Programa de Pós-Graduação em Ciências Biológicas
UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO

ATA DE DEFESA DE DISSERTAÇÃO DO CURSO DE MESTRADO EM BIOLOGIA ANIMAL DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS DO CENTRO DE CIÊNCIAS HUMANAS E NATURAIS DA UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO - ATA Nº 218 – 25/08/2022

No dia vinte e cinco do mês de agosto de dois mil e vinte e dois, em sessão pública, na sala 104 do prédio Bárbara Weinberg do CCHN/UFES, através de webconferência, conforme Portaria Normativa nº 08 da Pró-Reitoria de Pesquisa e Pós-Graduação/UFES de 01 de julho de 2021, procedeu-se a avaliação da dissertação do aluno **João Marcos Fausto Schuab Menario**. Às catorze horas, a Profa. Dra. Mercia Barcellos da Costa (UFES), Orientadora e Presidente da Comissão Examinadora de Defesa de Dissertação, deu início aos trabalhos, convidando a compor a banca a Profa. Dra. Mariana Beatriz Paz Otegui (Universidade de Buenos Aires), examinadora externa e a Profa. Dra. Teofania Heloisa Dutra Amorim Vidigal (UFMG), examinadora externa. A seguir, a presidente solicitou ao mestrando que fizesse uma explanação de seu trabalho intitulado **"Abundance of microplastic in different coastal areas using *Phragmatopoma caudata* (Kroyer in Morch, 1863) (Polychaeta: Sabellariidae) as an indicator".** Finda a apresentação, a presidente passou a palavra às examinadoras, que procederam à arguição do candidato. Ao final, a Comissão em sessão reservada deliberou pela **APROVAÇÃO** da referida dissertação nos termos do Regimento Interno do Programa de Pós-Graduação em Ciências Biológicas e alertou que o aprovado somente terá direito ao título de Mestre após entrega da versão final de sua dissertação, em papel e meio digital, à Secretaria do Programa. Encerrada a sessão, eu, Profa. Dra. Mercia Barcellos da Costa, presidente da Comissão Examinadora, lavrei a presente ata que vai com as devidas assinaturas (De acordo com a Portaria citada acima, membros de banca externos à UFES que não atuam como docentes permanentes ou colaboradores nos Programas de Pós-Graduação da UFES estão dispensados da obrigatoriedade de assinatura digital da ata. Caso o membro externo não assine a ata e, sendo o Coordenador o responsável final pela realização da banca, a assinatura do Coordenador via Lepisma assegura a legitimidade necessária do documento).

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Abundance of microplastic in different coastal areas using *Phragmatopoma caudata* (Kroyer in Morch, 1863) (Polychaeta: Sabellariidae) as an indicator

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Orientadora: Mércia Barcellos da Costa

Dissertação submetida ao Programa de Pós Graduação em Ciências Biológicas (Biologia Animal) (PPGBAN) da Universidade Federal do Espírito Santo como requisito parcial para a obtenção do grau de Mestre em Biologia Animal.

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Aos verdadeiros e verdadeiras cientistas que fazem ciência à duras penas nesse país, todos nós merecemos mais do que temos e fazemos mais do que podemos porque amamos a pesquisa genuína.

AGRADECIMENTOS

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A todas e todos que, de alguma forma, me ajudaram a estar aqui hoje. Obrigado.

SUMMARY

TABLE LIST

FIGURE LIST

Abundance of microplastics in different coastal areas using *Phragmatopoma caudata* (Kroyer in Morch, 1863) as an indicator

