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Analysis of the presence of microplastics in different brands of bottled water in region 6 - Ecuador

Abstract

Due to the excessive use of plastics in the human food chain, the aim of this investigation was to evaluate the presence of microplastics in bottled water in region 6 of Ecuador. For this purpose, 72 samples from four different brands of bottled water were analyzed, various plastic particles (fibers and fragments) were found, which were subsequently confirmed through FTIR (Fourier-transform infrared spectroscopy). A full range of fragment sizes, from 4.4 to 248.29 µm, and fiber sizes, from 102.64 to 840.20 µm, were identified, which were classified as secondary microplastics. A statistical analysis was conducted to determine the means of fibers and fragments found in each brand and it was established that brand B had the highest quantity of microplastics, with an average of 233.1 particles per liter. Finally, polyethylene was identified as the most recurrent type of particle detected by FTIR, suggesting that the bottles themselves may be a source of contamination.

Keywords: bottled water; FTIR; microplastics; polyethylene.

Análisis de la presencia de microplásticos en diferentes marcas de agua embotellada en la región 6 - Ecuador

Resumen

Debido al uso excesivo de plásticos en la cadena alimenticia humana, el objetivo de esta investigación fue evaluar la presencia de microplásticos en agua embotellada en la región 6 de Ecuador. Para ello, se analizaron 72 muestras de cuatro marcas diferentes de agua embotellada, se encontraron diversas partículas plásticas (fibras y fragmentos), las cuales fueron confirmadas mediante FTIR (espectroscopía infrarroja por transformada de Fourier). Se identificó un rango completo de tamaños de fragmentos, de 4,4 a 248,29 µm, y de fibras, de 102,64 a 840,20 µm, que se clasificaron como microplásticos secundarios. Se realizó un análisis estadístico de los promedios de fibras y fragmentos encontrados en cada marca y se estableció que la marca B contenía una mayor cantidad de microplásticos, con un promedio de 233,1 partículas por litro. Finalmente, se identificó el polietileno como el tipo de partícula más recurrente detectada por FTIR, lo cual sugiere que las botellas pueden ser una fuente de contaminación.

Palabras clave: agua embotellada; FTIR; microplásticos; polietileno.

Análise da presença de microplásticos em diferentes marcas de água engarrafada na região 6 - Equador

Resumo

Atribuível ao uso excessivo de plásticos na cadeia alimentar humana, o objetivo desta investigação foi avaliar a presença de microplásticos em água engarrafada na região 6 do Equador. Para isso, foram analisadas 72 amostras de quatro marcas diferentes de água engarrafada, e foram encontradas várias partículas plásticas (fibras e fragmentos), que foram posteriormente confirmadas através da FTIR (espectroscopia infravermelho por transformada de Fourier). Um intervalo completo de tamanhos de fragmentos, de 4,4 a 248,29 µm, e de tamanhos de fibras, de 102,64 a 840,20 µm, foram identificados e classificados como microplásticos secundários. Uma análise estatística foi realizada para determinar as médias de fibras e fragmentos encontrados em cada marca, estabelecendo que a marca B continha a maior quantidade de microplásticos, com uma média de 233,1 partículas por litro. Finalmente, o polietileno foi identificado como o tipo mais recorrente de partícula detectada pelo FTIR, sugerindo que as próprias garrafas podem ser uma fonte de contaminação.

Palavras-chave: água engarrafada; FTIR; microplásticos; polietileno.

Introduction

In recent years, plastics have aggressively irrupted into the food chain, impacting both human life cycles and ecosystems. The global demand for plastics has increased due to their physicochemical properties and low production costs, making them almost irreplaceable in the manufacturing of domestic and industrial goods [1]. Microplastics, solid particles ranging from 20 µm to 5 mm in diameter, accumulate in the environment and can be primary or secondary [2]. Primary microplastics, ranging from 5 to 1 mm, are used in the manufacture of industrial pellets and personal care products, while secondary microplastics, ranging from 1 mm to 20 µm, are generated by the fragmentation of plastics through photo-oxidative and mechanical processes.

