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High resolution X-ray microtomography as a tool for observation and classification of individual microplastics

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Abstract: Plastics are synthetic polymers, widely used due to their durability, resistance, and lightweight properties. However, their extensive production and long-lasting nature have led to environmental challenges, notably the accumulation of plastic waste, where they degrade into microplastics (MPs)—particles smaller than 5 mm. These MPs contaminate various ecosystems, including water bodies, soils, and even the atmosphere. Understanding the complex structures of MPs is essential, but their heterogeneity makes characterization challenging. This study investigates the use of X-ray microtomography (microCT) as a tool for characterizing MPs. By scanning plastic fragments embedded in sediment, microCT provides detailed internal and external morphological data in a non-destructive manner. The total volume measured by microCT was approximately 150.00 mm^3, accounting for 79% of the estimated theoretical volume, with a total surface area of 1061.00 mm^2. The analysis focused on morphometric parameters such as Feret diameter, anisotropy degree, and sphericity, which describe the shape and symmetry of individual particles. Results indicated significant variability in these parameters, reflecting the diverse nature of MPs. Additionally, microCT imaging detected slight variations in material composition, indicating potential heterogeneity within the polymers. The study highlights the need for standardization in MP classification and suggests that microCT, with its ability to detect subtle variations in material composition, holds promise for future environmental research. Further exploration of microCT's capabilities could enhance our understanding of MP behavior and impact, particularly in relation to their composition and environmental interactions.

Keywords: Microplastic pollution, X-ray microtomography, Shape characterization, Morphometric Parameters.

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Microtomografia Computadorizada de raios X de alta resolução como uma ferramenta para observação e classificação de microplásticos individuais.

Resumo: Os plásticos são polímeros sintéticos amplamente utilizados devido à sua durabilidade, resistência e leveza. No entanto, sua produção extensa e durabilidade resultaram em desafios ambientais, principalmente na acumulação de resíduos plásticos, que se degradam em microplásticos (MPs)—partículas menores que 5 mm. Esses MPs contaminam vários ecossistemas, incluindo corpos d'água, solos e até mesmo a atmosfera. Compreender as estruturas complexas dos MPs é essencial, mas sua heterogeneidade torna a caracterização desafiadora. Este estudo investiga o uso da microtomografia computadorizada por raios X (microCT) como uma ferramenta para caracterizar MPs. Ao escanear fragmentos plásticos embutidos em sedimentos, a microCT fornece dados morfológicos detalhados, internos e externos, de maneira não destrutiva. O volume total medido pela microCT foi de aproximadamente 150,00 mm³, correspondendo a 79% do volume teórico estimado, com uma área de superfície total de 1061,00 mm². A análise focou em parâmetros morfométricos como diâmetro de Feret, grau de anisotropia e esfericidade, que descrevem a forma e a simetria das partículas individuais. Os resultados indicaram variabilidade significativa nesses parâmetros, refletindo a natureza diversa dos MPs. Além disso, a imagem por microCT detectou leves variações na composição do material, indicando potencial heterogeneidade nos polímeros. O estudo destaca a necessidade de padronização na classificação de MPs e sugere que a microCT, com sua capacidade de detectar variações sutis na composição do material, promete ser uma ferramenta valiosa para pesquisas ambientais futuras. A exploração adicional das capacidades da microCT poderia aprimorar nossa compreensão do comportamento e impacto dos MPs, especialmente em relação à sua composição e interações ambientais.

Palavras-chave: Poluição por microplásticos, microtomografia computadorizada por raios X, Caracterização por forma, parâmetros morfométricos.

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1. INTRODUCTION

Plastics are synthetic polymers composed of hundreds to thousands of monomers linked by covalent chemical bonds [1]. These materials are cost-effective and highly engineered, offering a wide range of applications due to their durability, resistance, and lightweight properties. Over the past few decades, plastic production has rapidly increased, leading to a society heavily reliant on plastic materials [2]. The majority of plastics are derived from nonrenewable petrochemical sources, which contribute to their biological and chemical inertness, thus enhancing their durability [3].