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Abstract

Plastic consists of a synthetic or semisynthetic polymer and its molecular structure is built of long repeating chemical units composed of hydrocarbons. Plastic materials degrade very slowly and plastic pollution has been considered an emerging problem in modern society. Microplastic (MP) particles are highlighted among the countless types of plastic debris disposed of in the ocean, once they can trap chemical pollutants and be mistaken as food by marine organisms, mainly filter-feeders and suspension-feeders. The polychaete *Phragmatopoma caudata* (Kroyer in Morch, 1863) is a reef-building Sabellariidae widely found on the brazilian coastline and has the ability to trap MP particles in their colonies structures. In order to evaluate MP pollution in different coastal areas, we collected 12 samples of water and 36 samples of *P. caudata*'s colonies in 12 sampling spots on the Espírito Santo (Brazil) coast divided into three regions (North, Central, and South). These samples were, processed, washed, and sieved, and the resulting product stored at a petri dish for further analysis. For sorting the MPs, a stereomicroscopic with an attached camera were used and all MPs were counted regarding their type (filament, fragment, and 'other') and color. Statistical analyses were performed using the GrahPad Prism software considering significant results $p < 0.05$. All 12 sampling spots were contaminated with MP, thus the investigated beaches had a MP pollution rate of 100%. The Central region showed the highest numbers of MP, followed by the North and the South regions respectively. This pattern of MP pollution in the Central region could be due to the high anthropic occupation, once it is where the capital and its metropolitan region are located, presenting more traffic of people. The Central region also holds two busy port complexes with intense ship traffic. The North region takes second place due to a specific sampling spot in the mouth of a big hydrographic basin, the Piraquê-Açú Basin. The South region holds the second more urbanized region of the Espírito Santo State, however, showed small numbers of MP, probably because two of the four sampling spots are slightly away from city impacts. Thus, microplastic pollution is indeed ubiquitous and widely found in coastal areas. Therefore, *P. caudata* colonies are a trustable indicator of MP presence in intertidal zones once they can trap plastic particles inside the colony structure. However, it is essential to investigate the mechanisms of MP trapping and how long they take to be absorbed from the water column into the colony, and how long they can remain trapped.

Keywords: Bio indicator; Intertidal zones; Microplastic; Plastic pollution; Polychaeta.

1. INTRODUCTION

Plastic materials are relatively new, considering they were first produced in the early 20th century (Napper & Thompson, 2020). The first plastic-like material was ‘Parkesine’, a cellulose-based material that was made to substitute ivory in 1862. However, the first synthetic plastic was created in 1907, a material called ‘Berkelite’, and marked the beginning of the modern plastic era (Plastic Industry Association, 2022). After that, plastic began to emerge as a substitute for several materials due to its exceptional usage potential in versatility, strength, lightness, and cost and began being used in several sectors such as industry, food market, construction, and medicine (Cole *et al*, 2011). Nonetheless, it was after World War II that its production became massive, reaching 5 million tons of plastic in 1950 (Andrady & Neal, 2009).

Plastic consists of a synthetic or semisynthetic polymer and its molecular structure is built of long repeating chemical units composed of hydrocarbons derived from fossil fuel (American Chemical Society, 2015). Inside contemporary society, plastic became a fundamental part of everyday life, around 300 million metric tons of plastic materials were produced per year in 2015 (UNEP, 2015) up to 367 in 2020 (Tiseo, 2020) showing an increase in plastic consumption and therefore its disposal. A massive amount of this plastic is disposed into the marine environment every day. An estimate of 4.8 - 12.7 million metric tons of plastic was thrown away into the seas in 2010 (Jambeck *et al,* 2015), and around 5 trillion plastic pieces are floating in the oceans (Ericksen *et al,* 2014). Plastic disposal is one of the greatest threats to land and marine environments, in this scenario plastic pollution has been considered an emerging problem in modern society (UNEP, 2014; Wang et al., 2016). Plastic materials degrade very slowly due to their resistance to degradation by microorganisms (Yoshida et al., 2016) and can remain in the environment for a long period if wrongly disposed of (Andrady, 2015) which makes plastic a major threat to modernity and the future generations.

Plastic debris have been reported in the marine environment since the ’70s (Carpenter & Smith, 1972). Its presence has been appointed in the deep sea (Woodall *et al*, 2014; Bergman *et al*, 2017), the Arctic Ocean, the Polar Regions (Obbard et al., 2014), intertidal ecosystems (Cleassens et al., 2011; Mathalon & Hill, 2014) and other particular marine habitats. These plastic materials are introduced into the marine environment in several sizes (Hidalgo-Ruz *et al*, 2012), varying in shape, color, and chemical composition (Duis & Coors, 2016). Most plastic types are classified by their size into three categories: macroplastic (> 20 mm diameter), mesoplastic (5 – 20 mm), microplastic (< 5 mm), and nanoplastic (< 1000 nm).

