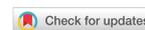


Research Article

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Occurrence of microplastics derived from tyres in bottom sediments of Guanabara Bay, Brazil: a form of pollution that is neglected or difficult to detect?

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Abstract

This study aimed to detect and characterize tyre wear particles (TWPs) in sediment samples from Guanabara Bay, an area where microplastics (MPs) have been extensively studied, but TWPs have not been specifically reported. Nine sediment samples were collected from different locations across the bay and processed using peroxide degradation to remove organic matter, followed by floatation and filtration to isolate MPs. TWPs were identified using binocular stereo microscopy, scanning electron microscopy (SEM), and Fourier transformed infrared spectroscopy. The results revealed significant quantities of TWPs in the bay sediments, although exact quantification was hindered by the particles' size variability and suboptimal sedimentation systems. These findings underscore the need for improved methodologies to accurately quantify TWPs in coastal environments, potentially through the development of specific TWP markers. Future research directions are also discussed.

Keywords: Marine contamination, chemical pollutants, microplastic analytical methods, urban runoff, coastal waters



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INTRODUCTION

The presence of microplastic (MP) particles has become one of the most serious forms of pollution in the aquatic environment worldwide^[1,2,3,4]; the slow degradability of these particles leads to their accumulation in the environment^[5,6]. Within this category, tyreMPs (tyre wear particles, TWPs) and, to a lesser extent, other related MP sources like recycled tyre crumb (RTC) and tyre repair-polished debris (TRD)^[7], have been indicated as one of the major contributors to MP pollution^[8,9]; there is, however, little information on the latter two particle types^[7]. Tyres consist of a mix of natural and synthetic rubbers, carbon black, steel cord, fibres, and other organic and inorganic components, which are used to improve their stability^[10]. Any of these could be present in TWPs, although not all qualify as plastics. In the EU alone, an estimated 500,000 tonnes of TWPs reach the marine environment every year and this is expected to increase with the introduction of electrically-powered and hybrid vehicles^[11], which are 24% heavier than others, leading to a 20-30% increase in TWP production^[12,13].

The number of tires produced annually reaches the mark of billions of tires produced each year around the world, making their disposal one of the most serious environmental problems today. The global production of tyres is estimated to reach 2.7 billion pieces of tyres by 2022^[14]; indeed, in China alone, this figure had reached 818 million in 2020^[15]. The highest numbers of automobiles are found in the USA, followed by Australia, Italy, Canada, Japan, Germany, the UK, France, Malaysia, and Russia^[5]. The increasing pollution is greater in developing countries, such as China, India, and Brazil^[16]. Due to exponential growth in the production and use of passenger cars, buses, motorcycle trucks and off-the-road vehicles in these countries, the amount of tyres manufactured and discarded has increased considerably. It is estimated that one billion tyres end their useful lives every year. Their improper disposal has become a major environmental problem worldwide. Few are recycled, and a huge volume ends up in landfill, often discarded in an uncontrolled manner in the environment, especially in developing countries. In countries like Brazil, tyre disposal has been a very serious environmental problem. It is estimated that thousands of tons of scrap tyres are abandoned in both marine and continental areas.

Before disposal, tyre particles originate from abrasion between the tyre and the road surface. They contain natural and synthetic rubber, fillers, and plasticizers. During the lifetime of a passenger car tyre, around 30% of its rubber is released into the environment^[17]. TWPs contain a broad range of chemicals, such as polyaromatic hydrocarbons (PAHs), sulfenamides, phthalates, thiazoles, guanidines, thiurams, dithiocarbamates, sulfur donors, phenolics, phenylenediamines and heavy metals, together with short fibers, that are potentially harmful to health and the environment^[18,19]. Many of these chemicals can pose risks to health. When tyre particles reach the oceans, various substances can be leached, including toxic metals (e.g., zinc, lead, cadmium), and organic compounds like hydrocarbons and benzothiazole derivatives^[19]. Thus, this type of particle is an important source of chemicals and metals in the environment^[20]. Particles derived from tyre wear have received more attention in recent years, mainly in relation to their analysis and methods for confirming their identity and quantification, the leaching of various compounds and their toxicity.

MPs have been identified in Guanabara Bay, both in the water column and the bottom sediments^[21-25], as well as in the bay sand beaches environments^[26,27] and in the Bay biota^[28]. This became a great problem, because it increased the inventory of pollutants present in this already so impacted bay. However, tyre fragments have never been considered. Guanabara Bay is located within the second largest metropolitan area of Brazil, with more than 7.500,000,000 automobiles, as well as being crossed by a bridge that connects two main cities in the metropolitan region. About 150,000 vehicles cross this bridge each day. Without a doubt, these various sources will contribute to the presence of TWPs in the bay.

