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# Case Studies in Chemical and Environmental Engineering

journal homepage: www.editorialmanager.com/cscee/default.aspx



# Simple and cost-effective method for microplastic quantification in estuarine sediment: A case study of the Santos and São Vicente Estuarine System



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#### ARTICLE INFO

Keywords: Microplastics Estuarine sediment Sieving Py-GC/MS analysis

#### ABSTRACT

Sediment is a useful environmental compartment in the evaluation and monitoring of microplastics (i.e., plastic particles between 1  $\mu$ m and 5 mm in length) in aquatic environments, since 70% of plastic waste is deposited on the ocean floor, in riverbeds, and on the bottom of estuaries. The techniques typically used to separate and quantify microplastics require extensive sample preparation and are often ineffective for estuarine sediment samples. In this study, we present a new method for separating and quantifying microplastics found in estuarine sediment samples. This procedure involves sediment collection, drying, sieving (2.0, 1.0, 0.5 and 0.25 mm mesh sizes), and stereomicroscopic examination of the samples retained in each sieve. The results were measured as microplastic abundance (particles  $g^{-1}$  of sediment). This method allows researchers to estimate total microplastic abundance and distribution at the sieve meshes tested, calculate thread/fiber and fragment proportions, and separate microplastic samples for subsequent Py-GC/MS analysis in order to identify their respective compositions. This method was found to be effective in precluding the need for solvents and reducing the amount of time required for sample preparation. For these reasons, this method is more cost-effective and generates less environmental impact than those currently available.

# 1. Introduction

The literature and media worldwide reflect the science community's concern with pollution caused by plastics and microplastics. This level of interest has sparked a search for experimental methods that can be used to assess this waste in different environmental matrices, particularly in cases of environmental monitoring and assessment.

Plastic waste reaches aquatic environments through multiple sources, most of which involve inadequate waste disposal and allow for plastics to be distributed to other compartments within these environments, from surface water to the water column to sediment [1]. Under these conditions, plastic waste undergoes fragmentation processes that lead to the formation of progressively smaller particles, or microplastics. Microplastics are plastic particles that vary between 1  $\mu$ m and 5 mm in length [2]. They are currently considered relevant and emerging contaminants as a result of their generalized presence in the

environment, as well as of new evidence of the risks they pose to aquatic environments [3].

Close to 70% of plastic waste is deposited in the sediment of water bodies, while almost the entirety of the remaining 30% settles on surface water and in the water column [4]. Microplastics can accumulate in the sediment and be ingested by benthic and epibenthic organisms, thus spreading through the food chain and eventually returning to humans through human consumption of mussels and shrimp. Thus, sediment is useful for evaluating and monitoring the presence of microplastics in aquatic environments. The use of sediment as a tool in environmental assessment is also a logical choice because of its ecological importance: it serves as a substrate and food source for countless species and is, therefore, an optimal indicator of aquatic ecosystem health [5]. However, wetland and swamp sediments exhibit specific characteristics, including increased levels of organic matter and nutrients, a large quantity of plant debris and fragments [6], and

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even seashell fragments, which hinder the identification of microplastics.

Until now, the literature has lacked an appropriate microplastic analysis method for estuarine sediments, since the methods available to date have not been effective in extracting microplastics using density separation with sodium chloride (NaCl) solutions, nor have they successfully removed organic matter and plant debris through the employment of hydrogen peroxide  $(H_2O_2)$  solution. When these steps are applied to estuarine sediments, microplastics are lost and quantities are underestimated, thus producing unreliable results. Organic and inorganic debris, present in sediment samples, float and persist after treatment by a variety of chemical reagents, thus hindering both the separation process, and the ability to stereomicroscopically analyze the microplastics present. Another limitation to these methods is the extensive time required for sample preparation when chemical substances are used to remove organic matter and other organic debris: this treatment step of the experiment may last as long as four weeks and may still not remove all relevant debris, thus making microplastic quantification both laborintensive and possibly inaccurate. There is therefore an urgent need for more effective and affordable ways to separate and quantify microplastics from estuarine sediment, particularly because these areas experience intense impacts from human activity.

