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Original Article

Occurrence and Assessment of Human Exposure to Microplastic Particles in Bottled Water From Southwest, Nigeria

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Abstract

Background: The investigation of microplastics (MPs) in different environmental media is well documented; however, the prevalence and possible human health risks of MPs in bottled water are not fully understood.

Methods: This study was designed to (*a*) determine the quantity, form, color, and size of MPs, (*b*) evaluate the influence of physicochemical properties on the release of MPs in bottled water, and (*c*) estimate the daily intake (EDI) of MPs in eight distinct varieties of bottled water from Southwest Nigeria. The Rose Bengal staining method and microscopic analysis were employed to identify, quantify, and characterize the MPs.

Results: The bottled water brands from Southwest Nigeria contained MPs of various shapes, colors, and sizes. The average abundance of MPs was 15.2 ± 2.9 particles/L, with the highest abundance being 21.3 ± 1.8 particles/L. Fibers (82%) were the most detected shape of MPs, followed by fragments (18%), with transparent and blue being the two colors observed. Particle sizes ranged from 0.2 to 2.5 mm. The concentrations of MPs in the bottled water were strongly correlated ($r = 0.789$, $P < 0.01$) with the pH of the water samples. Adults had an estimated EDI of 0.31 to 0.66 particles/kg bw/day, while children's EDI ranged from 1.13 to 2.36 particles/kg bw/day.

Conclusion: These findings provide insights for scientists to better understand the contamination of bottled water by MPs and help regulatory bodies formulate appropriate regulations for the production and bottling of drinking water.

Keywords: Microplastics, Drinking water, Human health risk, pH, Rose Bengal, Nigeria

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Introduction

Polymers used to make plastics can be synthetic or semi-synthetic and are produced from hydrocarbons or biomass sources.[1] Over the past 25 years, global plastic production has surged, growing from one million tons in 1945 to an astounding 390 million tons in 2021.[2] Plastics degrade through a photo-oxidative process into tiny particles called microplastics (MPs). MPs are plastic particles smaller than 5 mm, typically ranging from 1 to 5 mm in size.[3] Depending on their origin, MPs are categorized into two types: primary MPs, which are intentionally manufactured as microbeads in personal care products, and secondary MPs, which form when larger plastics, such as bottles, packaging, and synthetic clothing, break down under UV light.[3] MPs have been studied in marine environments[4] and freshwater systems,[5] and are not only found in environmental media but have also entered the food chain, raising food safety concerns.[6] MPs have been detected in various food products, including seafood,[7] sugar,[8] salt,[9] milk,[10] bottled water,[11-12] and tap water.[13] Water is an indispensable resource essential for human survival. However, population growth, industrialization, and climate change are depleting water resources.[14] Plastics, widely used in industries due to their durability, stability, low cost, and lightweight properties, have replaced materials[15] like glass in bottled water packaging.[16] The bottled water industry has grown steadily, producing over six billion gallons annually worldwide.[17] In Nigeria, the market is projected to reach 6.13 billion liters by 2027, with per capita consumption estimated at 27.50 L in 2023.[18] Bottled water is favored for its flavor, safety, and convenience[19] and is classified into three categories based on source and treatment: treated water, natural mineral water, and spring water.[12] However, the packaging process introduces MPs into bottled water, necessitating research into their presence and associated health risks. The World Health Organization (WHO) has emphasized the need to investigate MP concentrations in bottled water worldwide and evaluate potential health impacts.[20] While studies suggest that MPs can cause gastrointestinal obstruction, their toxicity in humans remains largely unknown. Numerous studies have confirmed the presence of MPs in bottled water.[11,12,15,21-24] For instance, Oßmann et al reported the highest MP concentrations in single-use PET bottles (2,649 ± 2,857 per liter) and glass bottles (6,292 ± 10,521 per liter) in a study of German brands.[23] Similarly, Zuccarello et al found the highest MP levels (5.42E + 07 ± 1.95E + 07 particles/L) in Italian mineral waters.[24] In Nigeria, only one study has reported the occurrence and pollution indices of bottled water in the country's Southeast region.[11] Given the rising MP pollution in drinking water and the high consumption of bottled and sachet water in urban areas, further studies are needed to assess the potential health hazards posed by MPs.