Transport has been identified as one of the most important sources of pollution for the environment^[29-31,18] and urban surface runoff is an important and complex form of diffuse contamination. Road traffic emissions comprise not only tailpipe exhaust gasses but also particles derived from wear and tear of vehicle parts, such as brakes, tyres, discs, etc., added to the vehicle-induced resuspension of dust deposited on the road. High concentrations of hydrocarbons^[32,33], heavy metals^[34-36], and calcium oxalate^[37,38] have been found at the roadside, demonstrating the complexity of this pollution. Surface runoff from roads is characterized by containing hundreds of different chemical compounds, making identifying potential toxicants far from straightforward^[39]. Since TWPs have never been separately considered in previous studies in Guanabara Bay, this article aims to, at least partially, rectify this omission by developing and applying a simple method to identify these particles in the bottom sediments of the bay.

METHODS

Area of study

Guanabara Bay is one of the largest bays on the Brazilian coast. It has an area of approximately 384 km² with a water surface of 328 km², due to its numerous islands; it is a very important coastal environment on the Brazilian littoral. It is one of the most beautiful scenery on the coast of the state of Rio de Janeiro, marked by the presence of Sugar-loaf Mountain and Corcovado, as well as many other natural beauties^[40]. Guanabara Bay is located between the coordinates 23°41'-23°56'S and 43°02'-43°18'W. Due to its physical characteristics, this shallow bay is classified as a partially mixed estuary^[41]. The humid tropical climate is marked by heavy rains in the summer (November to March) and drought in the winter (June to August)^[40,41]. The entrance and central channel of Guanabara Bay are marked by sandy sedimentation and dominated by tidal and wave processes. The inner part of the bay is characterized by a flat bottom and muddy sedimentation, dominated by fluvial processes. These muddy sediments are rich in organic matter and various pollutants. Between these two distinct sedimentation areas, there is a transitional zone, represented as the decrease in the speed of tidal currents; this area is characterized by a mixture of sandy and muddy sediments^[42].

Due to its location within the metropolitan region of Rio de Janeiro, Guanabara Bay is one of the most polluted environments on the Brazilian coast, where a large volume of untreated urban sewage is discharged, in addition to urban, industrial and agricultural effluents, urban surface runoff sediments are added to this. All this flow enters the bay through 45 rivers that drain a hydrographic basin of 4000 km²^[41], in a highly urbanized area and characterized by a significant low-income population^[40,43]. The vast majority of the rivers that drain into the bay are highly polluted. Since the rivers such as Guapimirim, Iguaçu, Caceribu, Estrela, Meriti and Sarapuí, alone account for 85.5% of all water flow into the bay^[41]. The drainage basin hosts 6000 industries, 2 airports, harbors, several shipyards, oil terminals and several untreated sewage

outlets around the coastline. The lack of planning for the development of urban centers around Guanabara Bay has led this environment to the current stage of degradation. Nowadays, this bay can be considered one of the most polluted bays on the Brazilian coast^[40,41,43-49].

