Research Article

A critical comparison of the main characterization techniques for microplastics identification in an accelerated aging laboratory experiment

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Abstract

Many studies have reported the occurrence of microplastics in different environmental compartments, through the description of their morphological characteristics and chemical identification, obtained mainly by spectroscopic techniques. However, the scientific community still lacks the implementation of standardized analytical methods that aim to assess not only the identification of the particle, but also its stage of degradation. It is understood that this information would be extremely useful in helping elucidate the main sources of pollution and contributing to strategies and mitigating measures for the management of solid waste and microplastics in the environment. In this respect, the aim of this study was to evaluate the efficiency of Fourier-transform infrared spectroscopy, Raman spectroscopy, carbon elemental analysis coupled with mass spectrometry, and scanning electron microscopy with energy dispersive X-ray spectrometry for the characterization of virgin and aged polyethylene and polypropylene microplastics samples. The degraded samples were subjected to accelerated aging in a QUV chamber in accordance with American standard for measuring accelerated weather testing (ASTM G-154). This work discusses the efficiency and limitations of each technique for the detailed chemical characterization of microplastic samples collected from the environment.

INTRODUCTION

Microplastics (MPs) are defined as small polymeric particles (≤ 5 mm) found in the environment[1]. The presence of these particles was first reported in marine environments in 1972[2]. More recently, these particles have been reported in various studies, in different geographical locations, and widely distributed throughout the different environmental compartments, including the hydrosphere, pedosphere, and atmosphere[3-8]. Considering their persistence, small size, and difficult removal from the environment, MPs can be identified as potential persistent pollutants in terms of negative impacts on ecosystems[9,10]. In the aquatic environment, because they are mistaken for food, MPs are easily ingested by various organisms, which can cause harmful effects through physical or physiological mechanisms[11-14]. MPs have also been reported in products intended for human consumption, such as foods and water[8,15,16]. Therefore, humans are also potentially being exposed to MPs through the consumption of these products and also through the contaminated air we breathe, which can cause oxidative stress, inflammation, neurotoxicity, and reproductive toxicity, as well as altering the structure of the intestinal microflora in cells or biota[8,17].

In this way, in order to accurately assess the possible ecological risks of these particles to the environment and potential impacts on human health, the scientific community has dedicated itself to gaining accurate knowledge of the main polluting sources of MPs, and their environmental concentration, composition, and morphology in different environmental matrices, food, drink, and biological samples[8]. It is known that the emission of MPs into the environment occurs throughout the life cycle of the polymeric material, including production, transport, domestic sewage disposal, industrial activities, atmospheric deposition from synthetic fabrics, and the improper disposal of larger plastic objects[18]. The scientific community classifies the entry of these particles into the environment as being from primary or secondary sources[19,20].

Primary MPs are those already produced in small sizes by the plastics industry, called pellets, that are released directly into the environment[18-21]. The industry uses these pellets as raw material for the production of plastic objects in the most varied sectors of society. Accidental loss to the environment occurs during transport and from processing plants[22,23]. In addition, these pellets are used as abrasive "scrubbers" to clean containers, machinery, and ships, and are often found near harbors, in industrial areas, on beach sands, and in the sea[19,24]. Primary source MPs, in micrometer sizes, are also part of the formulation of cosmetics and personal care products for abrasive effects[21,24]. These particles, of different shapes, sizes (from 4 to 2,000 μm), and chemical compositions, are transported to watercourses, for example, through domestic effluents[25,26]. Secondary MPs are small particles of plastic waste derived from the fragmentation of larger objects discarded in the environment. These plastic objects, when exposed to natural stressors, degrade through various processes: photochemical, thermal, mechanical, hydrolysis, and/or ozone-induced[19,27]. These processes are directly related to environmental conditions, the chemical composition of the polymer, and its production process[10,19,27-31].

Studies that report the occurrence of MPs in environmental samples must consider several important factors to provide and accurate analysis and potential exposure risks. These include the abundance of particles in relation to seasonality, and morphological and chemical characterization, as well as the stage of particle degradation, information that helps to elucidate the main pollutant sources. In order to qualitatively analyze the MPs collected, it is essential to use improved analytical techniques, as basic studies that identify MPs only use visual inspection and/or optical microscopy, which is an ineffective analysis and lacks conclusive information, contributing to false positives in the identification of MPs[32].

