



Combination of Micro-Raman and Infrared Spectroscopy to Identify Intriguing Case of Aged Microplastics of Estuarine Sediments

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The Atlantic Forest is one of the richest regions in biodiversity in the world. Originally the biome covered around 15% of the Brazilian territory. Currently, there are approximately 12.4% of forest remnants preserved in the country. Regarding mangrove areas in the state of São Paulo, there are around 223 km² according to the Brazilian Mangrove Atlas, with around 120.5 km² located in Baixada Santista. Analysis of sediments found in the estuarine mangroves of Santos shows a high concentration of microplastics (MPs), generated by industrial processes and human activity, that constitutes today one of the main environmental problems. The MPs presented in the sediment samples are quantified using a methodology that involves drying, sieving, quantification, and identification of these MPs through FTIR and micro-Raman spectroscopy. The two techniques complement each other to identify MP filaments and fragments through common polymer spectra. Furthermore, the micro-Raman technique also identified additives flexo blue (blue ink) and neolan green 8G (dye) in MPs. All identified polymers (< 5 mm) have wide applications and demands in various sectors, including packaging, construction, automotive, electronics, and textiles.

and São Paulo with 12.4% of original forest remnant preserved.^[1, 2]

Estuaries serve as transition zones where fresh water from a river mouth meets salt water from the sea. These are areas with high geochemical reactivity and high biological productivity, having a crucial ecological role in the environment. In addition to the dynamic processes of the estuary, irregular housing settlements without a basic sanitation system are present in that coastal region.

Irregular occupation^[3] and the presence of landfills and municipal dumps weaken the condition of the environment.^[4,5] Hydrodynamic dispersion, associated with high and low tides, may well explain the high contamination of the Santos-São Vicente estuary in addition to anthropogenic influences due to a high population density within the estuary.^[6] In a reported study Alencar et al.^[7] discussed the investigation and innovative methodological framework aimed at delineating focal points of

1. Introduction

The Santos-São Vicente estuary – SESS is in the Santos Basin within a sedimentary area that spans approximately 352 thousand km², covering the northern portion of the coast of the state of Santa Catarina, the southern portion of the state of Rio de Janeiro and the entire coast of the states of Rio de Janeiro, Paraná,

mismanaged plastic waste (MPW) within the geographic expanse of Brazil and its subsequent ingress into marine ecosystems. Drawing upon a multi-dimensional approach, they analyzed pertinent parameters including socio-economic populational metrics, municipal solid waste management strategies, and environmental variables. This meticulous analysis enabled the estimation of both plastic waste generation and the plastic type prevalence in thousands of municipalities in Brazil.

Another study of Belli et al.^[8], extensively examined the methodologies employed in monitoring, sampling, and identifying both macroplastics and microplastics (MPs) in aquatic environments such as water bodies, sediment layers, and organisms. It also delved into the regulatory frameworks across various nations alongside non-governmental efforts in this domain. Notably, according to Belli, Brazil emerged, specifically in the southeast region in São Paulo and Rio de Janeiro, as the leading contributor in terms of published research of MPs in sediments and oceanic waters. The analysis revealed that MPs constituted a significant portion (75%) of the selected literature, with blue microfibers emerging as the prevalent form, while polyethylene

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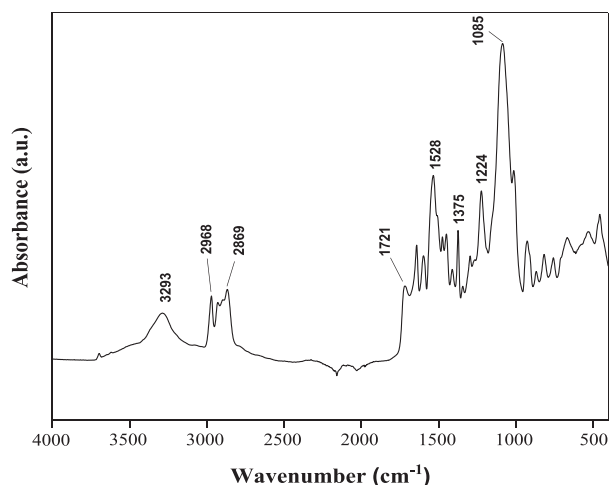


Figure 1. Infrared spectrum measured from a microplastic identified as polyurethane.

(PE) and polypropylene (PP) emerged as the most frequently encountered polymers. MPs fragments, multicomponent, aged under the exposition to environmental conditions and weather can be one challenger to the spectroscopic techniques of identification.

The present study is focused on the discussion on the complementarity of infrared and micro-Raman techniques for identification of MPs of difficulty identification. The utilization of two spectroscopic methods using the image tool of micro-Raman is necessary for a reliable result and to validate the structure and chemical composition of MPs.