Field work and laboratory analyses

In order to describe the quantity and distribution of different types of MP in the bottom sediments of Guanabara Bay, surveys were carried out throughout the bay. Nine sediment samples were taken from different areas inside of the bay, labeled “GB” in [Figure 1](#). They were collected from different depths, 3 to 7 meters on average [[Figure 1](#)], using a stainless steel Van Veen grab (Husky Duck Equipamentos e Serviços LTDA) from a boat. At each sampling station, 1-2 kg of sediment were collected. After collection, the samples were stored in sterilized polythene bags and were then sent to the laboratory. In order to eliminate the organic matter present in the bottom sediments of the bay, hydrogen peroxide was used. In this procedure, 20 mL (30% hydrogen peroxide) were added to the beakers and placed on a hot plate at 60 °C for 30 minutes. Every 10 minutes, the solution is mixed for one minute using a glass rod. If organic matter remains in the sample, the entire procedure is repeated as many times as necessary. The flotation method was used, using a saline solution (250 g NaCl/L distilled H₂O) to separate the microplastic from the sediments previously treated with hydrogen peroxide. 500 grams of sediment was placed in a beaker with 7 liters of saline solution, and then this mixture was continuously stirred for 3 × 2 min using a magnetic stirrer, with a rest period of 1-2 minutes between stirrings, depending on the observed clearance rate of the sediment from suspension^[50]. Using a 10 mL pipette, the supernatant was transferred onto 0.8-1 m pore-size, 47 mm gridded nitrocellulose filters (Millipore) using a vacuum system. After all the supernatant was collected, an additional 100 ml of saline was added to each sample, and the procedure was repeated to extract any remaining MPs. After saline extraction, the filters are placed in sterilized glass Petri dishes and dried in ovens at room temperature (21 °C). Once dry, the filters are taken to be examined under a microscope. For this work, a binocular stereo microscope (Zeiss STEMI 2,000 C) was used to count the PM, and categorize it by type and color and photographs were taken. SEM images of gold-coated plastic surfaces were obtained using a JEOL scanning electron microscope fitted with an energy dispersive X-ray fluorescent (EDXRF) spectrometer. For the chemical characterization, the samples were analyzed by attenuated total reflectance Fourier-transformed infrared spectroscopy (ATR-FTIR, Thermo Nicolet 6,700, Thermo Fisher Scientific). Identification based on infrared (IR) spectroscopy and known spectra was used in selected samples. The types of spectroscopy used for MP identification were IR, FTIR, and near-infrared. The chemical composition of the polymer was identified by comparing the entire range of the unprocessed spectra with published references. Spectra were identified with Open Specy, version 0.9.5^[51].

RESULTS AND DISCUSSION

The bottom samples collected at different sites within Guanabara Bay were very muddy and rich in organic matter. The processing for analysis of TWPs became very difficult, especially when hydrogen peroxide was not used. Even after the peroxide processing, black particles like those from tyres were difficult to identify [[Figure 2](#)]. We were only able to confidently identify them by the use of a binocular stereo-microscope confirmed by SEM [[Figure 3](#)]. The SEM images were compared to the works by^[52,53] because the authors used the same technique, and showed a similar pattern observed in this study. As soon as this method was developed, the TWP number became so large that it was impossible to determine accurately; for this reason, the numerical results are not shown. The fact that SEM is necessary to positively identify TWPs without chemical analysis perhaps explains why other MP investigations carried out in the bay did not identify the presence of tyre fragments^[21-27]. Two of the few studies worldwide to tentatively identify TWP pollution in the ocean are those of^[54] and^[55]. The first one^[54] hypothesized that synthetic rubber-carbon filled particles in the sediments of a stormwater floating treatment wetland were derived from tyres. They comprised 15-38% of all MPs. The second one used benzothiazole as a marker for TWPs in an attempt to determine the major pathways of TWPs to the ocean^[55].