This study employs a novel and innovative method to quantify microplastics in estuarine sediment samples from a site located within the Santos and São Vicente Estuarine System (SSES) located on the central coast of São Paulo State, Brazil. The region houses the Port of Santos and the Cubatão industrial cluster, as well as some of the country's largest coastal slum communities (which consist largely of settlements built on stilts in mangrove swamps) [7], landfills, and illegal dumping sites [8]. Thousands of people lack access to basic sanitation systems, unhealthy conditions which result in the constant disposal of solid waste (and the consequent accumulation of plastics) into local swamplands [9].

This study therefore sought to establish a simple, rapid, effective, and low-cost approach to collecting, quantifying, and separating microplastics from estuarine sediments by applying the technique developed herein to the highly impacted SSES.

#### 2. Experimental analysis

# 2.1. Sampling and collection site

Two sediment sampling campaigns were performed (April 2018 and July 2019). The sampling sites were located within the SSES and were chosen based on their proximity to large coastal slum communities (which consist mainly of settlements built on stilts directly in water bodies) that constitute the main source of domestic waste in this estuarine region. Some sites were also selected because of their proximity to potential sources of industrial waste. As detailed in Fig. 1, the sites chosen were as follows: SSES1 (the São Vicente Channel, located near the México 70 slum community); SSES2 (the Largo Pompeba region); SSES3 (Site I on the Bugres River); SSES4 (Site II on the Bugres River); SSES5 (Site III on the Bugres River, located near the former Sambaiatuba landfill); SSES6 (the mouth of the Bugres River); SSES7 (the Jardim São Manoel coastal slum community); SSES8 (the Casqueiro River); SSES9 (the mouth of the Piaçaguera Channel); SSES10 (Barnabé Island); and SSES11 (the Santos Channel, located near the Santa Cruz dos Navegantes coastal slum community).

The sediment samples were collected from each site with the aid of a Van Veen grab sampler. Three grab samples were taken from each site and then mixed together and homogenized in order to produce a single composite sample for each site [10] with each site's composite sediment sample weighing a total of 8 kg. The samples were stored in previously labeled plastic bags and kept refrigerated at 4 °C for approximately 3 months until their analysis.

#### 2.2. Sediment sample preparation

Despite the presence of organic matter and an excess of debris in this swamp sediment, the technique presented herein does not rely on the use of any chemical agents and is therefore faster than other protocols presented in the literature on microplastics.

The procedure was based on an adapted version of the method used in grain size analysis of clastic sediments. The size-frequency distribution of

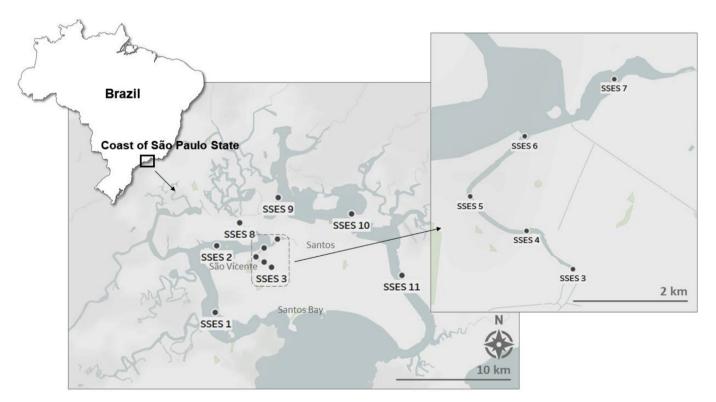


Fig. 1. A map of the sediment sampling collection sites within the Santos and São Vicente Estuarine System.

microplastics was analyzed with the use of a series of stacked sieve meshes with defined opening sizes, so that the successive sieves break up the sample into decreasing size fractions (0.5 phi). First, each previously stored sediment sample had to be homogenized, followed by drying at 100 g in an oven at 50 °C for 12 h to 48 h [11], depending on the moisture content of the sediment being analyzed. Next, three 20 g pseudoreplicates were separated for wet sieve analysis using potable water at sieve meshes of 2.0, 1.0, 0.5 and 0.25 mm. After the sieving process, the wet samples removed from each sieve were transferred to previously weighed Petri dishes. Next, the samples were dried in an oven at 50 °C for 12 h; once dry, they were weighed on an analytical balance to determine the mass of the sample obtained from each sieve.