Therefore, this research aims to: (*i*) examine the presence and characteristics (size, color, and shape) of MPs in eight bottled water brands from Southwest Nigeria; (*ii*) evaluate the influence of physicochemical parameters of bottled water on MP release; (*iii*) identify potential sources of MPs in bottled water; and (*iv*) estimate human exposure by calculating the estimated daily intake (EDI) for adults and children.

Materials and Methods

Chemicals, Samples Collection and Physicochemical Properties

Analytical reagent (AR) grade substances were used throughout this study. Rose Bengal dye was obtained from J.T. Baker (Phillipsburg, USA), and glass fiber filters were sourced from Whatman (Whatman GF/D, UK). Eight brands of bottled water, packaged in polyethylene terephthalate (PET) bottles, were purchased from a university water industry, retail shops, and supermarkets in Ilishan-Remo town and its environs in Ogun State, Southwest Nigeria. These brands were selected based on their accessibility and affordability, with each bottle containing 750 mL of water. To ensure durability, the expiration dates of the samples ranged from April to September 2022. Three bottles of each brand were collected for analysis. Also, the physicochemical properties of the water samples, including pH, electrical conductivity (EC), and total dissolved solids (TDS), were measured using a multi-parameter analyzer (EZ-9909A, China). Three reagent blanks were analyzed as controls alongside the water samples. All samples, stored at room temperature, were analyzed within a week. To prevent MP contamination, the samples were prepared and analyzed in a fume cupboard.

Sample Preparation and Extraction of Microplastics from Bottled Water

The extraction of MPs from bottled water was conducted using a modified version of the Makhdoumi et al method.[15] Each bottle was opened under a functional laminar flow fume hood, and 10 mL of Rose Bengal stain (200 µg/mL working concentration) was added to the water and incubated for 30 minutes. This stain adheres to the surface of organic fibers, aiding in the visual identification of MPs.[25] After incubation, the water samples were filtered in triplicate using glass fiber filters (Whatman GF/D; 90 mm diameter; 1.5 µm pore size, UK) and a vacuum apparatus (VP 180, Zhejiang, China). The MPs collected on the filters were dried in glass Petri dishes under a fume cupboard before microscopic analysis. MPs were visually examined for shape and color using an Omax optical microscope (Omax, A3550S, China) with a camera. Filter papers were observed at 40 × magnification, and the particle sizes of MPs were measured using ImageJ software (v1.53).

Microplastics Contamination Factor and Pollution Load Index

The microplastic pollution load index (MPPLI) and MP contamination factors (MPCF) were computed using the techniques of Ibeto et al and Enyoh et al.[11,26] The level of MP contamination in the analyzed bottled water is depicted by the MPCF. MPCF was divided into four groups by Ibeto et al. Low contamination is indicated by $MPCF < 1$, moderate contamination is indicated by $1 < MPCF < 3$, significant contamination is indicated by $3 < MPCF < 6$, and extremely high contamination is indicated by $MPCF \geq 6$. Equations 1 and 2 were used to compute MPCF and MPPLI, respectively[11] Where MPi is the number of MPs in sample i and MPs is the minimal baseline concentration obtained from the lowest MP abundance recorded in Praveena et al, who investigated the occurrence of MPs in Malaysian bottled water.[27] This study was carried out in a comparable environment and analytical frameworks.

$$MPCF = MPi/MPs \tag{1}$$

$$MPLI = (MPCF_1 \times MPCF_2 \times MPCF_3 \times MPCF_n)^{1/n} \tag{2}$$

Estimated Daily Intake for Bottled Water

The number of MPs consumed daily through bottled water can be evaluated using EDI.[15] The EDI of MP concentration in Nigerian bottled water (MP/kg/day) was calculated in equation 3 as follows:

$$EDI = (C \times IR)/BW \tag{3}$$

The ingestion rate (IR) is represented by the unit MP/kg/day for EDI, and it is assumed that it is 1.8 L/day for children and 2.2 L/day for adults. BW is the adult body weight of 70 kg and the children body weight of 16 kg. C is the concentration of MPs in (particles/L).[11]