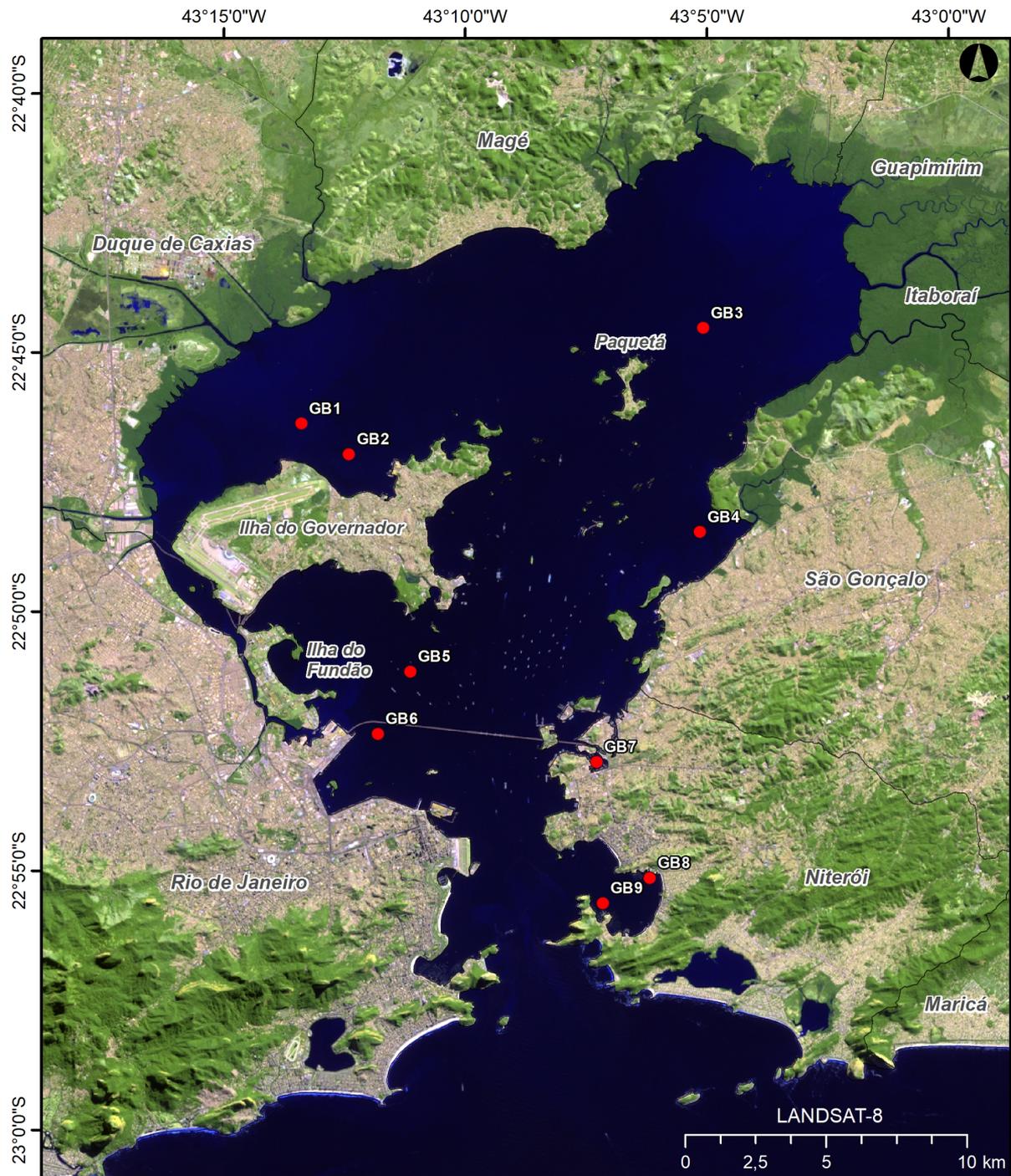


Figure 1. Sampling sites in Guanabara Bay, metropolitan area of Rio de Janeiro, Brazil.

The presence of TWPs in Guanabara Bay has not previously been proven, but recently a study carried out on the Niterói coastline^[56] observed high concentrations of abandoned tyres in an area close to Niterói Harbor. This “sea of tyres” [Figure 4] was so called because of the difficulty of counting them. The discarded

