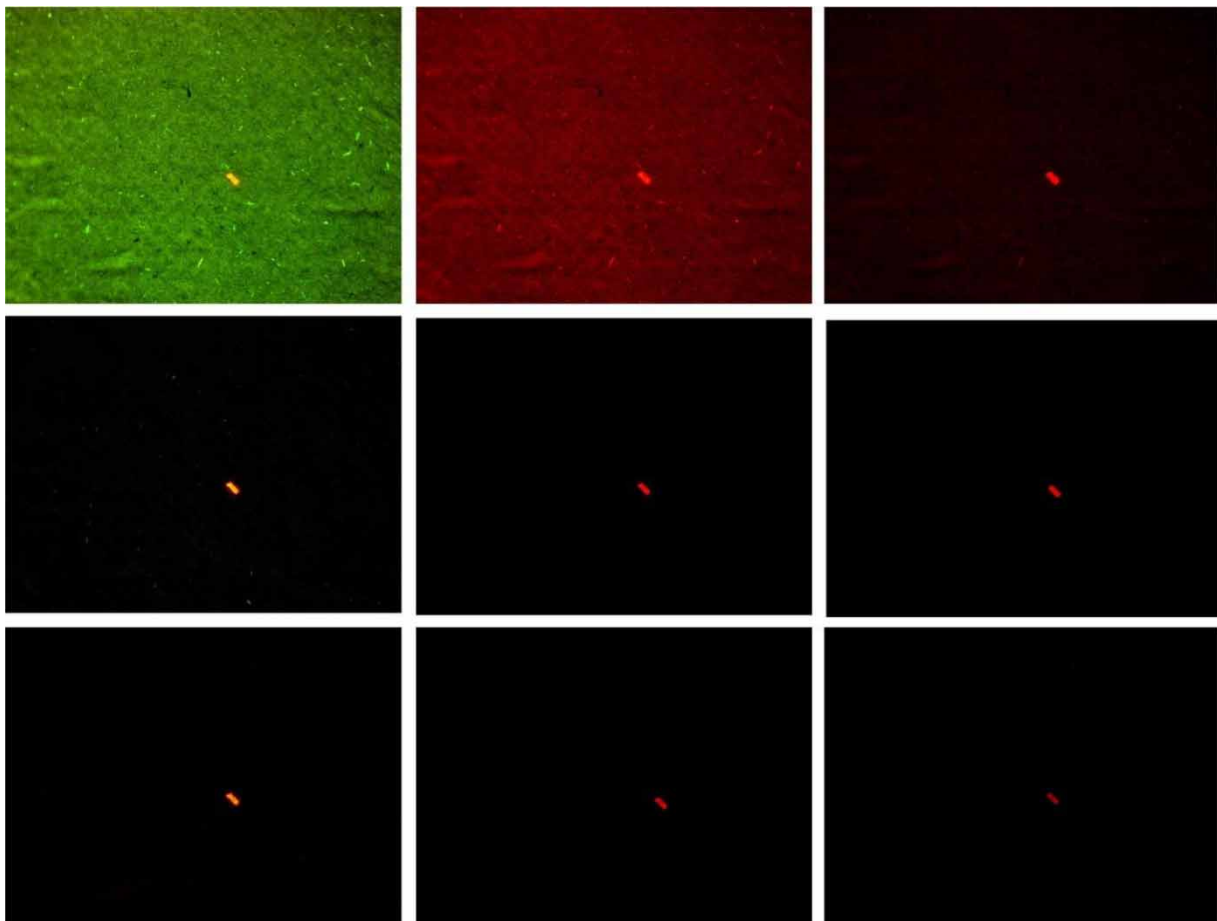
Figure 1 │ Photos from a single sample (17 × 13 mm² field of view) using 3 × 3 combinations of excitation wavelengths 440–450 nm (bottom row), 495–500 nm (middle row), and 520–525 nm (top row) with emission wavelengths >560 nm (left column), >580 nm (center column), and >600 nm (right column). Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.025.

top-left), whereas >600 nm emission wavelengths cut out most small particles. Based on these findings, the combination of excitation wavelengths of 520–525 nm and emission wavelengths >580 nm was used for the rest of this study.

