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Occurrence of Microplastics in Different Brands of Bottled Water and an Assessment of Human Exposure in Detroit, Michigan

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Abstract

A growing number of reports document microplastic (MP) contamination in bottled water, and because bottled water is consumed directly, it represents a plausible route of human exposure; however, published concentrations differ substantially across studies as a result of non-uniform detection approaches. Here we measured MP levels across five commercial bottled-water brands, plus an ultrapure water procedural blank, all purchased in Detroit, Michigan. After vacuum filtration, samples were stained with Nile Red and imaged with fluorescence microscopy; a custom ImageJ macro then recorded particle number, cumulative particle area, and average fluorescence intensity per sample, and each brand was compared statistically to the blank using *t*-tests. Every sample contained detectable MPs, with brand-level mean concentrations spanning 360–634 particles/L (180–317 particles per 500 mL filter); when brands were pooled, this exceeded the blank significantly ($p = 0.0001$), and total particle area was likewise significantly elevated relative to the blank for each individual brand ($p < 0.05$). Average fluorescence intensity reliably distinguished bottled-water samples from the blank, supporting the method's ability to separate genuine particles from background noise. Together, these results establish bottled water as a quantifiable source of MP exposure that varies by brand. Estimated Daily Intake values spanned 2.2–3.9 particles/kg/day in adults and 7.2–12.7 particles/kg/day in children, pointing to bottled water as a persistent exposure route and underscoring the need for standardized measurement protocols and greater regulatory scrutiny of packaging as a contamination source.

Keywords: microplastics; bottled water; Nile Red staining; fluorescence microscopy; ImageJ; estimated daily intake; human exposure; PET packaging

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1. Introduction

Plastic manufacturing has grown steeply since the mid-twentieth century, rising from under 2 million tons in 1950 to global output exceeding 320 million tons per year by 2016 [1–6] and, by some estimates, to roughly 800 million tons today. Derived mainly from fossil fuels such as coal, oil, and gas, plastics have driven a great deal of industrial and technological progress. However, inadequate waste management and a rapidly expanding industry have pushed pollution to critical levels, with annual plastic waste projected to exceed 40 million metric tons by 2030 [5,6]. Because so much of this material is disposed of or managed improperly, it has accumulated across land and water environments alike, contaminating landfills and ecosystems and harming both people and wildlife, fueling

concern over how plastic debris behaves and what harm it may cause [1–6]. Among the resulting pollutants, microplastic synthetic polymer fragments generally defined as ranging from 1 µm to 5 mm (MPs), are especially widespread [1–6]. They arise both from the fragmentation of larger plastic items and from deliberate manufacture for products such as textiles; cosmetics; cleaning supplies; and containers for water, soft drinks, tea, beer, and energy drinks. Within beverages, contamination can enter at several points during ingredient handling, from the packaging itself, or from the source water, and microplastics have by now turned up in virtually every environmental compartment examined, from soil and air to lakes and oceans [1–4].

Because water is a core ingredient in beverages, the beverage sector has drawn particular scrutiny in microplastic research, given how readily its products can pick up contamination [1–3,7]. Plastic packaging is used widely for food containers and for bottling soft drinks along with mineral and natural water [1–4]. Worldwide, more than 450 billion liters of bottled water were consumed in 2020, led on a per capita basis by the United States and, close behind, Mexico; in parts of the world where tap water cannot be safely consumed, bottled water functions as a necessity rather than a convenience [3,4,8]. Surveys of consumers point to perceived purity, taste, portability, and the range of flavored options as common reasons for choosing bottled over tap water [3,4,9–11].

The sheer volume of microplastics now present in the environment, and specifically in bottled water, is concerning because drinking water offers a direct and sustained exposure route with plausible consequences for health. People may also be exposed indirectly, for example by eating contaminated fish or shellfish [1–3,12]. Whatever the route, MNPs have now been detected in human blood, urine, breast milk, gastrointestinal tissue, stool, placenta, heart muscle, testis and semen, sputum, and blood clots. Particles larger than about 100 µm tend to become trapped in mucus, whereas those smaller than 10 µm can cross biological membranes, which may allow slow, symptom-free harm to accumulate over many years. In one study, about 89% of participants had MNPs in their blood, suggesting that these particles have become a routine part of human biology, whether or not they cause disease [5,6].