Currently, the most frequently reported chemical characterization techniques in studies involving MPs are spectroscopic, such as Fourier-transform Infrared spectroscopy (FTIR) and Raman spectroscopy[33]. However, despite the numerous studies involving analytical methods for identifying MPs and the extensive knowledge already clarified about the main characterization techniques used in the analysis of degraded plastics, the scientific community still lacks studies that consider samples of degraded MPs in the implementation of analytical methods[8,27,30]. The qualitative analysis of samples of degraded MPs is extremely important information for assessing the impact of these MPs on the environment and clarifying their classification in terms of the origin and main sources of pollution. This practice could encourage the discussion and comparison of the results of different studies and geographical locations, in order to understand, in greater detail, the extent and distribution of environmental contamination by MPs.

The current study aimed to evaluate the efficiency and limitations of the main techniques used to characterize synthetic organic polymers; FTIR, Raman spectroscopy, carbon elemental analysis coupled with mass spectrometry, and scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDS), using samples of virgin and aged polyethylene (PE) and polypropylene (PP) MPs. These polymers were selected because they have the highest percentages of production and consumption worldwide[34] and also because they are the most representative of those reported in the environment[35]. In the aquatic environment, for example, due to the low density of these polymers, they float and remain at the top of the photic zone, where they are directly exposed to sunlight, i.e., photochemical degradation. Therefore, the condition of the polymer sample in the aged stage was also considered in this study, in order to be closer to the real condition in the environment and also to include MPs of secondary origin[6,36,37].

METHODS

Materials
High-density polyethylene (HDPE, HE150) and polypropylene polymers (PP, H117) were supplied in the form of pellets by Braskem (Triunfo, Brazil). The density and fluidity index values are 0.948 g·cm^{-3} and 1 g/10 min for HDPE and 0.905 g·cm^{-3} and 45 g/10 min for PP, respectively.

Sample preparation
The PE and PP pellets were used to prepare specimens with dimensions of 105 mm × 75 mm × 2 mm in a heated press (Marconi, model 098) at 185 °C and 38.1 kgf·cm^{-2} for 2 min and cooled to 27 °C. These specimens were subjected to accelerated aging in an accelerated aging chamber (QUV - Accelerated Weathering Tester) in accordance with American standard for measuring accelerated weather testing (ASTM G-154), 2012[38].

After exposure, the specimens of both polymers were ground in a mill (Marconi, Model 580) to obtain MP particles, and the same procedure was also carried out with the virgin pellets. These virgin and aged MPs were then transferred to a stacking system with 1,000 μm (top) and 106 μm (bottom) mesh sieves to ensure their classification by size on the MPs scale (≤ 5 mm).

Accelerated aging
The samples were subjected to thermal and photochemical aging in an accelerated aging chamber (Accelerated Weathering Tester, Q-Lab), which simulates natural weathering, in accordance with the guidelines of ASTM G-154 [Standard Practice for Operating Fluorescent Ultraviolet (UV) Lamp Apparatus] for the exposure of non-metallic materials[38]. The radiation sources were Philips 80W lamps (Cleo Performance WR80 model) with spectral distribution in the UVA region (340 nm). The samples were subjected to the following cycle: 8 h of radiation (irradiance 1.55 W/m²/nm) at 60 °C, controlled by a cooling system, followed by a 2-h condensation cycle. The samples were collected for characterization, in

triplicate, after 1,240 h for PP and 2,000 h for PE. It was necessary to reduce the exposure time of the PP polymer due to the accelerated fragmentation and formation of MPs of the specimen inside the aging chamber at 1,240 h.