The significant issue with large-scale plastic production and its extended durability lies in the generation of plastic waste. Despite the existence of recycling programs and awareness campaigns regarding waste management, only a small portion of discarded plastic is properly processed. In 2019, for instance, merely 9% of discarded plastic was recycled, 12% was incinerated, and the remaining 79% persisted in the environment or was deposited in landfills [4].

Plastics in the environment are exposed to ultraviolet light, heat, wind, and various other chemical and physical conditions, which contribute to the degradation of large plastic pieces into smaller fragments. This degradation process leads to the formation of MPs, plastic particles smaller than 5 mm in size [5]. Due to their small size and light weight, these particles can easily disperse and contaminate diverse environments, including freshwater bodies and oceans [6-8], soils [9], and the atmosphere [10], with the potential to reach remote regions of the planet [11] and enter the food chain [12, 13].

MPs exhibit complex structures with a wide range of sizes, morphologies, and chemical properties. This extensive heterogeneity arises from the diversity of original plastic materials and the processes involved in MP formation [14]. Given this complexity, MPs are distinct entities, and treating them as homogeneous particles is not feasible. Furthermore,

their intricate nature poses significant challenges for accurate characterization. Extensive research has been conducted to detect and understand MPs and their environmental impacts. Recently, microCT has emerged as a promising tool for studying MPs, showing potential in addressing critical questions related to these particles [15–19]. The ability of microCT scans to provide detailed internal and external morphological information in a non-destructive manner, coupled with the capability of modern microCT equipment to perform investigations at the micrometer scale, makes this technique a powerful tool for MP analysis.

Particle characterization is crucial in various scientific fields, as many physical and chemical properties are dependent on particle shape [20, 21]. In this study, we propose the application of microCT to investigate shape-related characteristics of individual MPs. To achieve this, we analyze three-dimensional structural models generated from scans using a commercial microCT system, followed by the application of morphometric parameters to characterize MP samples.

2. MATERIALS AND METHODS

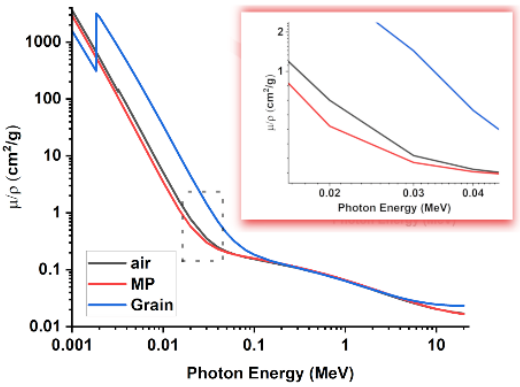
2.1 MicroCT Assay

To evaluate the potential microCT for investigating MPs, plastic fragments with a median diameter of 2.0 mm were enclosed in plastic Eppendorf tubes mixed with sand grains free of impurities. The maximum diameter of the MPs was determined using sieves (Granutest) with mesh openings [17]. The plastic fragments comprised a mixture of different polymers with unknown proportions. The theoretical volume of the plastics was estimated based on the total mass and the average density of the plastic material. Consequently, we estimated a theoretical volume of approximately 190 mm^3.

The challenge in this step arises from the low density and small diameter of the plastic fragments. The radiation absorption of plastic is comparable to that of air (Figure 1), making

it difficult to distinguish between them at the energy levels used in this study [22]. Microtomographic imaging was conducted using the X-ray microtomography VTomex/300M (General Electric). The equipment was optimized to balance image quality with acquisition time. A magnification setting was chosen to achieve a voxel size of 12.0 μm, with 1400 projections captured at an exposure time of 333 ms. The X-ray tube was operated at 110 kV and 120 μA. A 0.1 mm aluminum filter was placed in front of the X-ray source to eliminate low-energy photons and consequently reduce beam-hardening artifacts [23].

Figure 1: The energy-attenuation curves for air, polyethylene and sand: There is low Differences in the energy-absorption curve for air and plastic fragments. It is generating a limitation in microCT scan.



Source: Elaborated by authors (data from Xcom [22]).