While the sieving and drying processes were taking place, two blank samples were prepared on small Petri dishes; one with a membrane filter (0.45  $\mu m$ ), while the other was prepared using potable water in the sieve analysis. Both blank samples were dried under the same conditions used on the samples. The blank samples served as a control sample and aided in the identification of sample contamination by synthetic fibers and other microplastics present in the laboratory that could generate false positives [12].

#### 2.3. Microplastic quantification

The Petri dishes containing the dry material removed from each sieve were examined under a stereomicroscope at 0.7 to  $1.5\times$  magnification in order to count the number of plastic threads and fragments present in the sediment samples obtained from the sieves with sieve meshes of 2.0, 1.0, 0.5 and 0.25 mm. As part of the quantification method, the total average count of the three replicates of each sample was calculated and is presented as plastic particles per gram of sediment (particles  $g^{-1}$ ). The blank samples were read before the field samples. Plastic fragments and fibers identified in both types of samples (blanks and field samples) were not included in the microplastic counts. The procedure allows for the correct quantification of plastic particles from the sediment environment itself, without the addition of any plastic particles resulting from laboratory contamination.

The patterns used to identify types of microplastics were based on the descriptions provided by Nor and Obbard [13] and NOAA [14], as well as on visual counts obtained stereomicroscopically. Stainless steel tweezers were used to aid in the active search for microplastics and to separate them from other sediment fragments in order to make them more visible. The plastic fragments were categorized as colorful, opaque, transparent, irregular in shape, rigid, and flexible. Colored threads, transparent threads with uniform thickness, flexible threads, and those bunched together with other waste were also identified. Under the microscope, the fragments were manipulated or dragged around with the aid of tweezers in order to confirm the makeup of the plastic particles. If the materials crumbled or were easily crushed, they were not considered plastic compounds. If the particles kept their shape, they were separated out and their plastic content was confirmed [14]. An important characteristic of plastic is its static electricity [15], meaning that plastic is attracted to the tips of tweezers; this procedure was applied to aid in plastic fragment and thread quantification.

After the microplastics in the sediment samples were counted, the fragments and threads present on the 2.0, 1.0 and 0.5 mm sieves representing each sample site were removed using the tweezers and separated for subsequent polymer identification analysis by pyrolysis gas chromatography mass spectrometry (Py-GC/MS). It is important to note that the particles retained on the 0.25 mm sieve could not be counted or extracted due to their small size and invisibility without magnification.

# 2.4. Polymer identification

The microplastic samples (MPs) extracted from some of the sediment samples (SSES2 MP1; SSES2 MP5; SSES4 MP3) were analyzed to identify the types of polymers present. Py-GC/MS analyses were performed, as

were analyses of polyethylene, polypropylene, polystyrene, poly vinyl chloride, polyamide, and ethylene-propylene-diene rubber (EPDM) patterns.

In an effort to obtain the most representative sample, the three to five most frequently observed microplastics that also exhibited characteristics that differed most differently from one another were extracted from each sieve. From each of the microplastic types extracted, a sample less than 1 mm in size was removed using a retractable utility knife and was then transferred to the sampler of the pyrolyzer with a stainless steel sample cup in the Py-GC/MS system. In the GC system, the temperature of the column began at 40  $^{\circ}\text{C}$  for 2 min and reached 320  $^{\circ}\text{C}$  with a heating rate of 20  $^{\circ}\text{C min}^{\text{-}1}$  for 13 min [16]. The mass-to-charge ratio in the MS system was from 45-300 m/z, and the split applied was 45 with an electron impact of 70 eV. The gases resulting from sample firing were transferred by helium carrier gas through the Ultra ALLOY-5 (nonpolar) column, the dimensions of which were 30 m in length, 0.25 µm in thickness, and 0.25 mm in diameter. A Frontier Labs pyrolyzer (model EGA/PY-3030D) was used with a micro furnace in single shot mode, connected to a Shimadzu GC/MS system (model QP5000). The gases resulting from the firing were separated out and quantified using the GC/MS system.

Before each analysis, one blank sample was analyzed under the same conditions in order to determine any interference in sample retention time, and the chromatograms obtained were compared to those provided by Tsuge et al. [16].