Quality Control

To minimize contamination, all sample processing was conducted in a fume cupboard. Glassware was meticulously washed, rinsed with distilled water, and oven-dried. Laboratory benches were disinfected with 75% ethanol, and nitrile gloves along with cotton laboratory coats were worn during sample preparation. Blank filter papers were examined microscopically to confirm the absence of contamination. Control experiments were conducted using deionized water under the same procedures.

Statistical Analysis

Descriptive statistics and data analyses were performed using SPSS 24. Principal component analysis (PCA) and bivariate correlation analysis were employed to evaluate associations between physicochemical properties and MPs.

Results and Discussion

Physicochemical Properties

The levels of TDS, EC, and pH in several bottled water brands are presented in Table 1. The pH values ranged from 5.29 ± 0.01 to 7.78 ± 0.92. Half of the analyzed water samples complied with the WHO's recommended pH range for drinking water (6.5–8.5).[20] However, samples from certain brands, including A-C and H, did not meet this standard (Table 1). Only the water samples from brands A, F, and G exhibited higher conductivity values, exceeding the 100 µS/cm limit for drinking water. Nevertheless, the TDS in all bottled water brands were within the limits specified by the WHO. These findings align with those reported by Ibeto et al.[11]

Occurrence of Microplastics in Bottled Water

A total of 24 bottled water samples from eight different brands were analyzed for the presence of MPs using the Rose Bengal staining method and microscopy. Figure 1 illustrates the abundance of MPs in bottled water brands from Southwest Nigeria. The mean MP abundance was 15.2 ± 2.9 particles/L, ranging from 10.0 to 21.0 particles/L. Bottled water from brand H had the highest concentration of MPs (21 particles/L), while the lowest concentration was observed in brand C (10 particles/L). The variations in MP abundance across the brands may be attributed to multiple factors, including differences in the water preparation processes, workers' clothing, filling machinery, and water sources. Additionally, the mechanical strength and quality of the bottles could play a role. For instance, lower-quality bottles that deform more easily during handling, pressing, or opening might release a greater number of MPs compared to sturdier ones. These findings suggest that MPs in bottled water could originate from the water source itself—typically municipal water treated with modern drinking water processes—or from external contamination during production. The factory workers' fabrics and the packaging materials, such as PET bottles and polyethylene caps, may also contribute to the MP levels in the bottled water.

The MP abundance observed in this study (average: 15.2 ± 2.9 particles/L) is comparatively lower than that reported in previous studies on MPs in bottled water.[12,22,24,28] However, it is higher than the levels reported by other investigations[11,15,27,29] (Table 2). The contamination of bottled water with MPs in this study may originate from the manufacturing and packaging processes. Airborne MPs represent another potential source of contamination, as achieving an entirely MP-free water bottling environment is challenging due to the pervasive nature of MPs in the environment.[22] MPs in the water samples could also be attributed to the materials used in packaging. Bottles are manufactured from PET, while caps are made from polyethylene (PE). During the bottling process, MPs may be introduced into the water due to the high pressure exerted during injection molding of the bottles. Furthermore, MPs can be introduced during the filling process or through the repeated opening and closing of bottle caps.[15,30]

Figure 2 illustrates the shapes and colors of MP particles identified in the eight bottled water brands analyzed. Across all brands, fibers were the most prevalent shape, accounting for 82% of the detected MPs, followed by fragments at 18%. These findings align with previous research on bottled water, which also reported fibers as the dominant MP shape in drinking water.[12,22,23,33-35] However, some studies on bottled water have identified fragments, rather than fibers, as the most prominent MP shape.[11,15,36]