Macroplastics are plastic debris larger than 20 mm and are highly visible, which makes them perceived as the most critical plastic pollution. Due to their size, macroplastics can often be identified according to their usages such as bags, fishing materials, or clothes (Napper & Thompson, 2020). Most campaigns to clean up the ocean are focused on this kind of larger plastic (Nelms et al., 2019). Mesoplastics are plastic debris between 5 and 20 mm and are still visible particles, sharing with macroplastic the visibility appeal as critical pollution. Microplastic (MP) particles are highlighted among the countless types of plastic debris disposed of in the ocean. The name microplastic was first used to define plastic particles smaller than 5 mm differing in color and shape in 2008 (Arthur, Baker & Bamford, 2009) and has been used to define a great variety of plastic sizes as <10mm (Graham & Thompson, 2009), <5mm (Barnes et al., 2009), and <2mm (Ryan et al., 2009). Since then, MP is commonly used to define general small particles of plastic. This inconsistency represents a great problem when creating a standard scientific database for microplastic information and comparison (Costa et al., 2010; Cole et al., 2011).

MP particles are usually sorted according to its source (product fragments or industrial pellets), type (fragment, filaments, films, granules, and other categories), shape (cylindrical, disk, flat, rounded, elongated, irregular, etc.), erosion condition, and color (transparent, white, red, orange, blue, black, gray, brown, green, pink, and other variations). From those categories, color is used to preliminary identification of chemical composition and source of usage, once some colors have been related to specific chemical structures. Polypropylene (PP) is assigned to more clear and transparent colors while polyethylene (PE) is related to white plastic pellets. However, further analysis such as RAMAN Spectroscopy and Fourier Transformed Infra-Red (FT-IR) is needed to precisely identify chemical composition (Hidalgo-Ruiz et al., 2012).

Microplastics are divided into two types: primary and secondary MP (Cole et al., 2011; Auta et al., 2017). Primary MPs are manufactured as small plastic particles for specific use in domestic and industrial sectors. These MPs are present in cleaning products, synthetic clothes (Alomar et al., 2016), and in a massive amount of cosmetic products such as shampoos, facial creams, deodorants, baby products, lotions, makeup, and sunscreen (Fendall & Sewell, 2009; Costa et al., 2010; Cole et al., 2011). The use of microplastic in exfoliating creams is the most explored among cosmetics, they replace natural materials previously used in these products, including oatmeal, pumice (Derraik, 2002; Fendall and Sewell, 2009), and some Brazilian native seeds. On the other hand, secondary MPs are small plastic particles derived from the breakdown of larger plastic debris (Ryan et al., 2009; Norwegian Environment Agency, 2015). Over time, when disposed of in the environment, plastic debris goes through physical (Cooper & Corcoran, 2010), biological (Hodgson et al., 2018), and chemical processes that can reduce its structural integrity resulting in its fragmentation into smaller particles (Browne et al., 2007; Rios et al., 2007; Ryan et al, 2009). Secondary MP is mostly formed in the beaches and intertidal zones due to a greater incidence of ultraviolet light (UV), which causes oxidation of plastic material, weakening its structure, and physical degradation by wave turbulence (Cole et al., 2011; GESAMP, 2014).

Microplastics can act as a trap for chemicals from the surrounding waters such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichloro-diphenyl-trichloroethane (DDTs), metals (Bowmer & Kershaw, 2010; Rochman et al., 2014 a, 2014b; Gewert et al., 2015), and other chemicals from industrial waste, fuel and antifouling paints (Holmes et al., 2012; Brennecke et al., 2016). MPs have shown to be a better substrate to accumulate chemicals than larger plastic (Cole et al., 2011) and the environment itself (Gewert et al., 2015). When absorbing these pollutants they can carry them through the food chain causing contamination of biotic and abiotic variables in the sea and potentially affecting humans (Reisser et al., 2014; Wright et al., 2013; Yang et al., 2015). Microplastics have already been found in human tissues such as in the placenta (Ragusa et al., 2021) and the human blood (Leslie et al., 2022). However, their effect on the human body is still uncertain.

