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Article

Identification of microplastics in bottled water, a potential risk to human health

Identificación de microplásticos en agua embotellada, un potencial riesgo para la salud humana

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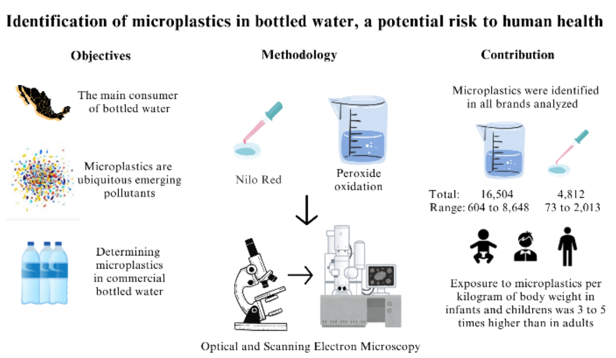
Abstract

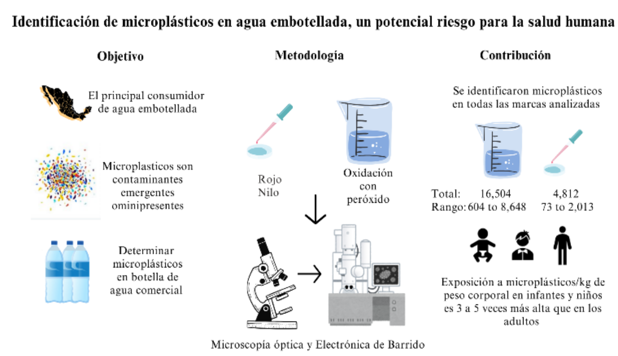
Mexico is the leading consumer of bottled water. Microplastic pollutants are emerging that are ubiquitous in the environment, posing a risk to human and environmental health. The aim was to determine the presence of microplastics in commercial bottled water using Nile Red staining, and 30% hydrogen peroxide oxidation. Microplastics were verified by optical and scanning electron microscopy. Microplastics were identified in all brands analyzed, with higher count observed in the oxidation method (16,504 range: 604 to 8,648) compared to Nile Red staining (4,812; range: 73 to 2,013), p = 0.04 (Mann-Whitney U test). Granules predominated in both methods. Exposure to microplastics per kilogram of body weight in children and infants was 3 to 5 times higher than in adults. The long-term effects of this exposure remain unknown, highlighting the need to implement monitoring programs for emergency contaminants to ensure access to high-quality water.



Microplastics, bottled water, health risks

Resumen

México es el principal consumidor de agua embotellada. Los microplásticos, son contaminantes emergentes omnipresentes en el ambiente, representando un factor de riesgo para la salud humana y ambiental. El objetivo fue determinar microplásticos en agua embotellada comercial por tinción de Rojo de Nilo y Oxidación con Peróxido de Hidrógeno al 30%. Se verificaron por microscopía óptica y electrónica de barrido. En todas las marcas se identificaron microplásticos, siendo mayor por el de oxidación (16,505; rango 604 a 8,648) que el Rojo de Nilo (4,812; rango 73 a 2,013), p = 0.04 (U de Mann Whitney), Los gránulos predominaron en ambos métodos. La exposición a microplásticos/kg peso en niños e infantes es mayor en 3 a 5 veces que en los adultos. Se desconocen los efectos a largo plazo, por lo que es necesario implementar programas para la detección de contaminantes emergentes para garantizar el acceso al agua de buena calidad.



Microplásticos, agua embotellada, riesgos para la salud

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Introduction

Bottled water (BTW) refers to water intended for human consumption, sealed in bottles or other containers with no added ingredients (U.S. Food and Drug Administration, 2023). In regions where water distribution systems are inefficient or of poor quality, EC becomes a crucial source of water. In cities with efficient and high quality distribution networks, consumption of EC is associated with the belief that it is healthier and safer, with better organoleptic properties. This perception has led to an increase in consumption worldwide, with Mexico being the country with the highest annual per capita consumption: 271.8 litres (71.8 gal) in 2017, increasing to 303.2 litres (80.1 gal) in 2022 (Aslani *et al*., 2021; Espinosa-García *et al*., 2015; International Bottled Water Association, 2024).