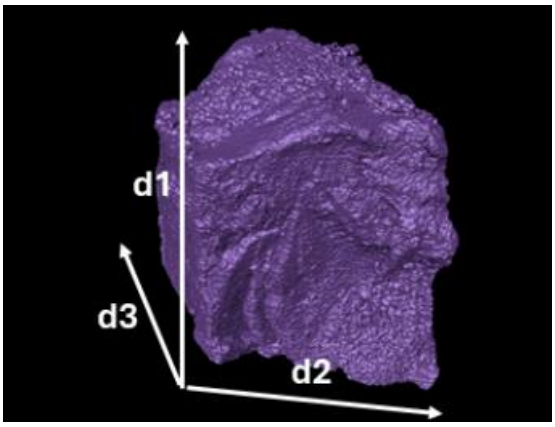
Subsequently, the X-ray projections were reconstructed into microCT images using Datos reconstruction software (GE, Sensing & Inspection Technology). The resulting tomographic images were analyzed with AVIZO software (VSG) and CTanalyzer (Bruker). Pre-processing procedures were implemented to reduce noise and enhance the contrast of the plastic material. Segmentation was performed by defining the Region of Interest (ROI) as the plastic. Slice-wise 2D comparisons between the original and segmented scans were conducted to ensure segmentation accuracy. The binarized image stack was utilized to explore various morphometric parameters of the samples, while the original images (in 256 gray levels) provided information on material composition.

2.2 Image analysis

In our studies, we utilized morphometric parameters to gain a deeper understanding of MP structures. Volume and surface area data provide crucial insights into plastic fragments found in the environment. We defined d1, d2, and d3 as the lengths of the maximum, intermediate, and minimum dimensions of the particles, respectively (Figure 2).

These data allow for the calculation of significant metrics related to particle dimensions, such as the Feret diameter (FD), defined as the longest distance between any two points along the particle's perimeter [24], and the anisotropy degree (AD), which describes the orientation of the object. These parameters can provide insight into key aspects of the mechanical behavior of three-dimensional structures.

Figure 2: Main three-dimensions d1, d2 and d3 used to characterize morphometrically plastic fragments.



Source: Elaborated by authors.

X-ray imaging relies on the attenuation coefficient of radiation interacting with matter, which characterizes the spatial distribution of material density. In this study, we employed a polychromatic X-ray source, where photons are generated at various energy levels, and their interaction with the sample does not provide a complete density characterization. However, we utilized an approach based on the grey level values of the microCT images to correlate voxel variations for comparative analysis. This property was leveraged to represent relative variations in material composition through a colormap.

3. RESULTS AND DISCUSSIONS

Parameters generated from microCT images have been extensively explored in medical research [25]. Over the past decades, microCT has been employed in various fields, with health science benefiting the most from advancements in the technique. The high-resolution imaging and the ability to generate 3D models have established microCT as the primary method for studying bone structure, and the main nomenclatures and tools developed have been tailored to these applications. However, these concepts can be exported and adapted to applications outside of medical science, as the analysis fundamentally starts from binarized images composed of voxels.

Evidence suggests that the analysis of morphometric parameters is influenced by microCT procedures, such as segmentation methods and voxel size. Moreover, currently, there is no universal standardization for microstructural parameters [26]. These limitations underscore the importance of utilizing microCT images for studying MPs exclusively when comparisons are made under consistent conditions.

In this study, we analyzed and characterized 61 fragments. The total volume measured by microCT analysis was approximately 150.00 mm^3, representing 79% of the estimated theoretical volume. The median volume per particle was 2.54 mm^3. The total surface area of the MPs was 1061.00 mm^2, with a median surface area of 18.00 mm^2 per fragment.