Positive control

The effectiveness of MP detection for this study was established using tests with three solutions prepared with known numbers of 75–90 μm PE particles added to 500 mL of DI water. NR tagged particles were measured using the Galaxy Count software. To partially correct for particles introduced by contamination in these control tests, any detected particles with shapes significantly different from spheres were excluded for the positive control tests. The results are plotted in Figure 2 and show a 70% detection efficiency.

Galaxy Count software threshold setting

Figure 3(a) shows a sample image of fluorescent particles captured as described previously. The effect of changing the threshold setting in the Galaxy Count software is shown in Figure 3(e). A threshold setting of 0.5 is optimal for this image. The software undercounts the number of particles for thresholds greater than 0.5 (as shown in Figure 3(d)) and counts noise as particles for thresholds below 0.5 (as shown in Figure 3(b)). The reported particle counts grow

rapidly when the threshold setting is reduced below the optimal value, due to the prevalence of image noise. Therefore, the optimal value for the threshold setting in the Galaxy Count software can easily be found from the 'knee' of the particle count vs. threshold setting curve. This method provides a repeatable and rigorous method for setting the threshold value and measuring the number of particles in images. The optimal value for the threshold setting was validated by the author for each image, and the total particle count for each sample is reported in (MPs/L) units.

Impact of open-close cycles on MP generation

The total particle counts for the experiments are plotted in Figure 4. It should be noted that the minimum number of bottle cap open-close cycles required to complete the experiment is 2. Each bottle was originally closed by the manufacturer after filling the water (this constitutes as a half cycle). The bottle was opened to add NR stock solution and then re-capped. Finally, the bottle was opened again 30 min later to filter the water completing two cycles. Similarly, the experiment with five open-close cycles prior to adding NR stock solution is reported as six total open-close cycles here.

The detected particle levels in all the experiments shown in Figure 4 are significantly above the lab blank samples (lab blanks compared with two open-close cycles: one sided t-test $p = 0.02$). A clear trend of increasing particle
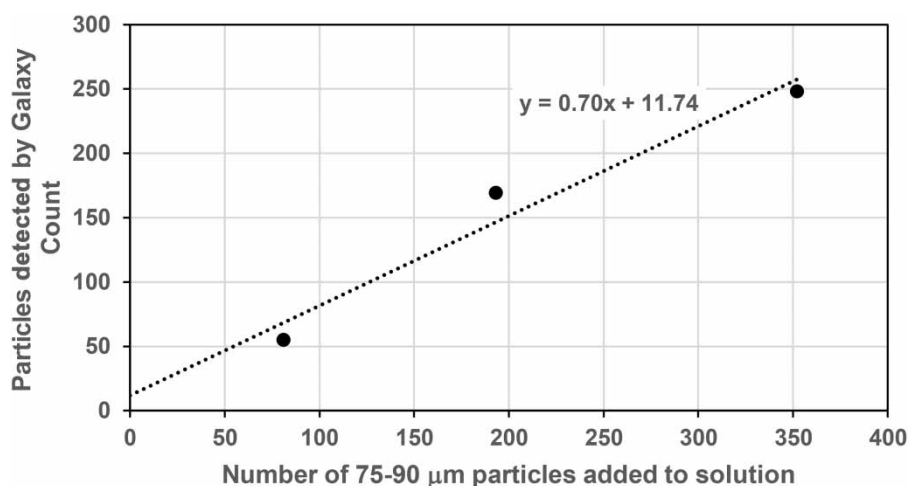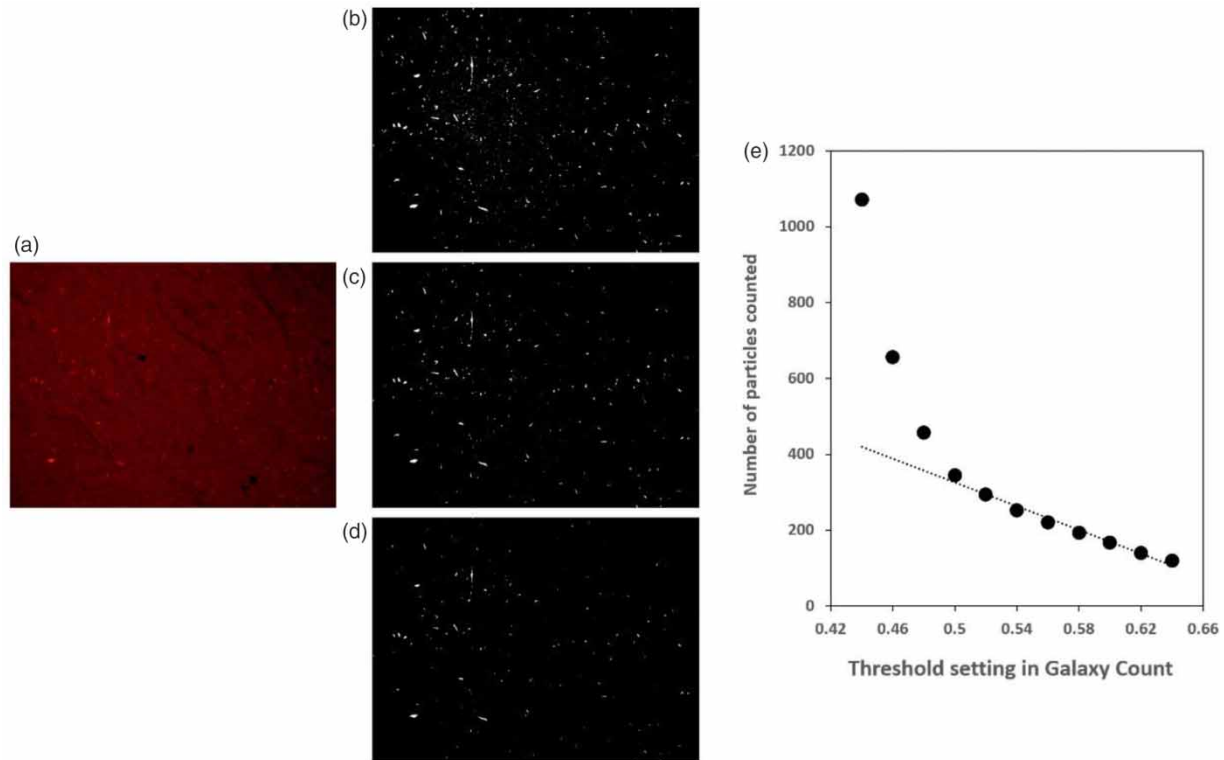