Table 1. Physicochemical parameters of the bottled water

Brand	pH	EC (μs/cm)	TDS (mg/L)
A	5.83 ± 0.18	105.50 ± 12.00	50.50 ± 6.36
B	5.63 ± 0.03	86.00 ± 1.41	40.00 ± 1.41
C	5.29 ± 0.01	60.00 ± 1.41	31.50 ± 0.71
D	7.42 ± 0.00	7.00 ± 0.00	3.00 ± 0.00
E	7.50 ± 0.05	64.00 ± 5.66	32.50 ± 2.12
F	6.60 ± 0.02	345.50 ± 2.10	179.50 ± 12.02
G	7.78 ± 0.92	207.50 ± 0.71	103.50 ± 0.71
H	6.09 ± 0.16	10.00 ± 0.00	5.00 ± 0.00
WHO	6.50 – 8.50	1000	1000

Source: World Health Organization[20]

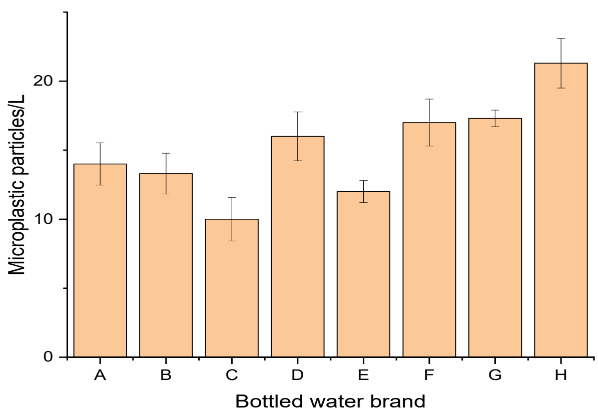

Figure 1. Abundance of microplastics in the bottled water

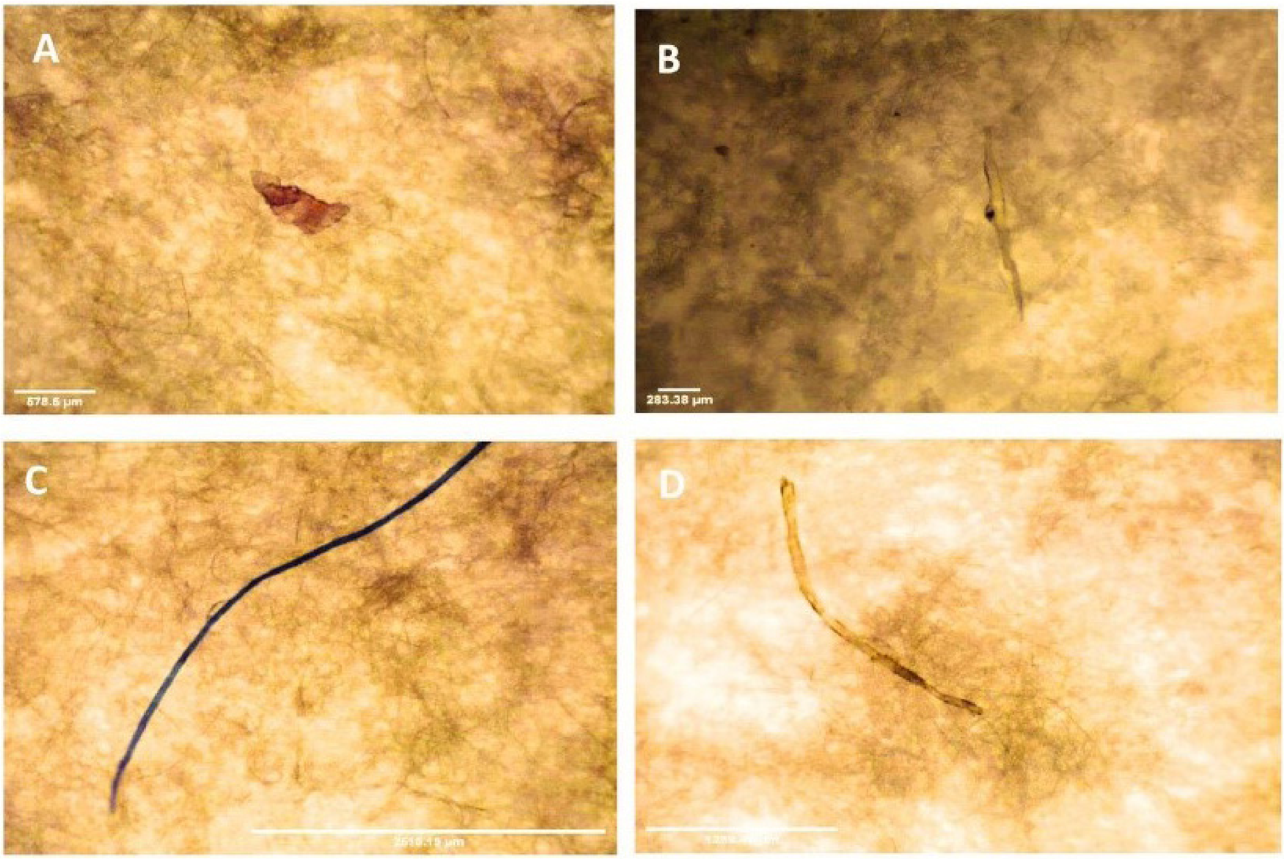

Figure 2. Microscopic Images of Microplastics Found in the Drinking Bottled Water: (a) Fragment; (b – d) Fibres

Table 2. Abundance of microplastic particles in the bottled water from Southwest, Nigeria compared with different studies

Country	Plastic-type	Morphology	Microplastics (Particles/L)	Size range (µm)	Consumption rate	References
Nigeria	PET	Fibre, Fragment	15.2 ± 2.9	200 – 2500	Adult: 0.314 & 0.66 particles/kg/bw/day Children: 1.13 & 2.36 particles/kg/bw/day	This study
Malaysia	PET	Fragment	11.7 ± 4.6	100 – 300	Adult: 0.068 & 0.19 particles/kg/bw/day Children: 0.089 & 0.25 particles/kg/bw/day	27