EC can be packaged in glass or plastic bottles, the latter being the most common due to its various characteristics: low cost, light weight, ease of transport, high strength and lack of taste. The most commonly used materials for bottles are polyethylene terephthalate (PET), polycarbonate and high-density polyethylene (HDPE), while caps are usually made of HDPE, low-density polyethylene and polystyrene (Brancaleone *et al*., 2024; Kirstein *et al*., 2021; Waghmare & Dar, 2024).

The perception that EC is a safer alternative, with fewer contaminants compared to tap water, is rooted in the belief that its production follows more rigorous standards. However, despite efforts to keep water safe and clean, the possibility of contamination during the various stages of production and consumption is undeniable. Emerging pollutants include microplastics (MP), defined as polymeric matrices with a diameter of less than 5 mm (Akhbarizadeh *et al*., 2020; Nacaratte *et al*., 2023).

One of the United Nations Sustainable Development Goals is universal access to safe drinking water. Recent studies have shown that aquatic bodies are becoming reservoirs of PM, resulting from the ubiquity of plastics in everyday life and the inadequate management of their waste, which end up being deposited in nature (terrestrial and aquatic) and in urban areas.

They are produced by biotic factors (biodegradation by micro-organisms) or abiotic factors (photodegradation by exposure to UV light or weathering resulting from wave or wind action). They have been found in the ocean, seas, soils and even the atmosphere as well as in various seafood, milk, salt and water (Li *et al*., 2023; Mason *et al*., 2018).

As a consequence, both marine and terrestrial organisms are exposed to microplastics, and thus enter food chains, representing a risk factor for various wildlife species and consequently humans. Due to their particle size they can enter tissues and organs, oxidative stress, chemical interference, cytotoxicity, inflammation, DNA damage, changes in the gut microbiome, metabolic disruption and increased immune disorders and cancer. Therefore, the World Health Organisation has called on the scientific community to study the possible short-, medium- and long-term health consequences (Brancaleone *et al*., 2024; Kirstein *et al*., 2021; Li *et al*., 2023; Waghmare & Dar, 2024).

Despite the increasing number of investigations on tap and bottled water in different parts of the world, risk assessment of PM in bottled water remains a complex task, due to the lack of data on exposure limits. The toxicity of PM is known to increase in smaller fractions, as they may move through physiological systems and accumulate in organs such as the liver and kidneys, as well as generate negative impacts at the cellular level (Kirstein *et al*., 2021; Nacaratte *et al*., 2023).

The objective of this study was to determine the presence of microplastics in bottled water from commercial brands in the Toluca Valley by comparing two methods.

Methodology

Sample selection

Eight commercial brands of EC, purchased in convenience stores located in the municipalities of Lerma, San Mateo Atenco and Toluca, in the State of Mexico, were selected. The selection of the brands was based on a consumer survey of 120 people, determining the inclusion of those with the highest and lowest frequency of purchase. Table 1 shows the characteristics of the samples, including batch, expiry date and cost.

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Box 1

Table 1
Characteristics of the samples

M	P	Vol (ml)	Precio (MXN)	Lote	FC
A	1	1000	7	A80228L192	21-05-25
B	2	1000	7	1006717RE	23-04-25
C	3	1000	6	L0331IZT310333	29-03-25
D	9	1000	5	40523	1-11-25
E	12	500	40	LPRD109122	19-10-25
F	15	1000	6	314401215	21-05-25
G	17	900	25	Z2039	23-05-25
H	19	1000	18	LT006230350	6-05-25

M: Brand, P: Popularity, FC: Expiry Date

Source: own elaboration

Characterisation of PM

Samples of 500 mL of water were obtained after homogenisation by shaking for 30 seconds. Subsequently, the safety seal was broken, the volume was measured and resealed, repeating the same procedure for a second sample. Each mark was analysed in duplicate. MP detection was carried out using two methodologies: staining with Nile Red (NR) and oxidation with 30% H_2O_2 (OXI) (Calvo Anglada, 2020; Quinteros Espinosa, 2022), for subsequent observation of the results with optical and scanning electron microscopy.