Animal studies have linked microplastic ingestion and buildup to localized gut inflammation [1–3,13] and to immune-mediated particulate toxicity [1–3,14]. Regardless of route, human microplastic exposure has been associated with outcomes such as oxidative stress, DNA damage, and tissue irritation, among other health effects. A review of 20 key articles additionally reported associations between MPs and disorders of the liver, arteries (atherosclerosis), blood vessels, blood clots, lungs, brain, and intestines, as well as gallstones in young people, rhinosinusitis, eye disease, and skin conditions; some authors have proposed that MPs may contribute substantially to several of these conditions, with toxicity depending on concentration and polymer type. Because exposure can go unnoticed for long periods, the resulting problems could resemble ordinary chronic neurological, metabolic, or cardiovascular conditions. Evidence from occupational and community settings points in a similar direction: a 2003 paper in *Annals of Global Health* described elevated risks of several cancers (leukemia, lymphoma, brain, breast, and mesothelioma), nerve damage, and reduced fertility among plastics-production workers; more deaths from interstitial lung disease and bladder and lung cancer among textile workers; higher rates of lung cancer, neuropathy, heavy-metal poisoning, and cardiovascular disease among recycling workers; and greater risks of lung cancer, low birth weight, asthma, childhood leukemia, heart disease, and chronic obstructive pulmonary disease (COPD) among people living near plastics plants or landfills [5,6].

This makes it important to characterize microplastic contamination across the range of water sources people are exposed to, including natural and mineral waters. Contamination

levels reported for bottled mineral water have trended upward in recent years, with cleaning and filling processes identified as a particular contributor [1–3]; a number of studies have found microplastics in up to 90% of the bottles they sampled [9,11,15–20]. In a survey of 32 mineral-water bottles across 21 German brands, Oßmann et al. [9] recorded an average of 2649 ± 2857 particles/L, with disposable plastic bottles reaching concentrations as high as 2649–6292 particles/L—notably higher than glass bottles—and identified PET as the dominant polymer. Working with Italian mineral waters, Zuccarello et al. [20] similarly found that a larger share of samples tested positive for MP contamination than in comparable studies. Kankanige and Babel [18], focusing on single-use PET bottles sold in Thailand, measured 140 ± 19 pieces/L at sizes of 6.5–20 µm, mostly fibers, with PE, PET, PP, and PA as the principal polymer types.

A broader survey by Mason et al. [15], covering 259 bottles from 11 countries—the United States, Indonesia, India, Mexico, the United Kingdom, France, Germany, Brazil, Lebanon, Italy, and China—identified fibrous polypropylene as the most common polymer, with per-bottle concentrations ranging from 0 to 10,000 particles/L and averaging 350 particles/L. In Iran, Makhdoumi et al. [19] sampled 11 mineral-water brands and found contamination in 9 of them, dominated by fragments (93% of particles) at an average of 8.5 ± 10.2 particles/L, with PET, PS, and PP as the leading polymers. Parveen et al. [21] examined eight Malaysian brands and reported concentrations of 8–22 particles/L, mostly in the 100–300 µm size range. Samandra et al. [22] estimated that over 28% of the Australian population faces elevated microplastic exposure risk, ingesting roughly 400 particles annually from bottled water alone.

Even with this expanding body of work, the field still lacks an affordable, standardized way to detect, identify, and quantify microplastics [1,4,23–25], largely because sampling and analytical procedures have not been harmonized across studies. Given its relative simplicity, bottled water offers a practical matrix for developing and testing such standardized methods [1,4,23–25]. Techniques applied so far include optical microscopy, (micro-)Fourier-transform infrared spectroscopy [11,15,18,19], (micro-)Raman spectroscopy [7,9,18,19,26], and scanning electron microscopy paired with energy-dispersive X-ray analysis [7,11,19,27].