Analytical methods

FTIR-ATR

In order to identify the polymers, the chemical characterization of the MPs was carried out by FTIR-ATR, in the attenuated total reflectance (ATR) mode, using a Bruker spectrometer (Massachusetts, USA). The spectra were recorded in attenuated total reflectance mode with a 15× Ge crystal objective on a Hyperion microscope coupled to a Vertex 80v. Spectra were acquired with a resolution of 4 cm^{-1} in 64 scans, in the spectral range from 4,000 to 400 cm^{-1}. The spectra were measured in a temperature and humidity-controlled environment and analyzed in absorption mode using Bruker's OPUS 8.0 software. The spectra obtained by FTIR-ATR were also used to characterize the stage of degradation of the surface of the PE and PP polymer MP particles by calculating the carbonyl index (CI) according to Equation 1:

$$CI = \frac{A}{B} \qquad (1)$$

where A corresponds to the intensity stretching mode of the carbonyl band between 1,735 and 1,715 cm^{-1} and B to the reference band at 1,471 and 1,460 cm^{-1} due to CH$_2$ scissor vibrations for PE and PP polymers, respectively[39].

Raman spectroscopy

Raman spectra were recorded using a Witec confocal spectrometer, model Alpha 300R, with a CCD (charge-coupled device) detector, using the exciting radiation at 632.8 nm (He-Ne laser), coupled to a Zeiss microscope. Measurements were acquired in the 500 to 3,000 cm^{-1} range, with a resolution of 4 cm^{-1} in 5 accumulations, with an exposure time of 20 s. Laser power varied from 1 to 5 mW depending on the sample. The spectra were processed using Fityk 1.3.1 software.

Elemental carbon analysis coupled with mass spectrometry

The percentage of carbon by mass of virgin and aged PE and PP MPs samples was determined using an elemental analyzer (EA 1110, Carlo Erba) coupled to a mass spectrometer (Finnigan - Delta Plus). The equipment operated in dynamic flash combustion mode, where around 1.0 mg of each sample was inserted into a quartz tube at 1,000 °C and subjected to combustion, followed by oxidation and reduction reactions. The resulting gases separated by gas chromatography were analyzed by thermal conductivity.

SEM-EDS

For the SEM-EDS characterization, the MP particles were deposited on carbon tape (PELCO Tabs™, Ted Pella, Inc; Redding) mounted on metal stubs. They were then sputter-coated for 180 s with gold to a thickness of 80 nm (Leica ACE 600, Vienna, Austria). Micrographs were taken on an SEM (Jeol JSM-IT300 LV, Tokyo, Japan) operating at 15 kV and the images were digitized. EDS analysis was carried out using the same microscope coupled to an X-ray detector (EDS, Oxford Instruments, Abingdon).

RESULTS AND DISCUSSION

Figure 1 shows the spectra obtained by micro ATR-FTIR for the MPs samples of virgin PE and PP polymers after accelerated aging. The typical spectral profile of PE can be seen in the presence of the bands at 2,915 cm^{-1} (υC–H), 2,845 cm^{-1} (υC–H), 1,472 cm^{-1} (δCH$_2$), 1,462 cm^{-1} (δCH$_2$), 730 cm^{-1} (δCH$_2$), and 720 cm^{-1}
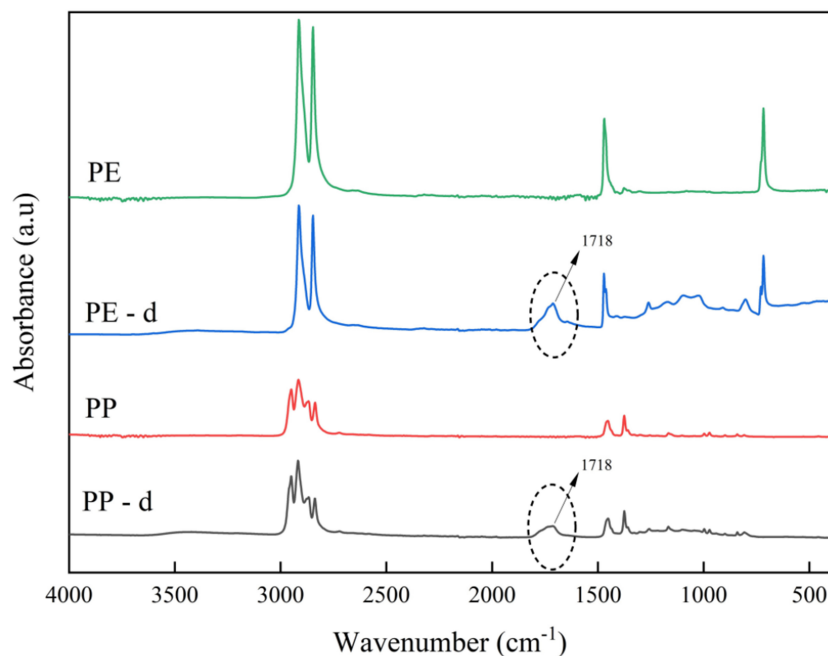
Figure 1. FTIR-ATR spectra of PE and PP polymer samples. PE and PP correspond to virgin samples and PE-d and PP-d correspond to degraded samples after accelerated aging in the QUV. FTIR: Fourier-transform Infrared spectroscopy; ATR: attenuated total reflectance; PE: polyethylene; PP: polypropylene.