Many microplastics arise from the decomposition of bulky plastics such as polyethylene (from bags and bottles), polystyrene (from food containers), nylon, polypropylene (from fabrics), and polyvinyl chloride (from pipes) [3]. Polyethylene, in particular, has been demonstrated to negatively impact human health due to its ability to release toxic chemicals. These substances, when ingested or absorbed over time, can interfere with the body's natural processes, potentially leading to disruptions in the endocrine system and impairments in immune function. Prolonged exposure to these chemicals raises concerns about their cumulative effects, as they may contribute to hormonal imbalances and weakened defense mechanisms, posing significant risks to overall health [4]. These particles have become ubiquitous, found everywhere from the deepest parts of the ocean, such as the Mariana Trench, to the highest peaks like Mount Everest, and even in the food we consume every day [5]. Despite South America being the region that produces the least amount of plastic, it ranks third, after Asia and Africa, in the amount of plastic that ends up in the ocean via rivers [6].

Microplastics are present in oceans and continental habitats, affecting marine animals and products such as sugar, salt, beer, and milk, as well as sources of drinking water [7]. These particles can act as disease vectors, impacting the health of terrestrial animals and soil, acquiring harmful properties as they degrade [8]. Fish, the most studied marine animals regarding microplastic ingestion, suffer from malnutrition, starvation, and population decline due to impacts on their reproduction [9].

The presence and impact of microplastics in aquatic environments is an emerging global issue. Although research on this topic is more advanced in marine environments, it has only recently begun to be studied in continental waters and those intended for human consumption. Current efforts focus on reducing primary production of microplastics by regulating their use in the manufacture of other products. However, the greatest challenge remains managing microplastics generated from the degradation of existing plastics in water. In response to this issue, legislation has begun to limit the use of single-use plastics [10].

Microplastics may contain toxic substances such as bisphenol A, phthalates, and heavy metals, which are risky if ingested [11]. These particles can introduce harmful elements into the food chain, leading to unpredictable ecological effects due to bioaccumulation and biomagnification [12]. Exposure can occur through ingestion of contaminated food and water, or inhalation of airborne particles [13]. Since water is essential for life, its contamination with microplastics raises concerns about long-term toxic effects on human health [14].

Several detection techniques have been successfully applied in research on microplastics. These include Fourier-transform infrared spectroscopy (FTIR), which allows the identification and characterization of microplastics by measuring the absorption of different wavelengths, and fluorescence microscopy, which is useful for detecting microplastics in complex samples such as water and food. These techniques are crucial for the accurate identification of microplastics and their characterization, enhancing our understanding of their presence and the potential risks they pose to human health and the environment.

This study aims to investigate the presence of microplastics in bottled water in region 6, Ecuador, and to increase awareness and understanding of the potential health and environmental risks associated with plastic pollution.

Materials and methods

Sample preparation

Different water samples (around 18 per brand) were collected from the most commercial areas of Azuay, Cañar and Morona Santiago, 500 mL of each sample were taken and heated to 60 °C in a water bath to improve their fluidity characteristics during the process. After this process, the samples were filtered through a 90 µm stainless steel sieve. The names of the brands have been replaced with A, B, C, and D to avoid issues with their privacy policies.

Oxidation

The residue from the 90 µm stainless steel sieve was rinsed with 500 mL of type I water before being placed in a glass container and treated with 50 mL of 30% hydrogen peroxide. The filtrate was placed in a 1000 mL Erlenmeyer flask with 50 mL of 30% hydrogen peroxide. Both containers were left to stand at room temperature for 72 h.

Vacuum microfiltration

After 72 h, the oxidized material was filtered through a polytetrafluoroethylene (PTFE) membrane with a pore size of 1 µm and a diameter of 47 mm. It was then left to dry at room temperature for 24 h.

Observation under inverted microscope and identification of microplastic profile

The PTFE membrane filter was placed between two glass slides, and the presence of microplastics was observed using a 10X lens [18]. Through the NIS image analysis program, the lengths and shapes of the fragments and fibers observed under the inverted microscope were recorded [19]. The identification of microplastic profile was carried out using the FTIR equipment with the attenuated total reflectance (ATR) analyzer, identifying the type of microplastics through the OMNIC Spectra software.