Of these methods, pairing Nile Red staining with fluorescence microscopy [15,18] looks especially promising as the basis for a protocol that is both quantitative and inexpensive enough for routine, high-throughput use. Nile Red's fluorescence intensifies when it partitions into hydrophobic environments, a property that has been leveraged to selectively label plastic particles for semi-automated image-based counting [23,28–34]. Even so, as far as we are aware, only two published studies have combined sample preparation, imaging, and fully automated image and data processing into a single efficient workflow [24,25], and neither calculated an Estimated Daily Intake (EDI).

The absence of an agreed-upon protocol for sampling, isolating, detecting, and identifying microplastics in bottled water makes it difficult to compare abundance figures directly across the literature. This study adds to that evidence base with a design built for greater statistical power, and it offers a refined methodologywith lower detection limits and higher precisionthat future quantification work can build on. A clearer picture of microplastic contamination in bottled water can help inform consumer-safety assessments, environmental policy, packaging design choices, and the development of regulatory standards. As global demand for clean drinking water keeps growing, keeping that water free of harmful microplastic contamination becomes an increasingly pressing goal.

Here, we describe an integrated method for quantifying microplastics in bottled water that combines Nile Red staining, fluorescence microscopy, and ImageJ-based image analysis (Figure 1). The study has two aims: first, to establish whether microplastic count, size, and

morphology differ across bottled water types; and second, to calculate the Estimated Daily Intake (EDI) of microplastics from bottled water for both adult and pediatric populations.

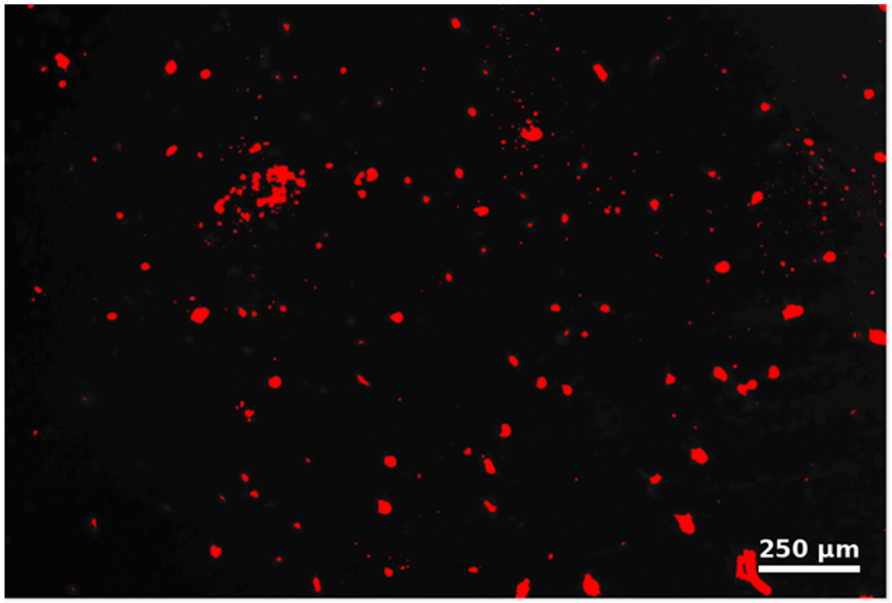


Figure 1. Fluorescence microscopy image of microplastic particles in bottled water stained with Nile Red.

2. Materials and Methods

2.1. Bottled Water Samples

Five commercially available bottled waters (500 mL each, designated A through E in this report) were bought from grocery stores in MI, USA; bottle characteristics are summarized in Table 1. All had shelf-life windows falling between September and December 2024, and any minor differences in bottle durability across that window were not expected to affect the results. To keep brand identities anonymous, each product was assigned a numeric label (1–5), and ultrapure water served as the procedural control/blank.

Table 1. General information about bottled water for the present study.