Staining with Nile red

A solution of Nile Red (Sigma Aldrich) was prepared at 1 mg/mL dissolved in methanol (J. T. Baker), stored refrigerated at 4°C in a 125 mL amber dropper bottle, covered with aluminium foil. 500 mL of water was placed in a 1 L beaker, lined with aluminium foil. In a laminar flow hood, 5 drops of the NR solution were added and allowed to stand for 30 minutes at room temperature. Subsequently, it was filtered using a vacuum pump and a glass funnel (Shamjina), with a refined, hydrophilic, non-toxic nitrocellulose membrane of 0.45 µm pore size and 50 mm diameter.

The filter was collected with round-tipped tweezers in an aluminium-lined Petri dish and dried for 1 hour at 60°C in a drying oven, for subsequent analysis under a microscope.

Oxidation with hydrogen peroxide

In a 1000 mL beaker with 500 mL of water, previously heated to 60°C in a magnetic grill, 50 mL of 30% hydrogen peroxide (J. T. Baker) was added. The beaker was covered with aluminium foil and left to stand for 72 hours. Afterwards, it was filtered as described above.

Observation of the samples

Optical microscopy

Filters were placed on slides using round-tipped forceps and observed at 4x and 10x magnification on an optical microscope (VELAB VE-B15), equipped with a VE-WUCAM0720PA camera (VE-WUCAM0720PA). The presence of microplastics and their morphology were identified by clockwise observations.

Scanning Electron Microscopy (SEM)

A square section of approximately 3 mm was cut from the membranes obtained from the water filtration process. Each section was placed on an aluminium disc holder and coated with a thin film of gold by sputtering (Denton Vacum, Desk V) for observation. Subsequently, the aluminium holder was mounted on the electron microscope (JEOL model JSM-IT 100, Japan) under high vacuum. Observations were made at an accelerating voltage of 8 to 10 kV, using a secondary electron detector (SED) or backscattered electron detector (REF).

Data analysis

Descriptive statistical analysis was performed, calculating measures of central tendency and dispersion. Methods were compared using a Mann Whitney U test, considering a $p < 0.05$, using MINITAB as statistical software.

Microplastics exposure assessment

The assessment of exposure to PM was carried out by estimating its presence in the daily consumption of EC (ECD, estimated daily consumption) and the daily intake (DI) for adults, children and infants, with equations I and II (Danopoulos *et al*., 2020; Rubio-Armendáriz *et al*., 2022).

$$ECD = MP\left[\frac{Partículas}{500\ ml}\right] * CD_{AE}\left[\frac{L}{día}\right] \quad (1)$$

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MP = According to Cox 2019, the means of the brands analysed per method were averaged (Cox *et al*., 2019). Because the results present outliers, the mean and standard error for the mean (SEM) were used to calculate minimum and maximum values.

CDAE= Daily consumption of bottled water.

Annual consumption data for Mexico were obtained from the Marker Report Findings from 2005 to 2022 of the IBWA (International Bottled Water Association, 2024) and a linear projection was made to determine the daily intake (DI) of MP until 2030.

$$ID = \frac{MP\ x\ Cd}{PC} \quad (2)$$

Cd = represents the average daily water consumption (1, 2, and 0.75 L per adult, child and infant) and BW = average body weight (70, 10 and 5 kg per adult, child and infant) as suggested by WHO (Danopoulos *et al*., 2020; Ravanbakhsh *et al*., 2023).

Quality control

All samples were processed in a laminar flow hood and glassware was used for storage. Staff wore 100% cotton gowns, and additional precautions were taken such as avoiding the use of make-up, nail polish and creams, as these products can release PM (Schymanski *et al*., 2018).

Results

Detection of microplastics by the Nile Red method and 30% Hydrogen Peroxide oxidation in bottled water

The detection of PM in drinking water has been subject to several studies in different parts of the world as it is considered a risk to human health (Hossain *et al*., 2023). The analysis revealed its presence in all brands of EC tested, using both detection methods (Table 2). The oxidation with hydrogen peroxide (OXI) method identified a higher amount of PM, with a total of 16,505 particles, ranging from 604 to 8,648 (C, E). On the other hand, the Nile Red (NR) staining method detected 4,812 particles, ranging from 73 to 2,013 (B, H).

The results indicate a significant difference in the amount of PM found between the applied methods (p = 0.04, Mann-Whitney U, Median RN = 247.5, OXI = 961).