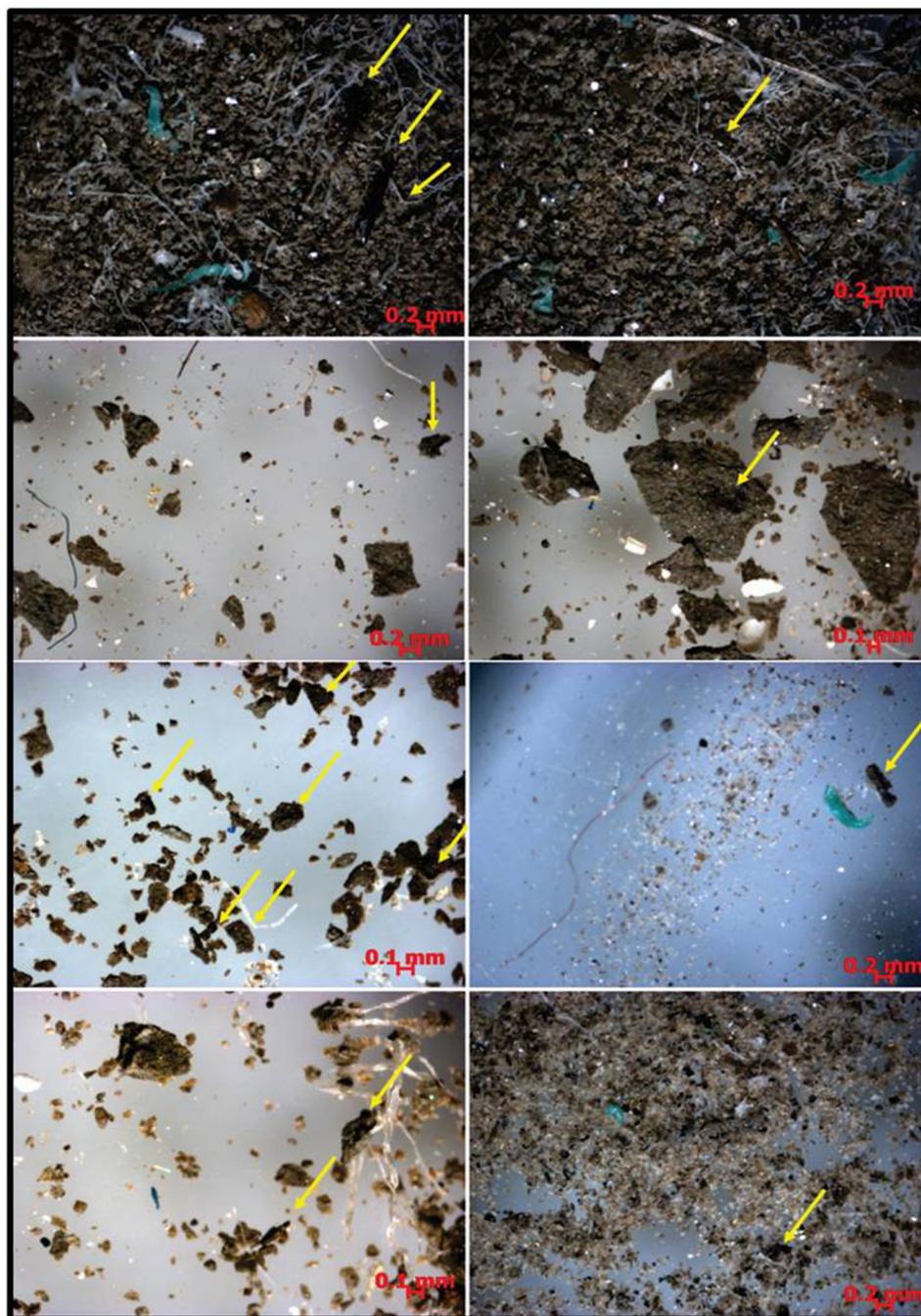


Figure 2. Binocular microscope images of MPs and tyre fragments indicated by the yellow arrows in the bottom sediment of Guanabara Bay.

tyres are used for mooring and as a protection for boats. This tendency in harbor areas has been extant for many years, with boat owners recognizing the worth of these unwanted and otherwise unusable tyres. Whether these discarded tyres may also, under some in situ conditions, produce MPs similar to TWPs is not known. It doubtless depends on the surrounding environment and its degrading potential. However, some of the particles we detected were rather large [Figure 4], and it seems possible that these are products of discarded tyres, degraded by other factors rather than direct road contact. In addition to low cost, old