The structure of MP is a complex mix of polymers with chemical and organic additives. These particles can interact with several marine organisms without apparent effect, but can easily be mistaken as food (Auta et al., 2017) with lethal or sub-lethal effects when ingested (Guzzetti et al., 2018). However, MPs cannot be digested once most marine organisms do not have an enzymatic or physiological pathway to digest the polymers, and then MPs can remain inert in the tissues or in the digestive tract

(Andrady, 2011). Consumption of MP has been reported in a wide range of marine organisms such as mussels, barnacles, birds, sea cucumbers, lugworms, amphipods, zooplankton, turtles, and some mammals (von Moos et al., 2012; Avio et al., 2016; Rehse et al., 2016; Cole et al., 2013; Goldstein & Goodwin, 2013; Ferreira et al., 2016; Batel et al., 2016; Fossi et al., 2016; Caron et al., 2016). After consumption, MP particles can cause weight loss, feeding rate decrease, reproductive activity decrease (Lusher et al., 2017), and also molecular damages such as immune system response activation, phagocytosis activity disturbance, DNA damage, oxidative response activation, and even cell death (Galloway et al., 2017). Filter-feeding, suspension-feeding, and deposit-feeding animals such as some polychaetes may be the first affected by MP pollution in the marine environment, once they are susceptible to whatever particles are present in the water column. Furthermore, some of these polychaetes are important bioengineers, building biogenic constructs in the marine environment that portray an important role as microhabitats in intertidal zones.

Polychaeta is a wide group of annelids that colonized several environments, particularly marine habitats. They are one of the most abundant in consolidated substrates as rocky shores, comprising 1/3 of the total richness both numerical and in species number (Giangrande et al., 2003; Chintroglou et al., 2004; Antoniadou et al., 2004). Several species of polychaetes live in self-constructed tubes made of sediment from the bottom of the oceans or dispersed in the water, fragments of shells, and other particles present in the water (Rabaut et al., 2008). Some of these species build reef-like structures that can extend for kilometers (Caline et al., 1988; Naylor and Viles, 2000). Some Sabellariidae species, commonly known as sandcastle worms, are great reef-building polychaetes (Capa et al., 2012), and their colonies are built of thousands of individual tubes side by side with each other. These tubes are made from sand clasts and calcareous particles from shell breakdown gathered in many sizes and shapes from the water column with their ciliated tentacles (Dubois et al., 2005; Fournier et al., 2010). Grains collected by these polychaetes are glued together with a biomineralized mucoprotein cement secreted by specialized glands (Vovelle, 1965; Vovelle and Grasset, 1990; Zhao et al., 2005). These sandcastle reefs control intertidal sands' texture and distribution once they can trap and well select grains by size (Fournier et al., 2010).

The sandcastle reefs work as an important microhabitat on the rocky shore of intertidal zones. These biogenic constructions have a complex structure that helps increase diversity and intra and interspecific interactions (Pinheiro et al., 1997; Dubois et al., 2002; Noernberg et al., 2010; Ataide et al., 2014; Nunes et al., 2017) due to increased oxygen availability, food resources, shelter, surface for larval settlement and protection against predators (Bell, 1985; Levin et al., 1986; Holt et al., 1998; Dias & Paula, 2001; Borthagaray & Carranza, 2007; Norling & Kautsky, 2007). The sandcastles can shelter several other organisms like decapods, isopods, amphipods, other polychaetes, bivalves, gastropods, bristles, sipuncles, bryozoans, and echinoderms (Lane-Medeiros et al., 2021; Bosa & Masunari, 2002; Gore et al., 1978; Narchi, 1973; Souza 1989). Some algae species also inhabit the sand reefs, highlighting these microhabitats' diversity, complexity, and importance.