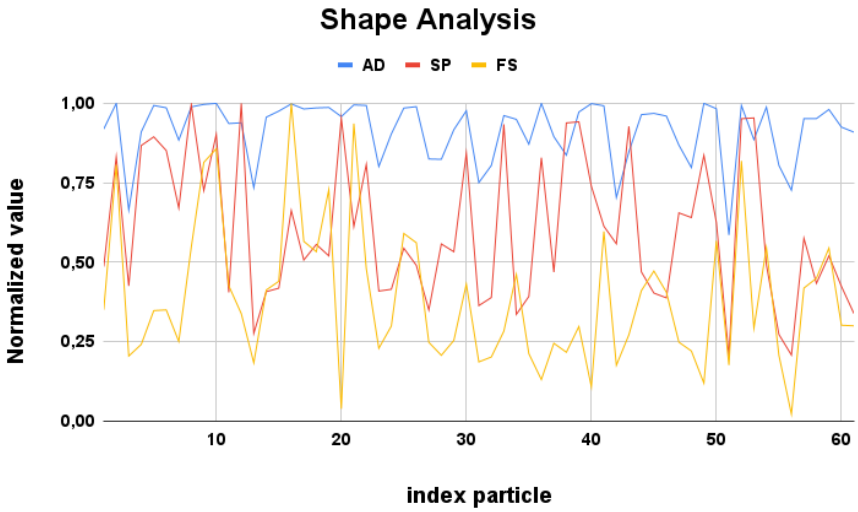
Morphometric parameters can be obtained for each particle individually. Due to the irregularity of the particles, it is not possible to directly define the size of MPs. However, microCT offers the ability to measure diameter through alternative methods. The Feret shape (FS), frequently used in particle size analysis as a descriptor for evaluating particle size distribution, is defined as the ratio between the minimal Feret diameter (FD) and the FD closest to the normal of the minimal FD. This method relies on sampling across a distribution of different orientations [27]. In this study, 31 orientations were used to calculate the Feret diameter of the MPs.

Another widely used morphometric parameter for characterizing particle shape is the anisotropy degree, a dimensionless value calculated by subtracting the ratio of the smallest dimension to the largest dimension from 1. Lower values indicate that the structure is closer to a spherical shape.

Shape symmetry may be based on relationship of Volume and Superficial area. Sphericity (SP) is defined as the ratio of the surface area of a sphere (with the same volume as the given particle) to the surface area of the particle [28]. In our approach, values close to zero means fragments more symmetric such as sphere.

In our study, we assessed the shape distribution of the particles based on these parameters. The fragments were labeled sequentially from 1 to 61, and the AD, FS, and SP values were computed and normalized for comparison. Figure 3 presents the distribution of these three parameters across all samples.

Figure 3: AD, SP and FS descriptors comparation.



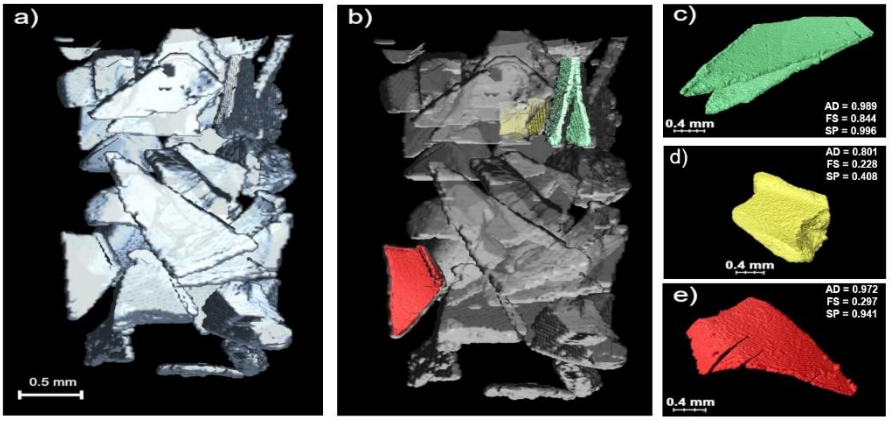
Source: Elaborated by authors.

The objective of this phase of the study was to compare the regularity of particle shapes as determined by the AD, FS, and SP parameters and to evaluate their consistency. As shown in Figure 3, there are significant variations in the parameter values. To ensure

consistency, we employed a strategy where the same segmented image stack was used to extract shape parameter data, thereby minimizing the influence of the dataset on the results. These variations can be attributed to differences in the evaluation methodologies. Although some concordance points are observed in the plots, a more detailed examination of individual particles is necessary to determine the dominant characteristics that define the shape parameters.