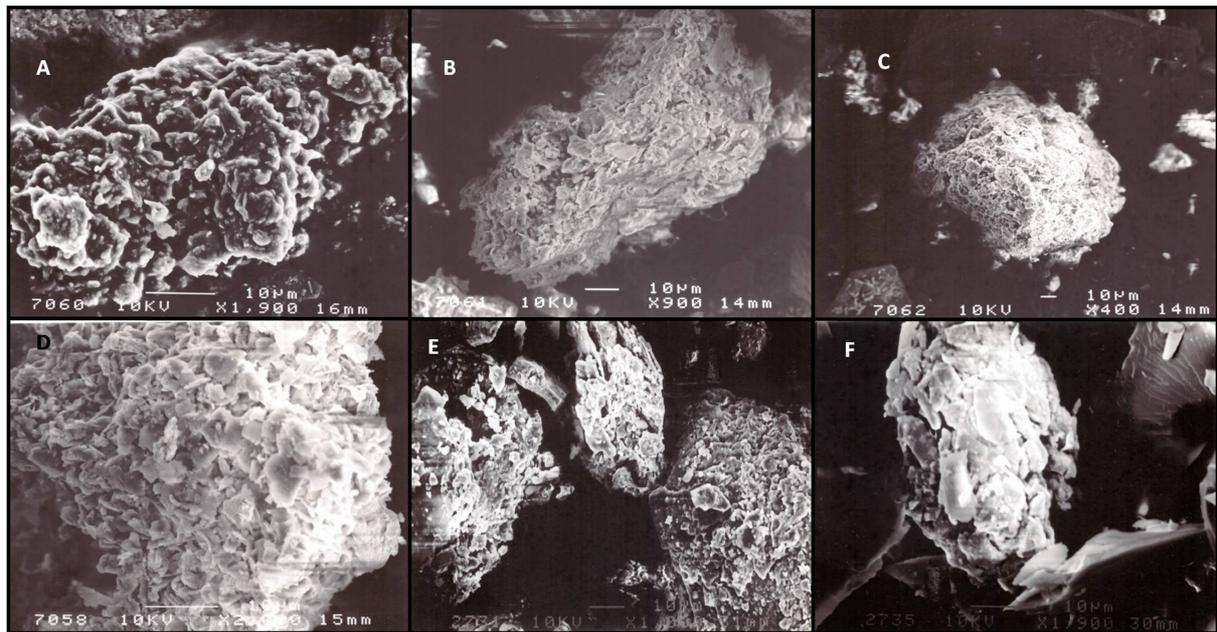


Figure 3. SEM image of tyre fragments from Guanabara Bay

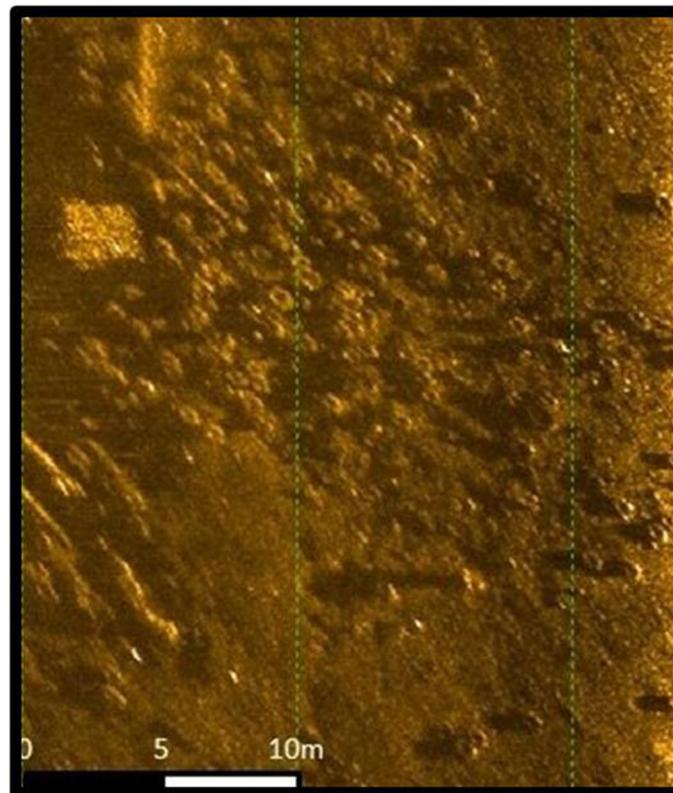


Figure 4. SideScan image of the “Sea of Tyres” near Niterói Harbor, Guanabara Bay [Modified from Oliveira *et al.* (2021)^[56]].

tyres are easy to obtain and there is little caution regarding their use in Brazil^[56], although a number of European countries have been attempting to introduce legislation about their disposal^[57].

Urban runoff in Guanabara Bay is largely responsible for the contribution of tyre particles to its water; it is surrounded by busy highways and is crossed by a bridge that unites the Cities of Niterói and Rio de Janeiro, with vehicle movement reaching 150,000 per day. Research using benzothiazole, detected by pyrolysis GC-MS^[48], as a marker for TWP, determined that surface runoff drainage was the major contributor of these MPs to the marine environment from conurbations off the southern coast of England. Runoff was also determined to be the major contributor to TWP in a rural highway environment in Sweden^[58]. They analyzed sieved fractions by SEM-EDX and single particle analysis, using a machine learning algorithm to classify them.

Tire wear is a major source of micro- and nanoplastics, which are often difficult to detect^[59]. In the case of tyres, in addition to the composition of the rubber and the braking process, the production of PM particles is influenced by the structure of the tire, tire age, climate, type of road surface, vehicle speed, vehicle weight and distance traveled, and style^[60].

The great problem in identifying TWP is the fact that they have the same color as organic sediments and heavy minerals. This makes it difficult to separate them from the rest of the materials present in the sediments. The difficulty of identifying tyre particles due to the lack of appropriate reference standards has been highlighted^[31]. Tyre particles are difficult to detect using conventional (micro)plastics methods such as FTIR; one problem is that they contain carbon black, which absorbs in the infrared region. In our study, we were, however, able to identify high density polyethylene as a component of some of the tyre particles [Figure 5].