ID	Volume (mL/Item)	Water Type	Type
1	500	Electrolyte water	PET
2	500	Sugar water	PET
3	500	Drinking water	PET
4	500	Carbonated and flavored water	PET
5	500	Flavored water	PET
6	500	Ultrapure water	

2.2. Quality Assurance and Control

All filtration and staining work was carried out inside a laminar flow biosafety cabinet (BSC) that had been wiped down with acetone beforehand to guard against cross-contamination by stray microplastics. Before each new sample was processed, every piece of equipment used inside the BSC scissors, tweezers, beakers, glass pipettes, and the Büchner funnel was rinsed first with 75% ethanol and then with ultrapure water. Control and sample

beakers were kept entirely separate, and filters remained inside the BSC from the moment filtration finished until they were imaged. Everyone handling samples wore cotton lab coats to minimize the shedding of synthetic microfibers. Both the ultrapure water blanks and the bottled-water samples were analyzed in ten replicates per group ($n = 10$ for each of the five brands and for the blank), for a total of 60 samples (50 bottled-water samples plus 10 blanks). The blanks were processed using the same procedures and under the same conditions as the experimental samples to account for potential contamination introduced during sample processing.

2.3. Extraction of Microplastics

Our extraction procedure builds on the approach described by Reference [35], adjusted to fit the equipment and materials available to us. Five commercial bottled-water brands were bought at retail, with ten 500 mL bottles purchased per brand (50 bottles in total; $n = 10$ per brand), and each bottle was processed as an independent sample. Before handling, each bottle was wiped down externally with laboratory-grade detergent, rinsed with deionized water to remove surface contamination, and had its label removed. Each 500 mL bottle was gently inverted to mix its contents, and its entire contents (500 mL) were filtered as a single sample, giving one filter per bottle (bottles were not filtered in replicate), using vacuum filtration through 0.45 µm Whatman paper (GVS, 47 mm diameter) following the membrane-filtration extraction approach described previously [36]. Sterilized forceps were used to move each filter onto a clean microscope slide. Particles were then stained by adding 70 drops (Table 2) of a 10 µg/mL Nile Red solution (in methanol) dropwise from a Pasteur pipette, chosen specifically to avoid disturbing the particles on the filter. Slides air-dried fully inside the laminar flow hood before a coverslip was placed over each filter and the slide was taped shut and labeled. Every step of this process filtration, staining, and slide preparation took place inside the same sterilized BSC used for quality control, and ultrapure water-only procedural blanks (sample 6) went through an identical sequence of steps alongside the experimental samples.

Table 2. Sample preparation and Nile Red staining parameters.

ID	Water Sample Size (mL/Item)	Nile Red Added Drops	Dry Time (Hours)
1	500	70	24
2	500	70	24
3	500	70	24
4	500	70	24
5	500	70	24
6	500	70	24

2.4. Data Collection

The interval between sample preparation and imaging matters for accurate counting [34] as dye fluorescence and particles themselves can degrade over time, and delays risk both under and over-counting. To limit this, we imaged and photographed every filter the day after it was prepared. Fluorescent microplastics were viewed at 10× magnification on an Olympus BX-60, Evident, Needham, MA, USA using an orange filter (~529 nm) under 450–470 nm blue-light excitation. This wavelength was chosen because Reference [33] had shown that nearly all common polymers and textile fibers nylon being the main exception fluoresce strongly under 470 nm illumination.

We selected 10× magnification specifically because the particles in our samples tended to run smaller than those typically reported from marine sampling, which meant a lower size detection threshold was appropriate here. To keep counting consistent, filters were topped with well-etched glass slides that served as a fixed visual reference during photography, and a Nikon DSLR mounted on the microscope captured an image of every fluorescent object visible, irrespective of its shape or size.

Particle counting from these fluorescent images was automated in ImageJ (v1.50i) via a custom macro that (1) calibrated the spatial scale, (2) removed background signal with a 1500-pixel rolling-ball radius, (3) converted each image to 8-bit grayscale, (4) applied brightness thresholds of 29 (lower) and 175 (upper), and (5) counted particles with an area cutoff of 400 μm^2. This 400 μm^2 threshold was set deliberately so that pores in the 10 µm-diameter filter membrane would not be mistaken for particles.