Figure 2 | Number of detected particles tagged by Nile Red in 500 mL of DI water with 81, 193, and 352 polyethylene (PE) 75–90 μm spherical particles.

Figure 3 | (a) Photo under microscope with excitation wavelength of 520–525 nm, imaged through a 580 nm LP filter. The Galaxy Count software transformation of the image in (a) for 0.44, 0.50, and 0.56 threshold settings are shown in (b), (c), and (d), respectively. The number of particles counted by Galaxy Count vs. threshold setting is shown in (e). A threshold setting of 0.5 is found to be optimal for this image. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.025.
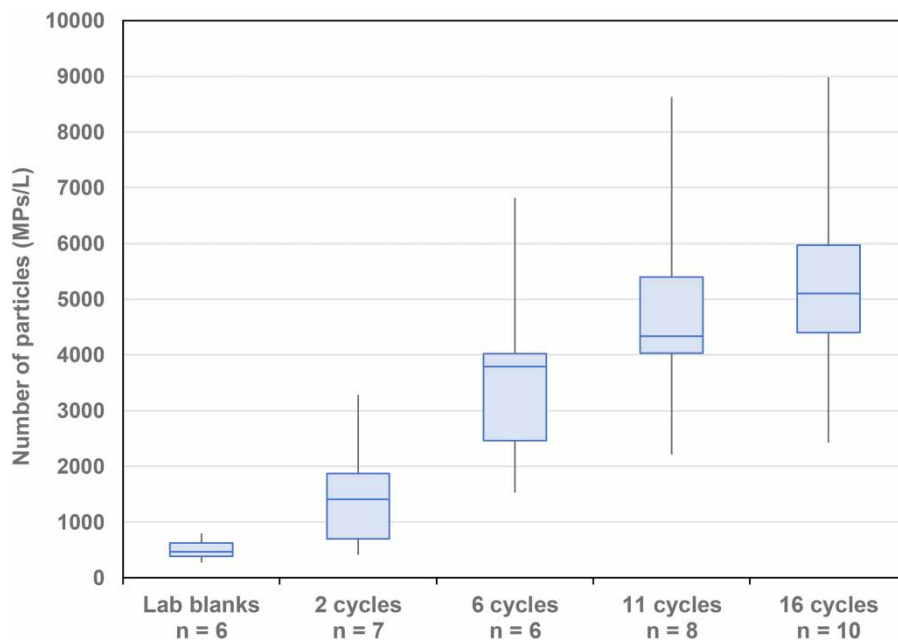
Figure 4 | Box and whisker plot of particle counts (MPs/L) measured in the bottled water as a function of the total number of bottle cap open-close cycles (whiskers show the maximum and minimum values, *n* is the number of samples).