It is likely that the polyethylene component of the TWP is derived from the carcass, or casing, of the tyre. This is a textile-based material of 2-3 layers that keeps the tyre in shape under pressure and helps transfer the workload. Among the plastic fibers that are used to make the carcass is polyethylene^[61-63].

A review of the ecotoxicological effects of tyre wear particles in the environment discussed the toxicity of tyre leachate, determined using various leaching procedures and test organisms^[19]. More recently, the growth of marine phytoplankton was shown to be affected by these leachates^[64]. Exposure of clams and ragworms to artificially produced TWP (0.2% - 10% in estuarine sediment) indicated that both animals were adversely affected, showing reduced protein content after 3 days at 10% and various negative effects on feeding and burrowing^[65]. In fact, most studies are still simulations, with almost no investigations of “real-life” situations. The former often use conditions removed from those actually found in the natural environments. The problems associated with studies on the toxic effects of TWP are discussed by^[66]. Several studies have pointed out that zinc and organic compounds are among the most important tyre constituents that can leach out in the environment^[67]. Zinc, as ZnO, is used as an activator for the rubber vulcanization process for the manufacture of vehicle tyres, along with various sulfur-containing organic complexes (e.g., dithiocarbamates and thiazoles) as vulcanization accelerators^[68]. The finished product typically contains up to about 1%-2% of the total weight of tyres, mainly as excess ZnO but also as ZnS (20), and the leaching of this element into water represents an important environmental concern. Considering the large concentrations of zinc found in the bottom sediments of Guanabara Bay by several investigators^[43,49], tyre fragments clearly play a large role in the inventory of zinc concentrations in the bay. Other chemical compounds present in automobile tires include polyaromatic hydrocarbons (PAHs), phthalates, sulfenamides, thiurams, thiazoles, dithiocarbamates, sulfur donors, phenolics, phenylenediamines and heavy metals^[18,69-72]. These chemicals not only pose a threat to the environment, but also have a major impact on the biota and are a risk to human health.

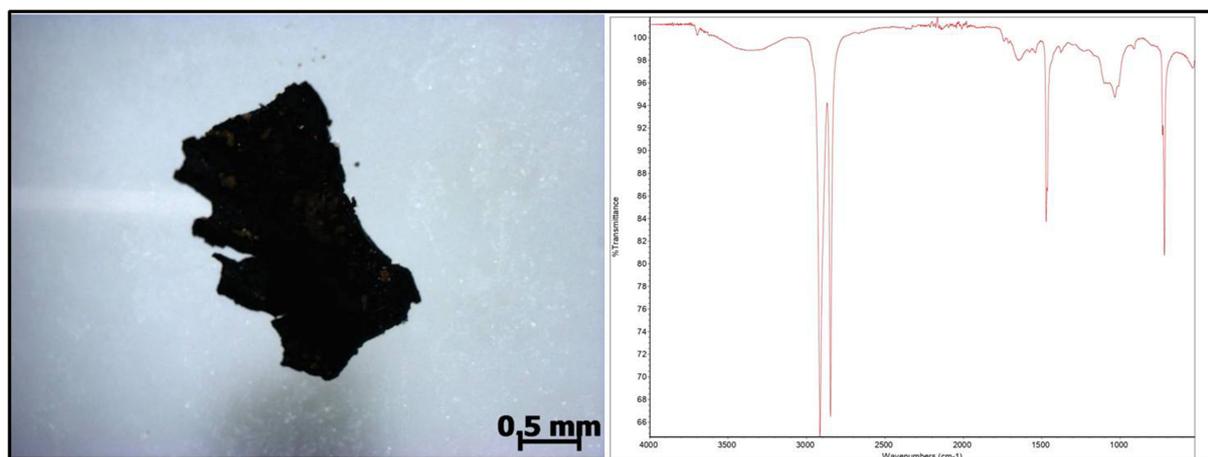


Figure 5. A tyre wear fragment and its FTIR spectrum, identifying it as HDPE

Not only chemicals, but also the specific biofilm associated with TWPs, are identified as a health hazard. It is of interest to note that the TWP biofilm is different from those on other substrates. The biofilm on TWPs has been found to be less diverse than those on HDPE and wood particles, with a relatively high proportion of putative hydrocarbon degraders^[73]. Differences in biofilm thickness have also been found; in a freshwater laboratory system inoculated from a wastewater treatment plant, TWPs encouraged the growth of a thick biofilm containing rapidly growing and potentially pathogenic bacteria, different from PET particles, which did not encourage bacterial growth^[74].