2.5. Estimated Daily Intake for Bottled Water

Estimated Daily Intake (EDI) highlights the amount of microplastics ingested daily via bottled water consumption [19,20]. The EDI for bottled water was calculated using microplastics in drinking water and daily water consumption (Equation (1) below). We used the reported ingestion rate (IR) values available for bottled water in the United States of America; IR values for bottled water consumption (0.49 L/day for adults and 0.36 L/day for children) were adopted from Reference [37], while body weight (BW) for adults and children in USA was 79.35 kg [38] and 18 kg [38]. Particle counts were obtained per filter, and each filter represented one 500 mL (0.5 L) bottle; counts are therefore reported per filter (N, particles per 500 mL) and were converted to concentrations in particles/L as C = N/V, where V = 0.5 L (equivalently, C = 2 × N). For example, a mean count of 180.2 particles per filter corresponds to 180.2/0.5 = 360.4 particles/L. The converted concentrations C were used in Equation (1).

$$\text{EDI (particles/kg/day)} = (\text{C} \times \text{IR})/\text{BW} \quad (1)$$

where C = microplastic concentration in bottled water (particles/L); IR = ingestion rate (L/day); BW = body weight (kg).

2.6. Data Analysis

Descriptive statistics of microplastic particle data (maximum, mean, minimum and standard deviation) were analyzed to reflect the microplastic particle data (number of microplastic particles, particle size, shapes and colors) in different bottled water brands. Furthermore, a one-sample *t*-test was performed to find any statistically significant differences between the number of microplastics particles in bottled water and blank samples. A value of p less than 0.05 was considered statistically significant. All statistical tests were carried out using SPSS 25.0 for Windows.

3. Results and Discussion

3.1. Occurrence of Microplastics in Bottled Water

Microplastics turned up in every bottled-water sample analyzed ($n = 50$ across the five brands, alongside 10 ultrapure water blanks), confirming that contamination was not confined to a single product. Fragments were the dominant particle shape, with fibers and films appearing less often—a pattern that matches earlier reports [15,26]. Because the ultrapure water blanks contained far fewer particles than the bottled-water samples, procedural contamination appears to have been minimal [27].

Figure 2 shows considerable brand-to-brand variation in per-filter particle counts (particles per 500 mL sample): Brand 1averaged 224.6 ± 165.4 (n = 10), Brand

2 305.0 ± 120.8 (n = 10), Brand 3 254.3 ± 133.9 (n = 10), brand 5 180.2 ± 159.0 (n = 10), brand 4 316.8 ± 263.6 (n = 10), against an ultrapure water blank average of 40.6 ± 15.0 (n = 10). Converting to particles/L (count ÷ 0.5 L), these correspond to mean concentrations of 449.2 (Brand 1), 610.0 (Brand 2), 508.6 (Brand 3), 360.4 (Brand 5), and 633.6 (Brand 4) particles/L, i.e., 360–634 particles/L across brands, versus 81.2 particles/L for the ultrapure water blank. Individually, brand 3 (t = 4.30, p = 0.005), brand 5 (t = 2.77, p = 0.022), brand 4 (t = 3.31, p = 0.009) each differed significantly from the blank; Brand 1 (t = 2.05, p = 0.071) and Brand 2 (t = 1.90, p = 0.089) did not reach significance on their own, largely because each contained one unusually high-count sample (225 particles for Brand 1; 305 for Brand 2) that inflated its variance. Pooling all bottled-water samples together and comparing them to the blank as a single group, however, produced a clearly significant difference (t = 4.19, p = 0.0001; n = 50 vs. 10). This brand-level variability echoes other work suggesting that manufacturing practices, packaging choices, and source water can each shape microplastic content. Studies using Nile Red and FTIR imaging have shown that opening and closing a bottle cap alone generates hundreds of particles per liter, with abrasion between the cap and bottleneck identified as a dominant source at a generation rate of roughly 553 ± 202 microplastics/L/cycle, and separate work has demonstrated that repeated cap opening significantly increases microplastic abundance in bottled water [15,26,39]. Carbonation likely adds physical stress to packaging, since dissolved CO_2 forms carbonic acid that lowers pH while bubble formation agitates the container walls, and researchers have proposed that the mechanical action of bubbling and pressure changes can dislodge micro fragments from the container, contributing to elevated microplastic content in soft drinks. For brand 1 drinks and brand 2 specifically, the extra manufacturing step is worth flagging even though I could not find a study measuring it directly: these products pass through additional processing lines (brand 1,4, and 5 concentrate dosing, mixing nozzles, in-line blending) that plain bottled water simply does not go through [40].