levels with the number of bottle cap open-close cycles is observed.

The average, maximum, and minimum particle counts vs. bottle cap open-close cycles are also plotted in Figure 5 on a cardinal x-axis for clarity. The rate of the average particles' increase is 553 ± 202 (SE) MPs/L per cap open-close cycle between 2 and 6 cycles and is 112 ± 171 (SE) MPs/L per cycle between 11 and 16 bottle cap cycles. Extrapolating the curve showing the average number of particles to zero bottle cap open-close cycles yields an intercept of 358 ± 875 (SE) MPs/L.

DISCUSSION

### Source of microplastic particle detected in the water

A linear extrapolation of the average number of particles in Figure 5 yields a y-intercept value of 358 MPs/L. This represents an estimate of the total particles present in the water from all sources, except for particles generated upon cap opening and closing. Therefore, particles generated during bottling, particles liberated from the inside surfaces of the bottle during bottling, transportation, and handling, and any contamination introduced during these experiments (e.g., ambient air contamination, particles in reagents used in this study, and particles from glassware), all add up to give the y-intercept value.

Comparing the y-intercept mean value of 358 MPs/L in Figure 5 to the average lab blank particle density of 506 MPs/L leads to the conclusion that on average experimental contamination (lab blanks) can completely account for y-intercept since the difference of y-intercept and lab blanks is -148 MPs/L. Therefore, the particles observed during all these tests (corrected for lab blanks) are likely generated during bottle cap open-close cycles. Microplastic particle density in this study is taken to be 553 ± 202 (SE) MPs/L when the cap is opened once based on the slope of mean particle density in Figure 5.

Comparison to previous studies

As noted previously, significant variation in particle levels reported in various studies remains a concern for MP research (Koelmans et al. 2019). MP levels reported in this study (553 MPs/L) for particles sizes $>4.7\,\mu m$ are higher than those reported in previous studies. Mason et al. (2018) reported an average of 325 MPs/L for particles $>6.5\,\mu m$
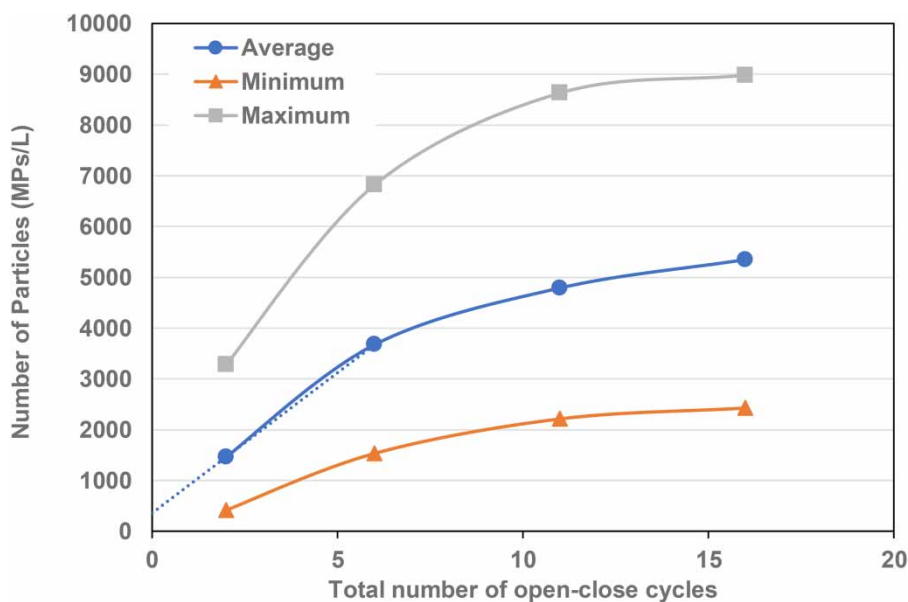
Figure 5 | Average, maximum, and minimum particle counts measured as a function of the total number of bottle cap open-close cycles. The dotted line shows the extrapolation of the average number of particles to zero open-close cycles to find the intercept.