Statistical analysis

To improve the accuracy and clarity of the results, Excel and R were used, enabling the creation of tables, graphs, and the execution of relevant hypothesis tests adjusted to the specific context of this study.

Results and discussion

Determination of the size of observed particles

The dimensions of the observed particles were then determined. Using an inverted microscope with a 10x magnification, plastic particles, fragments, and fibers were observed. With the aid of the NIS image analysis software, the dimensions were obtained as shown in **figures 1** and **2**.

With the measured dimensions and the dispersion of the particles, the range was calculated to indicate the interval between the maximum and minimum values of the samples. The values should be between 1 and 250 µm [20].

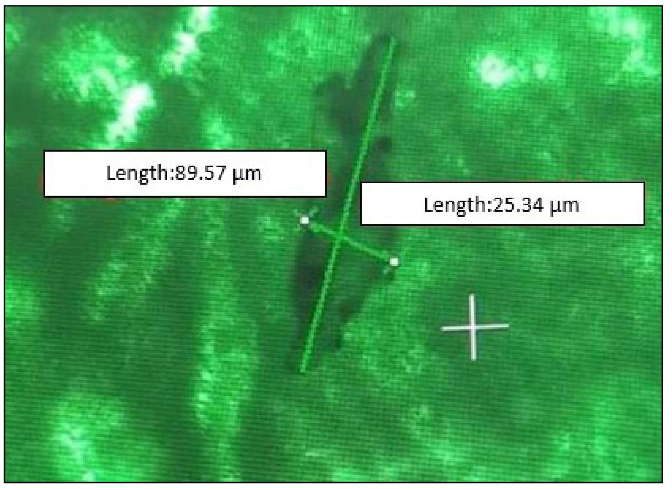


Figure 1. Measurement of a microplastic fiber using an inverted microscope (10× magnification). The fiber dimensions were obtained with NIS image analysis software. The sample was analyzed under fluorescence illumination.

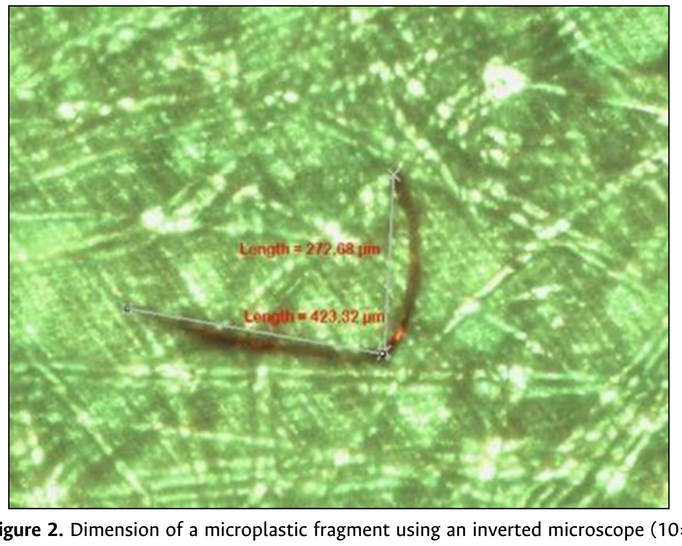


Figure 2. Dimension of a microplastic fragment using an inverted microscope (10× magnification). The fragment dimensions were obtained with NIS image analysis software. The sample was analyzed under fluorescence illumination.

Table 1 shows four brands of bottled water along with the size of the fibers and fragments found. The size of the fragment ranges from 4.4 to 248.29 µm, confirming that they fall within the specified range (1 to 250 µm). Conversely, a specific range for fibers was not determined due to their variable length and thickness of a few microns. However, they are considered microplastics because they are smaller than 5 mm [21].

In a study, three liters of bottled mineral water were analyzed, revealing synthetic fibers ranging from 1 µm to 5 mm [22]. In contrast, the samples from the brands studied in the present research contained fibers ranging from 102.64 to 840.20 µm, concluding that they fall within the established range (< 5 mm) as in other studies [21].

Table 1. Size distribution range of microparticles found.