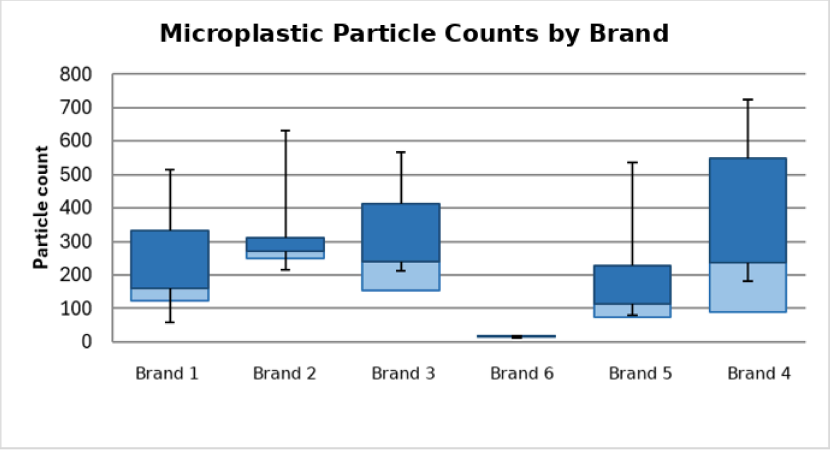


Figure 2. Distribution of microplastic particle counts by bottled water brand.

The two most extreme counts came from a single brand 2 sample (305 particles) and a single brand 1 sample (225 particles); once these outliers are set aside, the remaining brand-to-brand differences are smaller and track closely with the overall pattern in Figure 2. Conventionally treated drinking water can pick up microplastics from both point sources, such as leachate from poorly managed landfills, and non-point sources like scattered solid waste [41]; even reverse-osmosis treatment does not fully keep microplastics out, since some particles are small enough to pass through the membrane [42]. Our particle counts were higher than those reported by References [11,18,19,26,43], a discrepancy several groups have traced to the bottle cap and inner wall as contamination sources. Physical stress during

shipping, handling, and manufacturing can cause the PET used in bottle construction to shed polymer fragments from its inner surface [18,26,43]. Reference [44] found that reusable PET bottles carry higher microplastic loads than single-use ones, presumably because repeated use abrades the inner wall more; along similar lines, high storage temperatures or long storage times can accelerate fragmentation and increase migration of particles from the cap, bottleneck, and packaging [18,45]. Reference [43] additionally linked greater microplastic release to repeated opening and closing of the bottle, which generates friction at the cap and bottleneck. Our observed particle-size distribution is broadly consistent with these earlier findings.

Group means followed the same brand-level pattern seen in Figure 2: brand 2 and brand 1 had the highest average counts, while brand 5 water had the lowest among the bottled brands. The pooled comparison against the ultrapure water blank remained significant ($t = 4.19$, $p = 0.0001$), reinforcing that the particles detected reflect genuine contamination rather than laboratory artifacts.

One notably consistent finding involves the per-sample mean fluorescence value: all five bottled-water brands clustered tightly around 32–38, whereas the ultrapure water blank averaged 13—a gap significant at $p < 0.00001$ both brand-by-brand and in the pooled comparison. We interpret this consistent separation as supporting evidence that the imaging pipeline is successfully discriminating real particles from background signal. Total particle area told a similar story but with more brand-to-brand spread, ranging from a mean of 8962 μm^2 (brand 3) to 20,986 μm^2 (brand 2), compared with just 40.6 μm^2 for the blank; again, every brand differed significantly from the blank ($p = 0.002$–0.043 individually; pooled $t = 6.19$, $p < 0.0001$).