In 75% of the samples treated with OXI, a significant increase in the amount of PM was observed, with increases ranging from 297.9% in sample D to 2140.4% in sample E. However, in samples A and H, a decrease of 58.9% and 61.3%, respectively, was recorded (Table 2).

Box 2

Table 2

Comparison of the amount of microplastics detected by both methods used in bottled water samples. (0.5 L)

M	RN				OXI			
	No. MP	M ± DE	EEM	MIN - MAX	No. MP	M ± DE	EEM	MIN - MAX
A	208	52.0 ± 24.7	8.7	31 – 86	944	236 ± 133.8	47.3	110 - 404
B	73	18.3 ± 22.7	8.0	3 – 52	1198	299.5 ± 100.0	35.4	176 - 418
C	1469	367.3 ± 230.4	81.4	144 – 568	604	151 ± 47.0	16.6	98 - 199
D	246	61.5 ± 46.8	16.5	28 – 129	978	244.5 ± 28.4	10.0	206 - 270
E	386	96.5 ± 71.3	25.2	46 – 202	8648	2162 ± 672.9	237.9	1617 - 3059
F	249	62.3 ± 52.0	18.4	29 – 139	2514	628.5 ± 160.7	56.8	482 - 824
G	168	42.0 ± 28.8	10.2	17 – 73	840	210 ± 16.4	5.8	196 - 232
H	2013	503.3 ± 425.0	150.3	132 – 914	779	194.8 ± 37.7	13.3	146 - 227
Total	4812	601.5 ± 723.5	255.8	73 – 2013	16505	2063.1 ± 2726.0	963.8	604 - 8648

RN: Nile Red staining, Oxi: Oxidation Method, MP No.: Number of microplastics, M ± SD: Mean ± standard deviation, SEM: standard error of the mean and MIN-MAX: minimum - maximum.

Source: Own Elaboration

For the detection of PM in EC, two methods were used: RN staining and oxidation with 30% Hydrogen Peroxide, due to its low cost it is used to decompose organic matter and it is reported that an increase in temperature from 70 to 100ºC, can cause weight and size loss, in addition, combined with ultrasound or photocatalysis is used as treatment for degradation (Chen *et al*., 2022; Pfeiffer & Fischer, 2020), which is probably why I report a higher amount of 16,505 MP compared to 4812 with RN in 0.5L (p = 0.04, Mann Whitney U, table 2). RN, on the other hand, is a stain for rapid quantification and detection of MP given its selective absorption and fluorescent properties, without the need for spectroscopic analysis (Mason *et al*., 2018; Singh, 2021).

As in other reports, in both methods (table 2), a large variation in the number of PM was observed between the different brands (Singh, 2021), this is influenced by sampling and the complexity of identifying their source of origin.

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The detection of PM in freshwater, groundwater or wastewater has highlighted the potential contamination of the water being extracted. The pipes and filters used during EC production are often made of Polyethylene, Polyamide, Polyvinyl and PET, which due to mechanical and chemical factors can release them (Akhbarizadeh *et al*., 2020; Nacaratte *et al*., 2023).

Packaging is affected by photo-oxidation, ageing and cleaning processes, especially in reusable packaging (Brancaleone *et al*., 2024; Hossain *et al*., 2023). And finally the use, opening and closing the bottle, generates up to 553 ± 202 MP per cycle, or mechanical stress from squeezing the bottle (Akhbarizadeh *et al*., 2020; Singh, 2021).

Optical and scanning electron microscopy analysis

The shapes recorded for PM are fragments, films, pellets, granules, filaments and foams (Ebanks Mongalo *et al*., 2024). Microscopic observations showed that, irrespective of the method, the PMs present are granules and fibres, with variations between samples. In the case of the RN method, the highest percentage of granules was observed in samples C and H (92%), while for the OXI method, the highest percentage was in sample E (97%). Fibres represented 46% of the particles in sample G for the RN method and 36% in sample H for the OXI method. Sponges and films were the least frequent forms (Fig. 1).