India	PET	_	20 ± 5 to 127 ± 35	1 – 30	7 ± 2.6 mg/kg/day	31
Australia	PET	_	13 ± 19	77 ± 22	400 particles/year	
Saudi Arabia	PET, single used	_	1.9 – 4.7	200 – 500	1.7 – 1.9 particles/kg/bw/day	32
Iran	PET	Fragment, Fibre	8.5 ± 10.2	1280 – 4200	Adult: 5.35 particles/kg/bw/year Children: 23.43 particles/kg/bw/year	15
Nigeria	PET	Fragment, film, pellet	2.20 ± 1.85 P/0.75L	20 – 100	Adult: 0.019 & 0.193 particles/kg/bw/year Children: 0.040 – 0.800 particles/kg/bw/year	11
Thailand	PET, single used	Fibre, fragment	140 ± 19	6.5 – 20	_	22
Italy	PET, single used	_	5.42E + 07 ± 1.95E + 07	1.28 – 4.2	Adult: 40.1 µg/kg/bw/day Children: 87.81 µg/kg/bw/day	24
Germany	PET, single used glass bottles	_	2649 ± 2857 6292 ± 10521	> 1	_	23

The shape of MPs is a crucial particle characteristic due to its role in cellular uptake.[37] Research has shown that fiber uptake increases with smaller-sized fractions during displacement at the air-liquid interface and cellular interactions.[38,39] Given the enhanced cellular uptake of fibers and other small-sized particles, consuming water contaminated with MPs exposes humans to fibrous MPs, thereby heightening potential health risks.[22]