Nile Red staining was central to keeping false positives low and making particles easier to visualize. Reference [30] showed that the dye binds preferentially to synthetic polymers and fluoresces strongly on hydrophobic plastic surfaces, which gives it a reasonably reliable way of telling microplastics apart from organic or mineral debris. That combination of selectivity and sensitivity is why Nile Red staining has become a favored tool in recent microplastic monitoring work, especially when combined with fluorescence microscopy to catch particles as small as ~10 µm.

We did not attempt to identify the specific polymers present, but our method consistently visualized and counted particles across the 10–500 µm range, which fits current guidance on microplastic screening and the practical limits of Nile Red-based fluorescence approaches [30,44]. These results reinforce earlier evidence that bottled-water microplastics can originate from several places at once—the source water itself, the inner PET wall of the bottle, or friction at the cap during processing and storage [43]. Even without FTIR or Raman confirmation of polymer identity, the visual and quantitative data here make a strong case for microplastic contamination in commercially sold bottled water.

3.2. EDI Estimation for Adults and Children

Table 3 shows the EDI estimates for adults and children, calculated from the lowest and highest microplastic concentrations we measured in bottled water. Adult EDI values ranged from 2.2 to 3.9 particles/kg/day, and children's ones ranged from 7.2 to 12.7 particles/kg/day. These figures depend on the ingestion-rate estimates from Reference [37], but even so, they suggest that the potential health risk from microplastic exposure warrants continued attention rather than dismissal. Part of that risk comes from the additives and residual monomers embedded within plastic polymers themselves [46]—substances like plasticizers, stabilizers, and pigments that can leach out and accumulate in fatty tissue, where they may contribute to inflammation and cellular toxicity [47,48].

Table 3. Input values and calculation of the Estimated Daily Intake (EDI) of microplastics from bottled water, using the lowest and highest brand-level mean counts.

Group	Concentration Basis	N (Particles per Filter)	V (L)	C = N/V (Particles/L)	IR (L/Day)	BW (kg)	Calculation: EDI = (C × IR)/BW	EDI (Particles/kg/Day)
Adults	Lowest brand mean (brand 5)	180.2	0.5	360.4	0.49	79.35	(360.4 × 0.49)/79.35	2.23
Adults	Highest brand mean (brand 4)	316.8	0.5	633.6	0.49	79.35	(633.6 × 0.49)/79.35	3.91
Children	Lowest brand mean (brand 5)	180.2	0.5	360.4	0.36	18	(360.4 × 0.36)/18	7.21
Children	Highest brand mean (brand 4)	316.8	0.5	633.6	0.36	18	(633.6 × 0.36)/18	12.67

Note: N = mean particle count per filter; each filter represented one 500 mL (0.5 L) bottle, so C (particles/L) = N/0.5 L. EDI = (C × IR)/BW (Equation (1)). IR = 0.49 L/day (adults) and 0.36 L/day (children) from Reference [37]; BW = 79.35 kg (adults) and 18 kg (children) from Reference [38]. Values are rounded to 360–634 particles/L, 2.2–3.9 particles/kg/day (adults), and 7.2–12.7 particles/kg/day (children) in the text.

Both References [19,20] reported EDI values that diverge from ours in opposite directions. Reference [20] calculated approximately 1,531,524 particles/kg/day for adults and 3,350,208 particles/kg/day for children, while Reference [19] reported much lower figures of 0.015 and 0.065 particles/kg/day, respectively. Our estimates (2.2–3.9 particles/kg/day for adults; 7.2–12.7 particles/kg/day for children) fall several orders of magnitude below Reference [20] yet well above Reference [19], a middle position consistent with our measured concentrations (roughly 360–634 particles/L across brands), which sit within the range reported elsewhere in the bottled-water literature (e.g., [9,15]). Much of this spread across studies likely traces back to differences in both the measured particle counts and the ingestion-rate assumptions each group used. Future work refining EDI estimates should account for variables such as bottle-material quality, individual consumption patterns, storage conditions, and how often a bottle's cap is opened and closed.