for 11 brands of bottled water. However, 19% of the lots in that study had higher MP levels than this study. Winkler et al. (2019) reported 148 ± 253 MPs/L for particles >6.5 μm. Inherent variability in MP levels in bottled water, along with differences in procedures and detection methods between researchers, have highlighted the need for standardization of testing protocols (Koelmans et al. 2019).

Oβmann et al. (2018), Mason et al. (2018), and Schymanski et al. (2018) reported that 99, 70, and 69% of the particles detected in the water from single use plastic bottles had the same composition as the bottle (PET) and the plastic cap materials (PP and PE), respectively. It is likely that the contribution of background contamination (lab blanks) may explain the differences between the amounts of bottle and cap materials detected in these studies. Although the composition of particles generated by bottle cap open-close cycles was not measured in this study, the primary conclusion of this study that particles are generated from abrasion between the bottle and cap is consistent with findings from the aforementioned studies. This finding that bottle cap open-close cycles dominate particle generation is also in excellent agreement with Winkler et al. (2019), who detected >60,000 particles on the surface of single use PET bottle caps after 100 cap open-close cycles and detected no significant increase of particles in the water upon mechanical stressing the PET bottle body for 10 min.

The procedure used in this study for detecting MPs with NR tagging is based on the method used by Mason et al. (2018). Two procedural improvements in this study are also likely to explain the higher level of MPs detected in this study compared with previous studies. The first of these improvements is related to the orientation of the bottle during the NR incubation period. Figure 6 shows the most commonly used bottle cap design for single use PET water bottles. A collar feature on the cap seals against the inner diameter of the bottleneck. As the results of this study

show, all the MP particles detected are likely generated due to cap open-close cycles. Therefore, it is reasonable to assume that many MP particles will be trapped at the interface of the collar and bottleneck. Laying the bottles on their side during the dye incubation period will ensure that NR can tag particles still at the collar-bottleneck interface, whereas keeping the bottle vertical will eliminate this possibility. Previous studies do not mention bottle orientation during the incubation time.

The second procedural improvement in the study is to rotate the bottle about its axis four times as the water is being poured out for filtration. Winkler et al. (2019) have imaged particles on the bottleneck, and it should be expected that there are particles present all around the inner circumference of the bottleneck, due to the abrasion caused by the rotation of cap relative to the bottle. By rotating the bottle about its axis, all loose particles can be flushed out as the water is poured out. By contrast, pouring water gently from the bottle can result in only 30–50% of the bottleneck circumference contacting flowing water (see Figure 6(c)). Therefore, it is possible that variation in how the water is poured out of the bottle for filtration can induce a 2–3× variation in other studies' data, as previous studies do not mention a control on how water is emptied out of bottles.

Finally, it is likely that observed variations in various studies are simply related to the manufacturing tolerance of the bottleneck and cap. Whereas Winkler et al. (2019) observed significant differences in surface roughness and particle generation on bottlenecks from different brands, information on roughness variation within a lot is not available.

The data in Figure 5 show that the average, maximum, and minimum particles trend up with increasing cap cycles (though at different rates). Since neither external sources of variation (e.g. contamination, measurement
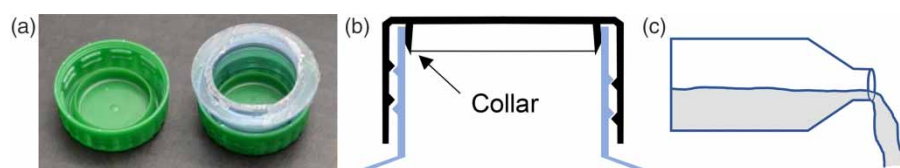
Figure 6 │ (a) Image showing a bottle cap and a bottleneck screwed into a bottle cap with the body of the bottle cutaway, (b) a sectional schematic showing a bottle cap screwed on bottleneck with a collar feature providing a seal against the inside of the bottle as seen in the schematic and the left image. (c) Illustration of pouring water from a bottle with <50% of bottleneck surface in contact with flowing water.