(δCH_2). On the other hand, the bands at 2,950 cm^{-1} ($\upsilon C–H$), 2,915 cm^{-1} ($\upsilon C–H$), 2,838 cm^{-1} ($\upsilon C–H$), 1,455 cm^{-1} (δCH_2), 1,377 cm^{-1} (δCH_3), 1,166 cm^{-1} (angular deformation CH and CH$_3$, stretching C–C), 997 cm^{-1} (δCH_3 and CH), 972 cm^{-1} (δCH_3 and $\upsilon C–C$), 840 cm^{-1} (δCH_2 and $\upsilon C–CH_3$), and 802 cm^{-1} (δCH_2, $\upsilon C–CH$ and $\upsilon C–C$) are characteristics of the PP.

Thus, the spectral profiles observed correspond to the chemical signatures of these polymers, which allows for quick, effective, and conclusive identification[40], where υ = stretching and δ = angular deformation.

The stretching bands present in the 1,700 cm^{-1} region ($\upsilon C=O$) in the PE-d and PP-d spectra are evidence of the photodegradation of the material when subjected to thermal and photochemical degradation in the QUV[39]. Knowing that these bands are not characteristic of PE and PP polymers, as polyolefins only have saturated carbon-carbon bonds in their composition, it is clear that the surface of the material has undergone photo-oxidative degradation, i.e., the introduction of oxygen into its polymer chain, due to exposure to UV radiation[41].

The absorption of sunlight by these polymers is due to other chemical groups present in the polymer chain. These could be chemical contaminants produced during the polymerization reaction, chemical compounds added as additives, polymer degradation products, contaminants present in the environment that have been sorbed onto the surface of the material, or even an anomaly in the macromolecular structure[20,42]. A small amount of radiation absorbed by these extrinsic chromophores and/or polymer structures is enough to initiate a free radical chain reaction[42]. This reaction, in the presence of oxygen, produces free radicals, leading to photo-oxidation, which consists of autocatalytic oxidation, with the formation of carbonyl groups such as ketones, aldehydes, and esters[27,42,43].

Due to these degradation reactions, polymers undergo changes in their chemical composition and morphological properties, such as the formation of carbonyl groups, chain scission with a decrease in molar mass, discoloration, and changes in mechanical properties. They become more fragile and break down more easily into small particles[19,20,27,44].

PE and PP polymers are widely used for the production of plastic packaging and utensils and are often reported as the main classes of solid waste disposed of inappropriately in the environment, which can suffer degradation, fragmentation, and the generation of secondary MPs[35]. These same polymers are also used, for example, as microspheres in cosmetics, meaning that they can reach environmental matrices through the discharge of domestic effluents[45].

Therefore, the presence of carbonyl in the FTIR-ATR spectra of the MPs of these polymers may be a relevant and decisive factor in classifying the polluting source. The aged PE and PP samples showed the same chemical alterations as those already mentioned and observed, and high values in the carbonyl index calculation were found at the end of each polymer's aging cycle [Table 1]. The evidence of degradation was more significant for PP than for PE; this is caused by the presence of tertiary carbons in PP with CH bonds with lower energy than those in the primary and secondary carbons. C–H covalent bond scission in the PP chain is thus favored over PE[27]. This explains the results found in the PP samples, with a high CI due to the insertion of the oxygen atom in its structure and faster fragmentation during the aging cycle. Despite the absence of tertiary carbons, the same degradative process of chain scission can also occur with PE, as indicated by the increase in the CI.