This study analyzed the colors of MPs in bottled water brands, an approach previously reported in other investigations.[15,34,40] However, several studies did not assess the colors of MPs in bottled water.[11,12,23,24,41] Two primary MP colors were identified in the sampled water, with transparent being the most prevalent, followed by blue (Figure 2). These findings are consistent with other studies that reported transparent, blue, black, and grey MPs in bottled water.[15,27,33,34,40] The transparent color is associated with the PET plastic bottles, while the blue coloration likely results from mechanical movement or abrasion of the bottle caps.[15] Assessing MP color is critical for identifying the source of the debris. Research indicates that colored MPs are more readily absorbed by aquatic organisms compared to non-colored ones.[34] In addition to color, determining the size of MPs is vital due to the potential health risks they pose. Figure 2 and Table 2 show the varying sizes of MPs detected in bottled water, ranging from 200 to 2500 µm. These size ranges are consistent with findings from previous studies.[27]

Microplastic Contamination Factor and Microplastic Pollution Load Index

Figure 3 shows the MPCF of the bottled water brands, which ranged from 1.25 to 2.60. According to the MPCF categorization, all water samples were moderately contaminated with MPs. The pollution load index was 1.82, suggesting that the bottled water samples analyzed from the Southwest area of Nigeria are polluted by MPs. This result aligns with the study by Ibeto et al,[11] who reported a pollution load index of 1.71 in bottled water from the Southeast area of Nigeria. These MPs tend to build up in the body, causing deleterious consequences. Therefore, contamination of bottled water by MPs is hazardous. Proper caution should be taken during the manufacturing and packaging of bottled water to reduce this contamination level. In addition, consumers should be careful when opening bottled water caps to minimize the consumption of this contaminant over time.

Estimated Daily Intake of Microplastics

The EDI for adults and children is presented in Table 3. The lowest and highest abundance of MPs obtained from this study were used to estimate the EDI. For adults, the EDI ranged from 0.314 to 0.66 particles/kg bw/day, while the EDI for children ranged from 1.13 to 2.36 particles/kg bw/day. The current report of the EDI is lower than those estimated by Zuccarello et al.[24] The team evaluated the EDI for adults to be 1,531,524 particles/kg bw/day and for children to be 3,350,208 particles/kg bw/day. The EDI in this present study, however, was higher than those reported by Praveena et al[27] and Altunışık.[33] Altunışık calculated the adult and child EDIs to be 0.009 and 0.040 particles/kg bw/day, respectively. The differences observed in the EDI estimations could be attributed to the varying number of MPs in the bottled water and the ingestion rate (IR) values utilized in each investigation. Additionally, the quality of plastic bottles, storage conditions, and the availability of drinking water sources could influence the EDI.

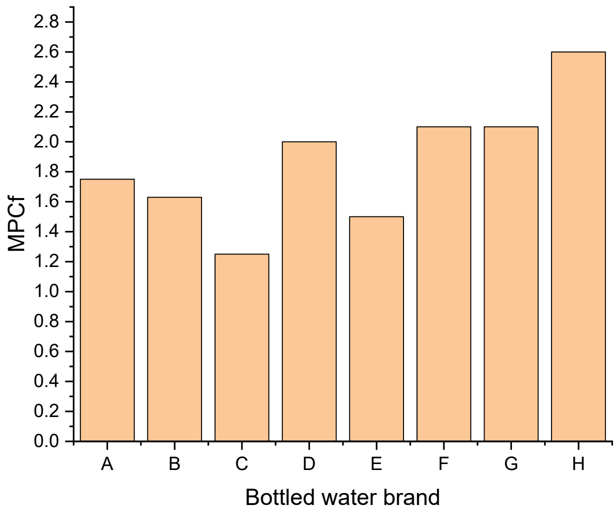

Figure 3. Microplastic Contamination Factors in the Bottled Water

Physicochemical Properties and Relationship with Microplastics in Bottled Water

PCA was employed to evaluate the association between the obtained values for physicochemical parameters and MPs. Table 4 shows the results of the PCA for physicochemical characteristics and MPs, whereas the component plot in rotated space is depicted in Figure 4. Two components were extracted with eigenvalue > 1. PC1 showed 53.05% of the total variance and was dominated by conductivity and TDS with a more significant positive loading value; however, PC2 was dominated by MPs and pH and accounted for 29.17% of the total variance. MPs showed a significant positive correlation ($r = 0.789$, $P < 0.01$) with the pH of bottled water. This indicates that alkaline pH could increase the abundance of small-sized MPs. This result aligns with those published by Zuccarello et al,[24] who reported a strong connection between pH and MPs in Italian bottled water.