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method variability) nor other internal sources of variation (e.g. water cleanliness) can generate the trends observed in Figure 5, the mechanism behind the variability must be impacted by the number of cap cycles. It is, thus, reasonable to assume that the size variation between the sealing collar on the bottle cap and the inner diameter of the bottleneck would have a significant impact on abrasion when the bottle cap is opened and closed. When the bottle cap collar's inner diameter is lower than the average, abrasion between the collar and bottleneck would be reduced. This would lead to lower levels of MP generation per cap cycle when compared with an average collar. Similarly, a larger collar diameter would result in an increase in MP generation per cap open-close cycle, in the pattern observed in Figure 5. The eventual smoothening of the surfaces that are in contact during cap motion or the yielding of the collar feature would reduce the abrasion force as the number of cap open-close cycles increase. This then reduces the rate of production of MPs after many open-close cycles, as observed in Figure 5.

Impact of airborne MP contamination

Concerns about airborne MP contamination in studies of MPs in bottled water have recently been highlighted (Koelmans et al. 2019). Various authors have used laminar flow boxes to minimize such contamination (Mason et al. 2018; Oßmann et al. 2018; Schymanski et al. 2018). In this study, exposure of bottled water and filters to ambient air was minimized and samples were stored in closed boxes. Furthermore, lab blanks were utilized as checks for ambient contamination. As noted previously, varying the number of bottle cap open-close cycles in this study allowed us to determine the absolute contribution of particles in the water, by conveniently eliminating the effect of all other sources of particles that can offset the particle counts (e.g. ambient contamination).

Although it is always preferable to minimize extraneous sources of contamination, the review of airborne MP literature suggests that the risk of MP contamination from the air is quite low in this study. Pratiwi et al. (2020) measured the greatest indoor airborne MP deposition rate of 1,186 MPs/m^2/day inside an office. Zhang et al. (2020) reported airborne MP deposition rates between 1,800 and 9,900 MPs/m^2/day in various indoor environments. Even when using

the highest reported airborne MP deposition rate from Zhang et al. (2020), airborne contamination can only introduce approximately one microplastic particle per experiment based on a 1 h exposure time for a 55 mm diameter filter paper. Compared with the measured density of 538 MPs/L/open-close cycles in this study, air contamination risk is negligible. Furthermore, the exposure time for bottles and filter paper to open room air was under 5 min. Any MP contamination falling on the filter paper after water filtration has been completed will not be detected in our study as these contaminants will not be tagged by NR dye.

CONCLUSIONS

In this study, two cases of single use bottled water from a major brand were used to study the contribution of bottle cap opening and closing on the levels of microplastic particles found in the water. NR dye was used to specifically stain microplastic particles, and tagged particles were detected using 520–525 nm light source and a 580 nm LP filter. Fluorescent detection of NR tagged MPs is a fast and effective method for MP detection in bottled water since the dye is selective for plastics. The opening and closing of the bottle cap of single use PET water bottles has been demonstrated in this study to be the primary mechanism for generating the microplastics detected in the water. The opening and closing of the bottle cap increased the number of MPs in the water at the rate of 553 ± 202 (SE) MPs/L/cap cycle. Therefore, controlling for the number of times a bottle is opened and closed is essential when comparing different test methods. NR tagging is usually performed inside the PET bottle, necessitating a minimum of two cycles of bottle openings and closings in the experiments. Considering the findings of this study, it is recommended that the number of bottle cap open-close cycles should be reported in future studies regarding levels of microplastics in bottled water. A procedural improvement on pouring out bottled water for studies of microplastics in bottled water is recommended as it could potentially reduce variability in reported microplastic levels in the literature by a factor of 2–3. Finally, the large variability of MPs observed in bottles from the same case suggests that

manufacturing tolerances for size differences in key features of the cap and bottleneck impact the number of microparticles that are generated in each bottle.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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