# 3. Results and discussion

The findings on the microplastic abundance in our estuarine sediment samples are presented in Fig. 2A, which shows the total amount of plastic particles found at each sample site, as well as the microplastics count on each sieve. These results provide information on the distribution of microplastics across different mesh sizes and show that the amount of smaller plastic particles (0.5 and 0.25 mm) is inversely proportional to sediment grain size; in other words, the smaller the mesh size, the larger the quantity of microplastics. The 0.25 mm mesh size exhibited a visibly larger quantity of particles per gram of sediment at all of the sample sites. The results show that abundance also differed between sites, ranging from fewer than 1,000 plastic particles  $g^{-1}$  to more than 30,000 particles  $g^{-1}$ 

In the study by Nor and Obbard [13], most microplastics in their intertidal estuarine sediment samples were found to be between 1.0 and 0.5 mm in size, followed by those between 0.5 and 0.1 mm in size; the authors also reported a tendency toward larger microplastic quantities in smaller mesh sizes (from 1.0 to 0.5 mm and  $<\!0.07$  mm). The authors used density separation and found that it provided results similar to those of our study.

A main cause for the prevalence of smaller microplastics in the SSES is the fragmentation of larger plastics presented in the estuarine environment [9]. Large amounts of plastic litter are discharged in the estuary and rapidly start to degrade into smaller fragments, as a result of physical variables (salinity, light, temperature, humidity) and microbiological degradation [1,4]. Thus, a large plastic fragment can produce hundreds or thousands of microplastics in the SSES. Moreover, sediments with the highest proportion of finer fraction grains ( $<250~\mu m$ ) tend to be more cohesive and flocculate regularly. It is expected that microplastics may be retained in sediment during flocculation of the particles [13] which are likely to affect the suspension and deposition behavior of microplastics [17].

In parallel to the technique presented herein, we suggest the use of sediment grain size distribution analysis, as well as analyses of the physical and chemical parameters of the sediment in order to complement the characterization of the sediment samples and to determine any potential sources of pollutants that may be contributing to pollution by microplastics. The use of grain size distribution analyses allows for the average grain size to be determined, which thus permits an estimation of the average plastic particle size likely to be present at the highest

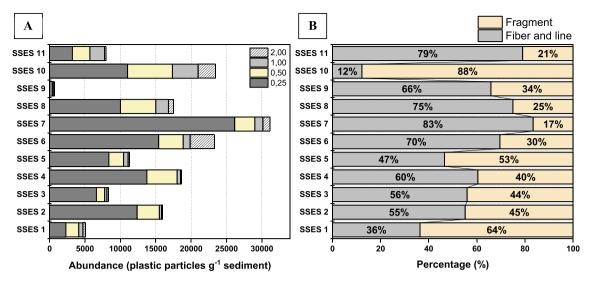


Fig. 2. A) Total microplastic abundance and abundance separated by sediment fraction for each sample site. B) Percentages of fragments and of threads/fibers quantified in each sample.

proportions in a given sediment fraction, as well as information on distribution. Given the fact that microplastics tend to accumulate in the smaller mesh sizes (0.5 and 0.25 mm), the risk of benthic and epibenthic organisms' exposure to these pollutants can be associated with quantities of microplastics.

Fig. 2B presents the relative percentages of each type of microplastic and distinguishes between fragments and threads/fibers. Overall, the proportions of the different types of microplastics were similar, with a greater presence of threads and fibers than fragments in most of the samples. Some authors [13,18,19] have also found plastic fibers to be the most common type of microplastic present in sediment samples.

The presence of plastic fibers probably is influenced by the proximity of some sources, such as raw sewage, garbage dumping sites and slums [8], and in special the release of lost fishing gear in the SSES. The fibers are preferentially removed from suspension as they are trapped between settling sand grains. Due to the elongated size of the fibers and the very large ratio of surface area to volume, it is more likely that they are impacted and dragged downward by settling sand grains which explains the enrichment of fibers in sediment samples. While the deposition of fragments is more strongly controlled by theirs low density, making fragments more likely to stay in suspension and therefore less prone to deposition [17].

The stacked sieve meshes analysis step is not always used in studies seeking to identify and quantify microplastics in sediments. Some studies [20] employed either one sieve or sieve cascades of two and three sieves (ranging from 4.75 to 0.038 mm) to separate the microplastics by size. The use of four different sieve meshes results in less sample material to be analyzed stereomicroscopically and prevents the overlap or accumulation of plastic particles with organic and mineral debris. The number of sieves, however, may vary depending on the study objectives, and may reach a sieve meshes of 0.063 mm when the polymer identification equipment available is able to assess this size.