In our study, these parameters varied across a wide range, reflecting the heterogeneity in the size and shape of the particles. Figure 4 (a-b) presents the three-dimensional models of MPs, while Figures 4 (c-e) highlight three specific fragments (particles 8, 23, and 39) as examples.

Figure 4: 3D models of fragments. Fragment spatial distribution is presented in (a) and in (b), where three fragments are highlighted: particle 8 (c), particle 23 (d) and particle 39 (e).



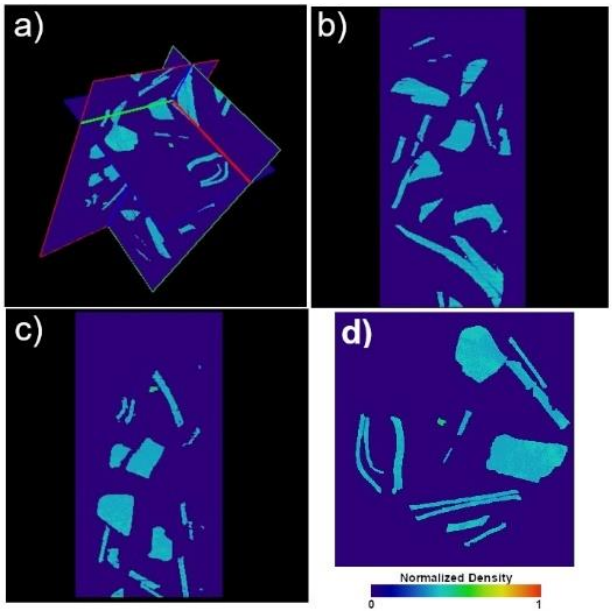
Source: Elaborated by authors.

Our samples represent only a small fraction of the vast array of structures found in the environment. Given the wide range of characteristics that MPs can exhibit, which significantly influence their behavior in the environment, it is erroneous to consider all particles as identical [29-30].

Figure 5 presents two-dimensional slices based on the normalized attenuation coefficient, where a value of zero (0) represents a low density associated with a detected grey level, and a value of one (1) represents a high value. The measurements in MicroCT is associated to attenuation coefficient and density such as described by Beer's law [38]. Our

result suggests that although polymers are predominantly homogeneous, they may present variation in material composition, and microCT can reveal it. In this preliminary study, only minor variations were observed in the samples. However, microCT shows promise as a technique for characterizing MP composition. This is consistent with its applications in geosciences and industrial materials, where it is used for material composition analysis based on radiation-matter interactions [31-35].

Figure 5: Density value distribution. Cold colors mean lower density values and hot colors means higher density values. In image is possible observe that the material studied presents little density variation. (a) three views; (b-d) sagittal, coronal and transaxial views.



Source: Elaborated by authors.

Furthermore, plastics in the environment undergo numerous physical and chemical effects that can alter their structure, and additional materials may adhere to them, transforming MPs into chemical and biological vectors [36-37]. In our research, we utilized conventional image acquisition methods; however, we believe that with appropriate calibration techniques, microCT has the potential to offer extensive information on density distribution.

4. CONCLUSIONS

This study confirms that microCT images can effectively evaluate the characteristics of individual MPs, even when embedded in sediments. In addition to detecting the majority of plastic fragments, shape descriptors provide valuable information regarding the irregularity of the fragments. However, relying on a single parameter is insufficient to fully describe a particle's characteristics. In this study, we examined three parameters related to symmetry. In some cases, the parameters did not correlate well, leading to unsatisfactory results. There is an urgent need to develop standardized methods for classifying MPs, and future research should explore additional parameters to address these limitations.

MicroCT images also demonstrated the potential to distinguish subtle variations in fragments. While homogeneity is predominant in polymers, a wide range of applications involves plastics with varying densities and chemical additives. Thus, microCT could serve as a valuable tool for characterizing MPs in the environment. In future work, we aim to further explore the potential of microCT for providing a comprehensive characterization of plastic fragments, focusing not only on shape but also on their composition.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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