Box 3

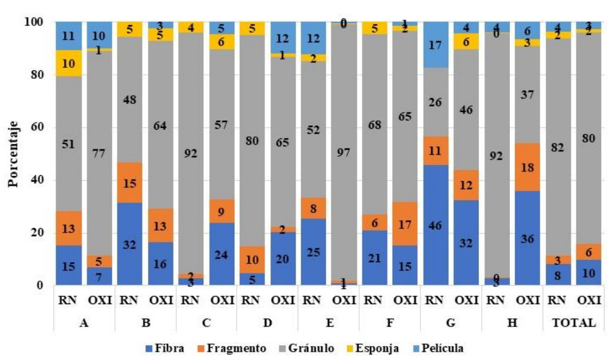

Figure 1
Distribution of microplastic forms in analysed bottled water simples
Source: Own Elaboration

Regardless of the method, the shapes and colours remain the same, the MPs observed (Fig. 2A) have different shades, the fibres (A and G) are mostly blue and grey. The granules (C and H) are transparent, while the fragments (F and B) are orange, brown or transparent, the sponges (D and C) are blue, brown to violet; the latter two forms are usually with irregular edges, finally, the films are transparent (E and F).

Also, scanning electron microscopy (SEM, Fig. 2B) observations revealed the presence of fragments of synthetic materials in almost all samples, confirming that they were microplastics. The dimensions of these fragments ranged from 3.98 to 7.70 µm in the NR-treated samples (C and H) and from 2.22 to 6.49 µm in the OXI-treated samples (E).

Box 4

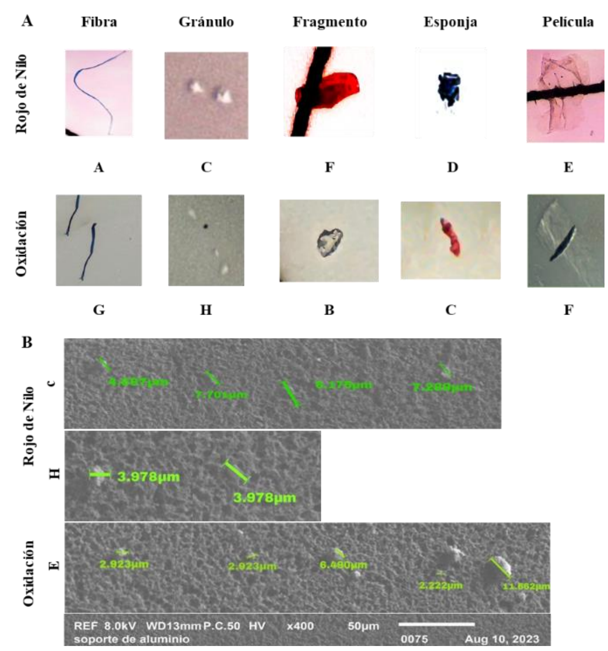

Figure 2
A) 10x optical and B) scanning electron microscopy of bottled water samples
Source: Own Elaboration

Human exposure to PM through drinking water is unavoidable, the size and form influences their absorption, adsorption and impact on organisms. The reported forms are fibres and fragments, followed by sponges and granules. What differs in this work, regardless of the method, is that granules predominate (Fig.1 and 2A), their origin is likely to be contamination of sources by industrial wastewater, and like fibres, they are difficult to remove during the treatment process (Hossain *et al*., 2023; Kye *et al*., 2023; Nacaratte *et al*., 2023; Rubio-Armendáriz *et al*., 2022).

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The size found by SEM varies between 2 and 11.6 µm, which is in agreement with other studies (Brancaleone *et al.*, 2024; Rubio-Armendáriz *et al.*, 2022), a size <5 µm with a considerable effect on human health (Taheri *et al.*, 2023).

Estimated consumption of microplastics via bottled water

According to the National Water Commission in Mexico, 78% of the population consumes bottled water (BW), placing the country in first place worldwide in this respect (Arriaga-Medina & Piedra-Miranda, 2021; International Bottled Water Association, 2024). Although the cost of EC is between 100 and 1000 times higher than tap water, the preference for EC is due to several reasons: firstly, the belief that it is healthier, safer and better tasting; secondly, distrust of drinking water systems, caused by insufficient, irregular and low quality supply in some households; thirdly, the ease and convenience of purchasing it; and finally, the lack of adequate regulatory frameworks and the power of multinational companies promoting its consumption (Nacaratte *et al.*, 2023; Sanei & Khodadad, 2024).