In this study, plastic particles smaller than 0.25 mm in size were not extracted for subsequent Py-GC/MS analysis due to their invisibility to the naked eye and the uncertainty of their visual identification under the stereomicroscope, since all of the fragments were identical in shape.

In terms of qualitative analyses using Py-GC/MS, three polymers making up the microplastics from the samples analyzed have thus far been identified: eight microplastic samples were identified as polyethylene, two microplastic samples were identified as polystyrene, and one was identified as EPDM. The respective chromatograms are shown in the Supplementary Material. Other authors have also reported polyethylene and polystyrene in their microplastic samples [19], even in

intertidal estuarine sediments [13] and subtidal marine sediments [17].

The importance of identifying microplastic polymer type lies in the ability to correlate to larger plastic waste, which may also be present at the same site, with the microplastics in the sediment samples, thus providing evidence of fragmentation and rendering it easier to identify the likely source of the waste. Py-GC/MS is one of the most commonly used methods in polymer identification. Though the technique is destructive in nature, pyrolysis eliminates the need for pre-treatment of the sample through its lack of chemical reagents. The advantage of Py-GC/MS lies in its use of chromatography, which is a technique that separates out each compound present in the sample. It is advised that pyrolysis be combined with other analytical techniques such as Fourier-transform infrared (FTIR) spectroscopy in order to validate the results.

In the method used herein, the study samples were first analyzed using density separation based on an NaCl solution to extract the microplastics, followed by vacuum filtration, sieve analysis, and stereo microscope examination [14]. However, many plastic particles were lost in the separation phase, which resulted in an underestimation of microplastic quantity. Some microplastics that are denser than salt water (>1.02 g cm<sup>-3</sup>), sink and accumulate in the sediment, while others, with lower densities, float to the surface of the water or into the water column. Different saline solutions, such as zinc chloride (ZnCl<sub>2</sub>, 1.5 - 1.7 g cm<sup>-3</sup>) and sodium iodine (NaI, 1.6 - 1.8 g cm<sup>-3</sup>) are also used as a way to more effectively extract high-density microplastics (those with densities between 1.32 and 1.56 g cm<sup>-3</sup>) from sediment samples [21].

In the current study, the method used produced results similar to those of other studies focused on quantifying and identifying microplastics. In addition to its efficacy, this method may be used in laboratories that lack sophisticated infrastructure or extensive options in terms of analytical equipment for polymer identification; this method may also be applied to marine sediment and freshwater sediment. It is important to note that more advanced techniques in polymer identification and/or quantification may be required to detect nanoplastics (from 1 nm to 1 µm) [2].

As this case study of an estuarine system on the São Paulo coast shows, the establishment of a simple microplastic analysis method that can be applied to estuarine sediment samples is of substantial importance. This region is economically and ecologically important but also highly impacted by human activity. The method proposed herein does not generate chemical effluents and may be applied in any laboratory with the basic tools necessary to perform research on sediment. Furthermore, this method may be incorporated into a larger microplastic analysis project, such as the Coastal Water Monitoring Program, run for

decades by the São Paulo State Sanitation Technology Company – CETESB.

#### 4. Conclusion

The method presented in this study for quantifying, separating, and identifying microplastics produced a variety of results based on a single sediment matrix: total microplastic abundance at each study site, microplastic distribution across different sediment fractions, and the proportions of different types of microplastics.

The method was found to be effective in this case study. Importantly, it does not require the use of chemical substances and it substantially reduces the amount of sample preparation time required relative to traditional analyses by at least one month. This method is, therefore, cost-cutting and sustainable than traditional methods. It is an ideal option for government-run environmental organizations and research institutes that require practical tools and routine analyses. It may also be used in environmental monitoring and assessment programs.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements.

The authors would like to thank the NGO known as the *Instituto Ecofaxina* for their help with sediment sample collection. GTG thanks the Coordination of Superior Level Staff Improvement (CAPES, grant number 88882.333464/2019–01). DMSA thanks the Brazilian National Research Council (CNPQ, grant number 308533/2018-6).

# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cscee.2020.100020.

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