In this sense, the possibility of assessing the aging stage of the samples by analyzing the intensity of the band referring to the $\nu(C=O)$ mode and calculating the index of the same group highlights the FTIR-ATR technique as a potential tool for non-destructive analysis of MPs.

However, as a disadvantage, qualitative analysis by FTIR-ATR presents a limitation in terms of particle size, as this method generally requires a minimum size of 100 μm in conventional equipment or, using μ-FTIR, MPs with a lower limit of 10 μm and with a spatial resolution of 5 μm[46,47]. In addition, during sample preparation, in conventional FTIR-ATR analysis, MP particles are analyzed individually, and the particle selection step needs to be performed manually, which requires a lot of work and time. Automated analysis of MPs samples on a filter, for example, is only possible with μ-FTIR and often requires the use of a focal plane detector (FPA) or an electron multiplier charge coupled device (EMCCD), which allow multiple points to be measured; these analyses are more complex and require sophisticated instrumentation and a qualified analyst[48]. Another disadvantage is related to the need for sample preparation; FTIR analyses must be purified and dried before detection, otherwise, interferences can occur in the spectra due to the humidity of the samples[49].

Figure 2 shows the spectra obtained by Raman spectroscopy for samples of virgin PE and PP polymers and after accelerated aging. The chemical identification of the polymers is possible and conclusive, due to the presence of two characteristic bands at 2,846 and 2,881 cm^{-1}, referring to the vibrational mode of the PE polymer. Similarly, PP is identified due to the presence of a set of characteristic bands from 2,800 to 3,000 cm^{-1}[50]. However, when comparing the spectra of the same polymers, PE with PE-d and PP with PP-d, in contrast to the spectra obtained by FTIR-ATR, it is not possible to clearly observe evidence of manipulation in the samples subjected to accelerated aging, as the vibrational transitions occurring with this technique do not include the introduction of bands characteristic of the degradation mechanisms occurring in the samples, with the mode referring to carbonyl stretching being much more intense in the infrared,

Table 1. Variation in the carbonyl index as a function of the accelerated aging time of the PE and PP samples

Exposure time QUV (h)	Carbonyl index	
	PE	PP
0	0.0 ± 0.0	0.0 ± 0.0
1,240	-	2.38 ± 0.1
2,000	0.71 ± 0.0	-

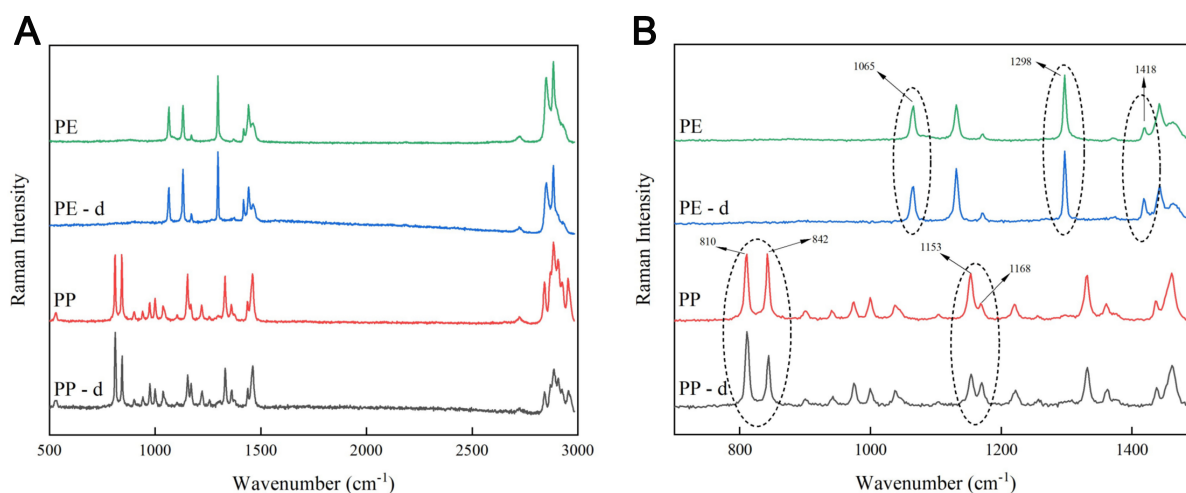PE: Polyethylene; PP: polypropylene.