2. Results and Discussion

The degradation of MPs in the aquatic environment occurs through physical, chemical, and biological processes.^[9–11] This degradation requires the presence of oxygen and sunlight^[10,12,13] and one of the most likely places for this degradation to occur is in coastal regions, where conditions are conducive to high rates of photodegradation.^[10] Floating plastics degrade at slower rates compared to terrestrial plastics due to biological interactions that cause a surface film to form protecting the surface from sunlight and lower temperatures.^[10,14,13,15] Lower temperatures and the absence of UV radiation further reduce the handling rate.^[9]

Infrared spectroscopy is a consolidated technique for identifying polymers, however, in this work, it was difficult to identify some samples because they were highly oxidized. The spectrum presented in **Figure 1** was identified as polyurethane microplastic showing –NH bonds representing urethane and urea groups. Polyamide also has N bonds in the absorption spectrum near 3200 cm^{-1} , referring to the amide groups.^[16] CH_2 groups show an asymmetric stretching peak at 2915 cm^{-1} ; CH_2 a symmetric stretching peak at 2853 cm^{-1} ; C = O carbonyl group at 1737 cm^{-1} ; – CH_2 bending at 1425 cm^{-1} ; 1025 cm^{-1} enlarged is attributed to stretching symmetric of NCO–O and stretching of –COC polymeric at 957 cm^{-1} to ether.^[17,18]

The same sample was analyzed by micro-Raman, a promising technique for identifying MPs smaller than one micron and exhibiting the capability to detect even smaller nanoplastics, down

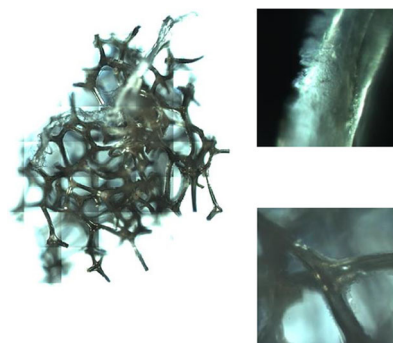


Figure 2a. Raman microscope image of the microplastic identified as polyurethane, with aspect of the fine foam, and another transparent thread polymer.

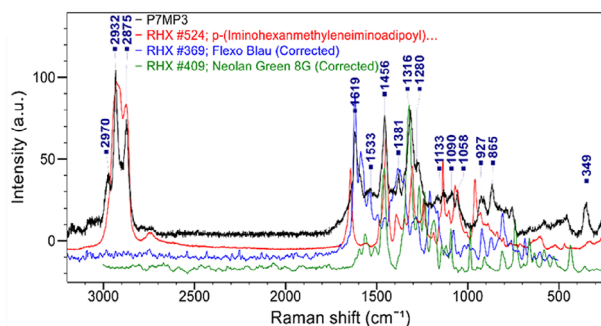


Figure 2b. Raman spectrum of fragment foam containing a polyamide (nylon), and other pigments, compared to reference spectra from Horiba database.

to sizes as small as 25 nm.^[19] The measured spectrum was analyzed by database comparison using the *Willey* and *Horiba* libraries available from *KnowItAll* software. The sample's spectrum was identified as polyamide. The two techniques, FTIR and micro-Raman, identified two different polymers. Furthermore, the high quality of the micro-Raman image permitted the observation of foam (polyurethane by FTIR) intertwined with fibers (polyamide by Raman). The micro-Raman also identified the additives *flexo blue* (blue ink for flexography) and *neolan green 8G* (dye), (**Figure 2a** and **b**).

It follows from the case studied that the polymeric filament trapped in the foam is composed of polyamide according to the Raman and the foam is polyurethane according to the FTIR identification.

Considering the size limitations of MPs that make FTIR identification difficult, the additives that limit the Raman identification of some polymers (such as fine filaments of foams), the oxidation state (aging)^[20] that limits the identification by Raman of mixtures containing more than one polymer component, and the quality of images obtained from microparticles by FTIR (which is improved by Raman), both techniques were useful for the correct identification of the microplastic in the case studied.

3. Conclusion

The sample preparation procedure involved sediment collection, drying, sieving (2.0, 1.0, 0.5-, and 0.25-mm mesh sizes), optical



microscope examination, and identification of the samples retained in each sieve. We employed the same method described by Gimiliani et al. for the identification of MPs. Among the MPs isolated, in Rio Casqueiro (latitude / longitude – 23°23'55"20,00"S / 46°24'7,56"O) one special case of difficult characterization was resolved by the careful observation of its image in micro-Raman and the interpretation combining the FTIR and micro-Raman spectra.

Comparing the two different vibrational spectroscopic techniques (FTIR and micro-Raman) it was observed additives combined with MPs in micrometric dimensions that can be identified by the micro-Raman technique. In the study were identified flexo blue (blue ink) and neolan green 8G (dye). In cases of pigments or dyes with high Raman scattering cross sections, it becomes difficult to identify the polymeric components for which the FTIR is a proper tool of characterization. Although the micro-Raman technique is more time-consuming, it provides more detailed information regarding contaminants and other additives that cannot unequivocally be identified only with infrared spectroscopy. The uncommon compositions of the two polymers Polyurethan and Polyamide were understood by image observation of a fishing line embedded in a foam fragment of the Micro-Raman. Considering the size limitations, the oxidation state (aging), and the multicomponent of the MPs, both techniques associated are essential for MPs identification and useful for future studies of environmental pollution by MPs.

4. Experimental Section

The methodology was established among international protocols for studies with MPs in sediments.^[21–23] Sediment samples from mangroves were collected with a Van Veen grab sediment sampler totaling approximately 8 kg at each site. Samples were collected from estuarine sites, among which, part of easiest identified results was previously reported. The sample preparation procedure involved sediment collection, drying, sieving (2.0, 1.0, 0.5, and 0.25 mm mesh sizes), optical microscope examination, and identification of the samples retained in each sieve. The same method described by Gimiliani et al. was employed for identification of MPs.^[8] Among the MPs isolated, in Rio Casqueiro (latitude / longitude – 23°23'55"20,00"S / 46°24'7,56"O)^[21] one special case of difficult characterization was resolved by the careful observation of its image in micro-Raman and the interpretation combining the FTIR and micro-Raman spectra.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

microplastic, micro-Raman, polymer, Santos estuary, sediment

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