One of the major problems associated with tyre particles is extraneous forms of pollution. Accumulation of organic materials and chemical compounds on the surface of TWPs occurs in various parts of the environment. This not only represents a risk to the environment, but also makes FTIR analysis difficult. Various extraneous chemical compounds have been associated with tyre degradation. Some authors^[75,76] observed the presence of bisphenol A leaching from tyres in the sea, indicating that TWPs can be important sources of this endocrine disruptor for the marine biota. Research carried out in Guanabara Bay, studying the same samples used in the current study^[77], found important concentrations of BPA in all sediment samples. Bisphenol A may have been adsorbed to the particle surface from external sources, although this compound is used, in small amounts, as a stabilizer of polymerization in tyre manufacture. Release of the latter from tyres is considered low environmental risk^[78].

Although there is considerable understanding of the adverse effects of chemicals leaching from MPs in general, and of the composition of tyres (and, therefore, of presumptive TWPs), there are, as yet, no well-defined methods for analyzing and quantifying these particles^[79]. The use of the sedimentation technique used by us and many others has been criticized^[80]; the authors found that only 63% of tyre and road wear particles were retained by this sedimentation method. There are two ISO technical specifications, ISO/TS 22687:2018 - Rubber, and ISO/TS 20593:2017 - Pyrolysis-GC-MS method, but these make assumptions on the standard composition of tyre rubber that are not valid^[81]. The same group as^[80] devised a method based on LC-MS/MS and pyrolysis GC-MS to evaluate the release of both TWPs and their additives in an Australian tributary^[82]. However, these methods presume that tyres contain homogeneous natural and synthetic rubbers^[83]. This can underestimate the TWP concentrations by a factor of 5 or even more^[81]. Research carried out recently^[84] suggested that Raman Tweezers (optical tweezers plus Raman spectroscopy) could be used as a reliable quantification method. However, the recently developed methods do not necessarily all produce the same results^[58]. The latter authors used automated single-particle SEM/EDX

analysis coupled to a machine-learning classifier in a mostly successful attempt to differentiate TWP, bitumen wear particles, road marking particles (paint and glass beads), metal particles, and mineral particles in environmental samples. However, the most common analytical method at the moment is the detection of a specific chemical, such as the benzothiazole mentioned previously^[55]. The same authors discuss the various markers and techniques that have been used to detect and enumerate these particles in the air, concluding that no current method meets the requirements sufficiently well^[55]. The development of improved methods is of immense importance for the determination of the real levels and environmental effects of TWPs.

CONCLUSIONS

There have been several reports of MPs in Guanabara Bay, but tyre wear particles (TWPs) have never been included as a separate class. The location of this bay, with the surrounding intense urbanization and road systems, indicates that these particles will be present, but their detection and identification are difficult because of the characteristics of the bottom sediments of the bay, which are very fine and organic, and of the dense nature of the particles themselves. Indeed, TWPs have been neglected in the literature because they are difficult to detect. The most commonly used techniques for MP detection do not transfer well to TWPs. Using a binocular stereo-microscope and confirming with SEM and FTIR, we were able to detect TWPs of various sizes in large quantities in the bottom sediment of Guanabara Bay. Despite Guanabara Bay receiving a great impact from urban surface runoff and being crossed by a bridge connecting two important cities, and there are already several articles published about microplastics in the bay, this article presents for the first time the occurrence and distribution of TWPs in the bay. Characterizing the ecological impact of TWPs requires further analyses and a standardized and improved methodology. This article presents preliminary methods and results in a continuing study to determine the impact of these materials on estuarine and coastal ecosystems. The most important future development in this area will be the development of a reliable and generally accepted method for the identification of TWPs. It seems likely that this will be based on the detection of a specific chemical compound, possibly based on polybutyldiene, although even this is found in some non-tyre-derived MPs. The current lack of a reliable analytical method for the detection of TWPs is considerably retarding the acquisition of knowledge of these particles.

DECLARATIONS

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Authors' contributions

Conceptualization: Baptista Neto JA, Gaylarde CC, da Fonseca EM

Methodology: Baptista Neto JA, Gaylarde CC, de Carvalho DG, da Fonseca EM

Formal analysis: Baptista Neto JA, Gaylarde CC, de Carvalho DG, Lourenço MFP

Investigation: Baptista Neto JA, Gaylarde CC, de Carvalho DG, da Fonseca EM

Writing-original draft preparation: Baptista Neto JA, Gaylarde CC, de Carvalho DG, da Fonseca EM

All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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