These particles have also turned up in the olfactory bulbs, in the arterial plaques (atheroma) that lead to cardiovascular events, and during pregnancy; they have also been associated with a wide range of diseases. At the same time, because plastics are produced and used on such an enormous scale, our age could be called the "Plastocene," a period in which any not-yet-explained illness might end up being blamed on plastic contamination. It would not be surprising if, for some conditions, these particles turned out to be protective rather than harmful [5,6].

Cleaning up environmental MPs would be a complicated and costly undertaking, but reducing how much the body absorbs, at least through the digestive tract, looks far more achievable. In this regard, retention findings from a pilot study showed a clear pattern. MPs with dense hydrogen bonding or rigid structures (CE and PF) stayed in the body longer, whereas polymers with smoother surfaces and functional groups that can form hydrogen bonds and electrostatic interactions (PE, PET, and RA) were cleared more easily. This "selective polishing" could help guide future diets aimed at lowering the MP burden inside the body. As a first, this pilot study found that a single 0.8 g dose of PCC given to people markedly increased MP excretion after a standardized meal (SM). That suggests PCC may reduce systemic MP exposure, even when taken with just one meal [5,6].

3.3. Study Limitations

This work offers useful insight into microplastic presence in bottled water sold in the Detroit area, but several caveats apply. We did not confirm polymer identity through spectroscopic methods such as FTIR or Raman, so although the fluorescent particles we detected are very likely microplastics based on their Nile Red staining pattern, they were not chemically verified, meaning we cannot rule out other hydrophobic particles that fluoresce similarly [23,24]. Our sample was also limited to brands purchased locally, which may not represent the full diversity of bottled water sold nationally or internationally; sampling more brands across a wider geographic area would strengthen how broadly these findings can be applied.

Nile Red staining, though efficient and widely adopted, is not perfectly specific to plastics, and non-plastic materials can sometimes stain as well, which risks inflating particle counts [29,30]. Particles smaller than 10 µm may also have escaped detection given the resolution ceiling of our microscope and analysis software. Because fluorescence signal fades over time, any delay between staining and imaging introduces a risk of undercounting [34]; we imaged samples promptly, but some minor signal loss cannot be entirely excluded. Finally, despite working inside a laminar flow hood with ethanol-rinsed equipment and cotton lab coats, background contamination remains a possible confounderthe ultrapure water blanks registered negligible counts, but stray airborne fibers or particles entering during processing cannot be fully ruled out [44].

4. Conclusions

This study examined microplastic contamination across bottled-water brands widely sold in Detroit-area retail stores. Every sample tested positive, reinforcing how pervasive microplastic contamination has become in this product category. Irregular fragments were the most common particle shape, followed by fibers and films, with most particles falling between 100 and 500 µm. Although we did not confirm polymer composition directly, combining Nile Red staining with fluorescence microscopy allowed us to detect and quantify particles that are very likely synthetic polymers. Chemical confirmation using spectroscopic or chromatographic techniques would be necessary to definitively determine the polymer composition of the particles; however, these analytical techniques were not available for the present study. Concentrations varied meaningfully by brand, a pattern probably shaped by differences in source water, packaging material, and manufacturing steps such as high-pressure filling or cap friction; bottled waters marketed with added features like electrolytes or carbonation tended to show elevated counts. Estimated Daily Intake came out to 2.2–3.9 particles/kg/day for adults and 7.2–12.7 particles/kg/day for children lower than some figures reported internationally, but still indicative of a real, ongoing exposure pathway. Contamination controls, replicate sampling ($n = 10$ per brand), and automated image analysis all strengthened this study's design, but future work should tackle its remaining gaps, particularly the lack of polymer identification and the exclusion of sub-10 µm particles. Expanding sampling across regions; adding detailed polymer characterization; and examining variables like storage conditions, consumer handling habits, and packaging wear will all help build a fuller picture of microplastic exposure from bottled water and what it may mean for human health.

Author Contributions: E.N.N., S.Q. and K.C.L. conceived the experiment(s); E.N.N., S.Q. and K.C.L. conducted the experiment(s); E.N.N., S.Q. and K.C.L. analyzed the results. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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