Bottled water brands				
Polymers found	**A**	**B**	**C**	**D**
Fibers (µm)	102.64 – 840.20	192.00 – 800.06	136.47 – 830.50	274.53 – 829.06
Fragments (µm)	4.50 – 131.60	10.36 – 146.87	4.40 – 248.29	6.03 – 240.05

During the research process, the statistical technique analysis of variance (ANOVA) was performed to determine which brand of bottled water contains the highest amount of microplastics (**table 2**). The obtained p-value is less than 0.05, thus rejecting the null hypothesis.

Table 2. Analysis of variance of bottled water brands.

	GL	Sum Sq	Mean Sq	F value	P value
Factor	3	390974	130325	72,86	0,000
Error	68	121636	17789		
Total	71	512611			

Consequently, the Tukey method was applied to identify which of the four brands behaves similarly or differently. After comparing the samples of each brand using the Tukey method, three groups were identified based on the means as shown in **table 3**. Group 1 includes brand B. Group 2 consists of brand A and C. Finally, group 3 includes brand A and D. The means that do not share a letter are significantly different, indicating considerable variability between brands.

Table 3. Comparison of the samples from each brand using the Tukey method.

	Brand A	Brand B	Brand C	Brand D
Mean	70.78	233.10	95.00	40.61
		G1		
Groups	G2		G2	
	G3			G3

G1: group 1. G2: group 2. G3: group 3.

Upon analyzing the samples from the four brands of bottled water in region 6, it was concluded that 100% of the samples contain microplastic particles, averaging 12.2 particles per liter of water. This finding indicates a ubiquitous presence of microplastics in bottled water within this region.

Comparatively, the study reported by Mason *et al.* [23] analyzed eleven brands of bottled water worldwide and found that 93% of the samples contained microplastics, with an average of 10.4 particles per liter of water. The results from our research, therefore, show a higher average of plastic particles per liter of water compared to the global study.

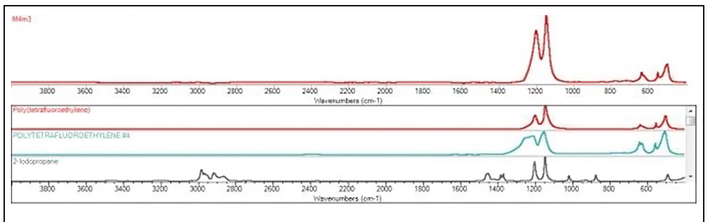
This discrepancy may be attributed to factors such as differences in the manufacturing processes, bottle materials, and environmental conditions. The higher average of plastic particles in our study suggests that bottled water in region 6 may be more susceptible to microplastic contamination.

The high prevalence of polyethylene (PE) observed in our samples (shown in the next section) suggests that local manufacturing practices may have a significant role in microplastic contamination. PE is a common material for bottled water packaging, and it can degrade or shed microplastics during production, filling, sealing, and transportation [24]. Variations in manufacturing standards or machinery quality between regions may lead to higher rates of plastic particle release in bottled water from region 6. In addition, the specific types of plastic and additives used in bottles may also influence microplastic prevalence. Some plastic additives can accelerate degradation of plastic, increasing particle shedding. Region variations in regulations around plastic manufacturing could contribute to these differences.

Moreover, this region is subject to unique environmental pressures that could contribute to increased microplastic levels. For example, higher temperatures and UV radiation levels could accelerate polymer degradation in the bottles during storage and distribution, particularly if stored in open or unshaded conditions [25]. Further investigation into manufacturing protocols and environmental exposure in region 6 could provide more insights into the sources and mechanisms of microplastic release in bottled water.

Identification of microplastic profile using FTIR

After observing the samples from each brand through the inverted microscope, particles larger than 130 µm were identified and characterized, as FTIR analysis is not suitable for particles smaller than this size because it may face challenges in detecting extremely small particles due to its limited spatial resolution and the efficiency of infrared light scattering [26]. The PTFE membrane composition was determined through the emitted spectrum, followed by spectral subtraction. Using the OMNIC software, the samples from the analyzed brands produced a spectrum different from that of the filter. These results were compared with the library in the OMNIC Spectra software, leading to the identification of various types of polymers as shown in **figure 3**.