Annual per capita consumption of EC in Mexico increased by 67.93% between 2005 and 2022, from 180.7 L/year (0.49 L/day) to 303.2 L/year (0.83 L/day), with an average annual growth of 3.2%. Linear projections to 2030 (ASM 3.94, MAD 9.2 and MSD 111.45) suggest an increase of 14.2%, reaching 348.95 L/yr (0.96 L/day), with an increase of 1.8% from 2022 (Fig. 3A, dotted black line).

Previous studies indicate that humans may be exposed to tens of thousands to millions of PM each year or several milligrams per day. Although ingestion is considered the main source through food and water, it is difficult to determine the content of PM in different foods, and other routes such as inhalation, especially in enclosed spaces, and dermal contact are also considered (Lee *et al.*, 2023; Olea, 2024).

Daily exposure to PM was estimated at 150 ± 64 particles (86 - 214.2 PM/0.5L) for RN and 516 ± 241 particles (275 - 757 PM/0.5L) for OXI.

Therefore, in 2005 it is estimated that an individual could have ingested 149 PM (85-213 PM) daily, in 2022 that figure rose to 250 PM (186-314 PM), and by 2030 it is projected to be 287 PM (223-351 PM) for NR. For OXI, these figures were 511 MP (270-752) in 2005, 857 MP (616-1098) in 2022, and are estimated to be 836 MP (595-1077) in 2030 (Fig. 3A).

Box 5

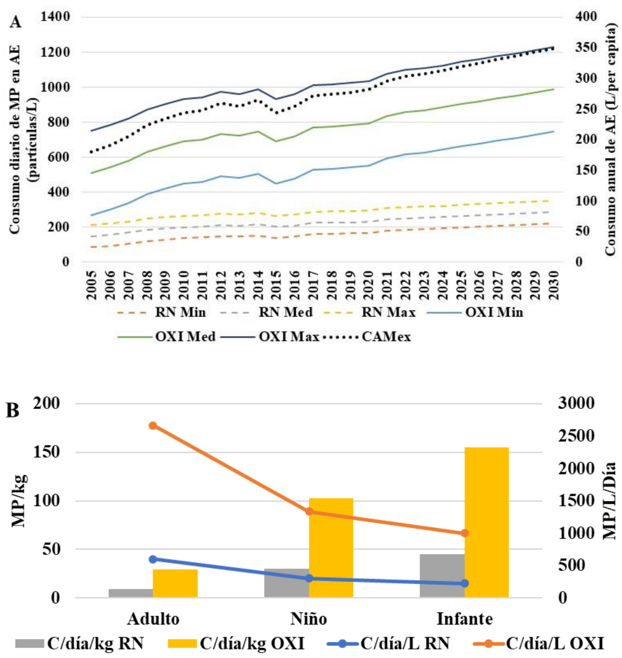

Figure 3
Projected Particulate Matter Consumption of PM/L through bottled water. A) Consumption of bottled water (right, black dotted line) and consumption per day of PM by the various methods (left, solid line oxidation method, dashed line Nile Red). B) Calculation of PM/L/day (right) and PM/kg (left) consumption in adult, child and infant.

Source: Own Elaboration

EC is a significant source of PM, such as PET, HDPE or Polypropylene (Hossain *et al.*, 2023; Nacaratte *et al.*, 2023), used in the manufacture of the bottle body or cap. Some authors claim that dietary exposure is higher than in tap water, the consumption per day for 2 L of water has been estimated at 2 x $^{10-5}$ - 33,626 particles (Danopoulos *et al.*, 2020). In this work, the per capita consumption for 2030 of EC will be 0.96 L/day, so exposure would be in the range of 223 - 351 for NR and 595-1077 for OXI, and infants and children is 5 and 3 times higher than in adults, suggesting a high risk for them (Ravanbakhsh *et al.*, 2023).

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Results in animal experiments and cell cultures have shown that PM can affect several systems of the human body, including the digestive, respiratory, endocrine, reproductive and immune systems. First, one route of exposure is ingestion, through food and water contaminated by them, whereby the digestive system may become irritated and the gut microbiome altered, eventually causing inflammation and gastrointestinal symptomatology (Brancaleone *et al*., 2024; Lee *et al*., 2023).