Figure 2. (A) Raman spectra of the PE and PP polymer samples PE and PP correspond to the virgin samples and PE-d and PP-d correspond to the degraded samples after accelerated aging in the QUV; (B) corresponds to the prominence of the altered bands after accelerated aging. PE: Polyethylene; PP: polypropylene.

which requires a more detailed analysis in the Raman analysis of the MPs samples collected in the environment. On the other hand, although the evidence of degradation in the Raman spectra is more subtle and difficult to interpret, it can be identified due to the relationship between the amorphous and crystalline regions, with the increase in crystallinity being related to the aging of the material. For the PE polymer, the intensity of the band at 1,080 cm^{-1}, characteristic of the C–C stretching mode of amorphous chains, decreases as the exposure time increases, representing an increase in crystallinity. Another characteristic indicating degradation is the change and increase in the area of the peaks and the C–C crystalline stretching modes at 1,063 and 1,130 cm^{-1} with the irradiation time. Changes in the 1,400 cm^{-1} region also show a change in the state of degradation, especially in the orthorhombic crystallinity band at 1,418 cm^{-1} and the amorphous band at 1,460 cm^{-1}[51,52]. Considering the PP polymer, the presence of tertiary carbon in its structure makes it more prone to degradation processes compared to PE. The main variations in crystallinity due to aging can be observed through changes in the peaks at around 810, 842, 973, 998, 1,153, and 1,168 cm^{-1}, for example[53].

The analysis of MPs using Raman spectroscopy requires a long measurement time and is more laborious than FTIR analysis, for example, as this technique involves more instrumental variables. It is also more sensitive to interference due to the possibility of chemical additives present in the plastic, such as dyes, or the presence of contaminants, which can cause fluorescence-induced spectral distortion and compromise the analysis, and does not occur in FTIR analysis. On the other hand, as well as FTIR analysis, Raman spectroscopy allows assertive chemical identification of the polymer due to the existence of a spectral

library, and also the analysis of MPs on an even smaller size scale than μ-FTIR, with a lower limit of 1 μm. In this respect, it can be considered the most promising technique, depending on the type of sample to be analyzed, for example, for drinking water samples[54]. Another advantage of this technique is the possibility of mapping a certain area with numerous MP particles, like a filter. Finally, like FTIR analysis, Raman analysis represents a non-destructive analysis of the sample[47,49].

Table 2 presents the average results of the mass percentage of carbon in virgin MPs samples and aged PE and PP MPs samples obtained by elemental carbon analysis. As a consequence of the degradation mechanism, it is possible to observe the loss of mass suffered by both polymers subjected to aging, which is more pronounced in PP than in PE. This is due to the presence of tertiary carbon in the PP structure, which facilitates the degradation mechanism by homolytic scission, which further favors the reduction in molar mass[41].

The carbon elemental analysis shows accuracy and good reproducibility of the results, as well as being a relatively low-cost analysis compared to other techniques. The equipment used for elemental analysis only provides %C, and this value, close to 80%, aids in the identification of both virgin and aged MPs, as well as making it possible to observe the loss of carbon mass in aged MPs. However, this analysis alone does not make it possible to discriminate between the chemical identities of the MPs, since they have similar carbon content. There is also the possibility of misinterpreting the result, knowing that other solid materials present in the environment such as biochar, commonly reported in the soil profile, also have a C content similar to that of MPs[55].

Another drawback of elemental carbon analysis is that it is destructive and requires at least 1.0 mg of sample to provide a reliable result. On the other hand, ATR-FTIR requires only one particle to fill the minimum space in the equipment's accessory, which consists of direct contact between the sample and the tip of the germanium crystal lens. In the case of MPs samples collected from the environment, the mass corresponding to 1.0 mg is very significant and often unfeasible.