	Match	Title	Range	Folder	Filename	Index
1	87.42	Poly(tetrafluoroethylene)	4000.0-400.0	HR Specta Polymers and Plasticizers by ATR	c:\my documents\omnic\libs\sea464.lbd	70
2	57.47	POLYTETRAFLUOROETHYLENE #4	4000.0-400.0	HR Polymer Additives and Plasticizers	c:\my documents\omnic\libs\sea450.lbd	1052
3	56.64	2-Iodopropane	4000.0-400.0	HR Hummel Polymer and Additives	c:\my documents\omnic\libs\sea406.lbd	1505

Figure 3. Spectrum search results from one particle found in sample 3 – Brand D.

In addition to **figure 3**, which serves as an illustrative example of the analysis process, the full data across all samples is provided in **table 4**. This table provides a comprehensive overview of the microplastic measurements obtained from each bottled water brand. Approximately four particles from each sample were analyzed, and it can be observed that PE is the most present polymer in all brands.

Table 4. Percentage of polymers found in different brands of bottled water.

	Bottled water brands			
Polymers found	**A**	**B**	**C**	**D**
Polyethylene	69.40	89.60	60.00	81.70
High density polyethylene			30.30	
Polypropylene		10.40		18.30
Polyamide			9.70	
Cellophane	20.83			
Nylon	9.77			

According to Zhou *et al.* [27], in the analysis of 23 brands of bottled water they identified the presence of various polymers, cellulose and polyamide were the most prevalent polymers found. In contrast, our research, as shown in **table 4**, indicates that PE is the most likely polymer present in bottled water samples, with a detection range of 60.0 to 89.6%. This significant presence of PE suggests that a substantial portion of the microplastics detected in the water may originate from the degradation or shedding of the bottle material itself. PE's prevalence highlights potential contamination sources from bottle materials, supporting the hypothesis that manufacturing practices significantly contribute to microplastic contamination in bottled water.

This discrepancy between our findings and those of other studies highlights the variability in polymer prevalence across different studies and geographic locations. The predominance of PE in our analysis aligns with the common use of this polymer in the production of plastic bottles that are distributed in the region, further supporting the hypothesis that bottle material is a primary source of microplastic contamination.

Conclusions

Microparticles are present in all brands of bottled water, appearing as both fragments and fibers. This finding underscores a significant concern regarding the purity and safety of bottled drinking water, highlighting the pervasive issue of microplastic pollution. The particle sizes determined using the inverted microscope and the NIS image analysis program showed that the fragments ranged from 4.40 to 248.29 µm, falling within the established range from 1 to 250 µm. This confirms the presence of microplastics within the expected size range.

Of the brands analyzed, brand B had the highest quantity of particles, fibers, and fragments, with an average of 233.1 particles per liter of water. In stark contrast, brand D had the lowest quantity, with an average of 40.61 particles per liter of water. This significant disparity highlights the variability in contamination levels across different brands of bottled water, pointing to inconsistencies in manufacturing practices and quality control measures.

Furthermore, the FTIR analysis identified PE as the most likely type of microplastic present in the bottled water samples from all four brands. This suggests that PE, a common secondary plastic, is a major contaminant, potentially originating from the decomposition of the bottle material itself. This finding is particularly alarming as it indicates that the very containers meant to provide safe drinking water are a source of pollution. PE has been shown to have adverse effects on human health, as it can release harmful chemicals into the body, potentially disrupting endocrine and immune functions when ingested over time.

Overall, the research hypothesis is confirmed: microplastics are indeed present in bottled water in region 6 of Ecuador. This finding highlights the necessity for stricter quality control and regulatory measures to ensure the safety and purity of bottled water. The presence of microplastics implies potential health risks, so addressing this issue is crucial for protecting consumer health, and immediate action is required to mitigate the impact of microplastic pollution on our water supply.

In addition to the findings, it is crucial to highlight the urgent need to review and modify manufacturing practices in the bottled water industry. A transition towards more sustainable production methods must be implemented, reducing the release of microplastics and other contaminants in the final products. This call to action aims to promote more responsible policies and practices in the manufacturing of consumer goods, in order to protect both public health and the environment.

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