Although 90% is excreted, particles smaller than 150 µm can cross the intestinal barrier by various mechanisms depending on size and type. Some may be digested by phagocytosis by M cells of Peyer's patches or dendritic cells, but MP are reported to resist gastric juices, and so, not being broken down, persist for a long time, favouring their bioaccumulation and causing chemical toxicity, if accompanied by heavy metals and polycyclic aromatic hydrocarbons. Their excretion will depend on the characteristics of the polymer matrix (Brancaleone *et al*., 2024).

PM can be absorbed and reach the bloodstream through the intestinal mucosa, perhaps because the inflammatory process increases permeability or simply because of their size. Once in the blood they can translocate and deposit in various organs such as the gut, liver, lungs, etc., as corroborated by findings in faeces, blood, lungs and placenta, and recently the brain and testes. With the potential for immunotoxicity, alteration of oxidative stress control gene expression and activation of nuclear factor E2 expression. In addition, their hydrophobicity favours exposure to heavy metals, organic pollutants and pathogenic microorganisms (Kirstein *et al*., 2021; Rubio-Armendáriz *et al*., 2022). Therefore, to prevent health risks and reduce potential exposure, it is necessary to implement monitoring strategies.

EC has transformed water consumption habits, but it is necessary to reflect on its implications. Annually, EC consumption generates tons of plastic waste, of which only 7% is recycled, contributing significantly to environmental pollution. Its production and distribution require a high energy demand, in addition to the fact that three times more water is consumed than is needed to fill the bottles.

With regard to the quality of EC, it is not necessarily better than tap water and may be associated with health risks, such as exposure to phthalates and other emerging pollutants such as PM (Aslani *et al*., 2021; Sanei & Khodadad, 2024).

Plastic pollution is a global public health and environmental problem, education on responsible use of EC is needed and proper management of plastic waste could generate economic and environmental benefits (Ebanks Mongalo *et al*., 2024; Olea, 2024), promoting more sustainable water management. Authorities should also improve water quality in areas where water quality is not efficient and ensure access to good quality water resources (Sanei & Khodadad, 2024).

Conclusions

In this study, both methods employed detected PM in EC, verified by optical and scanning electron microscopy. The RN method was used for rapid detection of PM, while the oxidation method showed a higher abundance of PM, due to its ability to expose PM by decomposing organic matter. The results suggest that children and infants are more exposed to PM through EC consumption, which highlights the need for further studies to establish minimum limits that do not pose a health risk and to improve the characterisation of PM. This underlines the importance of implementing permanent screening programmes for emerging contaminants such as PM in order to ensure access to good quality water.

Humans are exposed to PM by various routes such as oral ingestion through food and water, inhalation mainly in enclosed spaces and dermal contact, which is reflected in increased findings in various parts of the body. Long-term effects are unknown, it is important to emphasise exposure in infants and during embryonic development. Recent reports indicate that in addition to increasing the expression of inflammatory factors, inhibiting acetylcholinesterase activity, reducing germ cell quality and affecting embryonic development, it is speculated that they may be related to the development of several chronic diseases such as cancer, diabetes, cardiovascular disease, among others.

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Declarations

Conflict of interest

The authors declare that they have no conflict of interest. They have no known competing financial interests, or personal relationships that could have appeared to influence the article reported in this paper.

Author Contribution

Alva-Rojas, Elvia, idea, experimental design, analysis and interpretation of data, writing the article.

Rivera-Ramirez. Fabiola, idea, experimental design, analysis and interpretation of data, writing the article

Jiménez Rosales, *Angélica*, analysis of the samples in the scanning electron microscope, revision and approval of the final version.

Velázquez-Garduño Gisela, analysis and interpretation of data, writing and revising the article.

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Abbreviations

BTW: Bottled water
CDBTW: Daily consumption of bottled water
DI: Daily intake
HDPE: High-density polyethylene, MP: Microplastics
NR: Nile red
OXI: Oxidation with 30% H_2O_2,
PET: Polyethylene terephthalate,
SEM: Scanning Electron Microscopy
WHO: World Health Organization

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