Figure 3 illustrates the results obtained by SEM-EDS of the virgin and aged PE and PP MPs samples. Both the PE and PP MPs samples showed a loss of mass percentage of carbon and an increase in mass percentage of oxygen after accelerated aging, as well as the presence of some trace elements. The micrographs also make it possible to characterize the morphological alterations resulting from the thermal and photochemical degradation of the samples, with cracks and porosity being observed in the aged samples.

The advantages of SEM-EDS analysis are that it can be used to analyze the surface of small particles and requires only a small amount of the sample. However, this analysis is relatively expensive and does not provide the composition of the entire sample, but only of the elements that are actively selected at a given point, which can result in an inaccurate analysis of the chemical composition of the material. Therefore, it is not possible to identify the MPs with this method, added to which, this is a destructive form of analysis of the sample, and an unfeasible option considering the large number of MPs samples to be analyzed.

CONCLUSIONS

The techniques evaluated here are extremely relevant and are already widely known by the scientific community in the context of characterizing synthetic organic polymers. However, when considering the qualitative analysis of MPs collected in the environment, with the aim of assessing their distribution and identifying the main polluting sources, i.e., the stage of degradation of the particles, it can be concluded that carbon elemental analysis and the SEM-EDS technique are only useful as complementary techniques, which

Table 2. Mass percentage of carbon in PE and PP polymer MPs samples

MPs samples	Average mass (mg)	Average %carbon
PE	1.12 ± 0.01	85.72 ± 0.26
PE-d	1.13 ± 0.00	84.99 ± 0.08
PP	1.13 ± 0.01	86.67 ± 0.49
PP-d	1.13 ± 0.01	78.88 ± 0.39

PE and PP correspond to virgin samples and PE-d and PP-d correspond to QUV-aged samples. PE: Polyethylene; PP: polypropylene; MPs: microplastics.
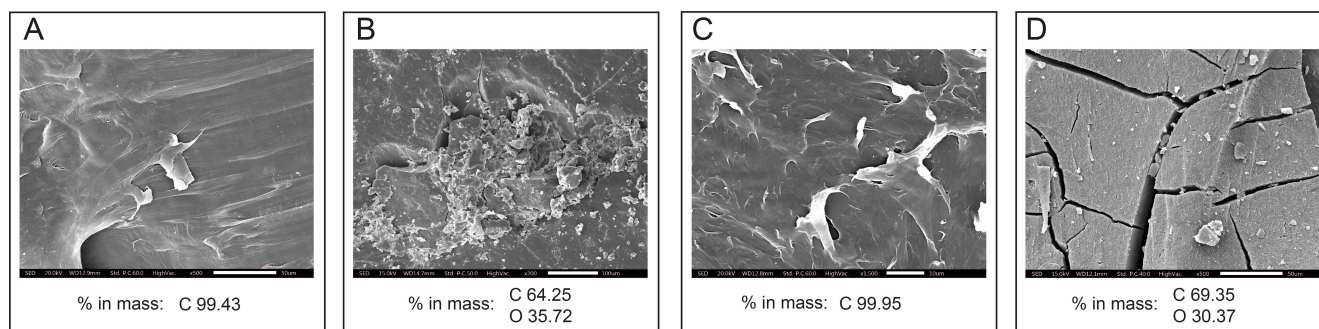

Figure 3. SEM and EDS images of the MPs samples: (A) virgin PE; (B) aged PE; (C) virgin PP; and (D) aged PP. SEM: Scanning electron microscopy; EDS: energy dispersive X-ray spectroscopy; MPs: microplastics; PE: polyethylene; PP: polypropylene.

can help in understanding the degradation of the particles, as shown by the current results from the loss of carbon mass resulting from photodegradation. It should be noted that only the FTIR and Raman techniques can contribute to the conclusive chemical identification of MPs, due to the characteristic spectra of each type of polymer, while μ-FTIR is the most suitable for the analysis of MPs samples in the size range of more than 10 μm, as it easily provides information on the stage of aging, through the quantification of the carbonyl index and, consequently, aids elucidation of the main polluting sources of MPs in the environment.

DECLARATIONS

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Authors' contributions
Conceptualization, data curation, figures, table, writing - original draft preparation: Olivatto GP
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