Study Limitations

This study provides the first proof of MP contamination in readily available bottled water brands in Southwest Nigeria.

Table 3. Estimation of EDI for the Both Adults and Children

Group	Microplastics in bottled water (particles/L)	Ingestion Rate, IR (L/day)	Body weight, BW (kg)	Particle/ kg/bw/day
Adults	10	2.2	70	0.31
	21	2.2	70	0.66
Children	10	1.8	16	1.23
	21	1.8	16	2.36

Table 4. Total Variance of Physicochemical Properties and Microplastics in the Bottled Water

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.122	53.053	53.053	2.122	53.053	53.053	1.993	49.835	49.835
2	1.167	29.175	82.228	1.167	29.175	82.228	1.296	32.393	82.228
3	0.710	17.758	99.986						
4	0.001	0.014	100.0002						

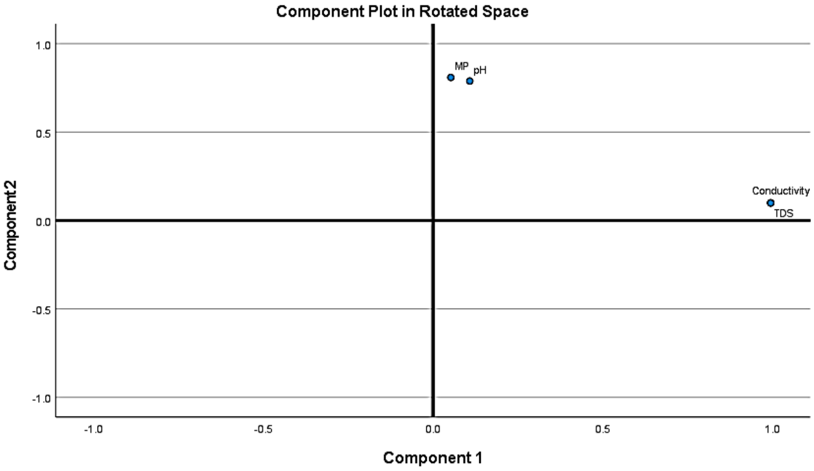

Figure 4. Principal Component Plot in Rotated Space

The approach has several drawbacks. The investigation of MPs in bottled water was conducted in this study using a straightforward and affordable technique called Rose Bengal stain and MP analysis. Therefore, not every suspected MP was selected for polymer identification using ATR-FTIR or micro-FTIR.

Conclusion

This is the first investigation of the abundance, shape, color, and size of MPs in bottled water from the Southwest region of Nigeria. In total, eight different bottled water brands from major supermarkets and retail stores in the Southwest were analyzed. Every water sample contained MPs. The average abundance of MPs was 15.2 ± 2.9 particles/L. The most prominent shape was fiber, followed by fragment. Transparent and blue were the most common colors observed in the samples. There was a significant correlation ($r = 0.789$, $P < 0.01$) between pH and the abundance of MPs in the bottled water. The water samples had a moderate level of contamination with MPs. The EDI values for adults (0.314 and 0.66 particles/kg bw/day) and children (1.13 and 2.36 particles/kg bw/day) estimated in this study indicate that consumption of bottled water contaminated with MPs over an extended period can increase the hazards these particles pose to human health. Therefore, it is recommended that regulatory agencies and the water industry closely monitor the bottled water production process, starting from the water treatment plant to the bottling process, to minimize MP contamination.

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Writing–original draft: Oluwatosin Sarah Shokunbi.
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Competing Interests

The authors declare no financial or personal conflicts of interest.

Ethical Approval

Not applicable.

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