The polychaete *Phragmatopoma caudata* (Kroyer in Morch, 1863) is a reef-building Sabellariidae widely found on the brazilian coastline, with an occurrence area extending from Santa Catarina (Brazil) to Florida (United States) (Occhioni et al., 2009). Commonly found on the rocky shore of intertidal and subtidal zones, they are extremely abundant in the Espírito Santo State seashore. Although ingestion of MPs has also been reported for other species of polychaetes (Knutsen et al., 2020; Hamzah et al., 2021; Jang et al., 2020), Costa et al. (2021) was the first study to investigate the capability of a reef-building polychaete to incorporate microplastic in its colony's structures. They collected one sample of *P.* caudata's colony in one site in Camburi Bay (Vitória – Espírito Santo - Brazil) and analyzed the digested tissues, washing water, and inner structure of these samples. In their results, they showed the capability of *P. caudata* to randomly trap MPs in the structure of their sandcastles, pointing to their usability as indicators for MP pollution.

The study of MPs' pollution, their presence, and distribution alongside seashores of Espírito Santo state is still poorly reported (Baptista Neto et al., 2019; Costa et al., 2021; Zamprogno et al., 2021). Furthermore, this work aims to elucidate the abundance of MPs on the coastline of Espírito Santo by expanding the number of sampling spots in Costa et al. (2021), collecting samples in beaches with different structures and anthropic impacts in order to correlate the MP pollution rate with these characteristics, and consolidate *P. caudata* as a trustable indicator for MP pollution in coastal areas.

2. MATERIAL AND METHODS

2.1. Study region

The Espírito Santo State (ES) is located in southeastern Brazil and is the smallest shoreline in the region. More than half of the ES coastline is located between two great hydrographic basins, the Doce River Basin in the north and the Paraíba do Sul River Basin in the south, which makes this coastline singular when compared to its surrounding neighbors, once the basins form a geographical barrier for many marine organism. For the aims of this study, we selected 12 beaches as sampling spots (S1, S2, S3… S12) alongside the ES coast (Figure 1) according to their anthropic impact, measured by proximity to cities or residential conglomerates, and structure, sheltered and exposed beaches. The diversity in sampling spots is important to test the difference in the MP pollution rate between coastal areas with different structures and different anthropic impacts and understand the behavior of *P. caudata* as an indicator in these different conditions.

We separated these spots in three major regions, North, Central, and South. Sampling spots S1 to S4 are located in the North, S5 to S8 in the Central, and S9 to S12 in the South. Spots S1, S2, S10, and S11 are distant from residential areas and therefore, receive less direct anthropic impact, while S3, S4, S5, S6, S7, S8, S9, and S12 are inside city areas or on their borders. The structural classification can be seen in Table 1 below.

Table 1: Sampling spot identifications, classification, coordinates, and sampling dates. Morphology symbols are (E) Exposed; (S) Sheltered; (SE) Semi-exposed; (I) Intermediate.

Area	Sampling spot	Morphology	Coordinate	Date
North	Portocel (S1)	E	-19.837786, -40.056252	28 June 2021
	Piraquê-Açú (S2)	E	-19.943109, -40.141864	
	Praia Grande (S3)	S	-20.037328, -40.174540	
	Manguinhos (S4)	E	-20.188610, -40.189980	
Central	Ponta de Tubarão (S5)	S	-20.272356, -40.253293	23 June 2021
	Iemanjá (S6)	SE	-20.292448, -40.287760	
	Praia do Meio (S7)	S	-20.315687, -40.288122	
	Pedra da Sereia (S8)	I	-20.333218, -40.273194	
South	Guarapari (S9)	E	-20.733499, -40.528574	09 August 2021
	Pontal de Ubu (S10)	E	-20.802683, -40.581376	
	Boca da Baleia (S11)	E	-20.838837, -40.630745	
	Itaipava Pier (S12)	S	-20.894527, -40.773811	

2.2. Sampling

Thirty-six colony blocks (15 cm x 15 cm x 15 cm) of *Phragmatopoma caudata* (Kroyer in Morch, 1863) were collected along 12 sampling spots (three replicates each) and 12 water samples. The blocks were separated from the rest of the colony using a stainless steel spatula. Each sample was stored in a bag for transportation and immediately stored at -20º C freezer when arriving in the laboratory for further analysis. A total of 500 ml of water was sampled in the water 1 m near the sampled colony by sinking the bottle into the water column at a depth of approximately 30 cm from the surface. Sampling was performed on three different dates of 2021 (28 July, 23 June, and 09 August) during the first low tide of the day, between 0.1 - 0.3 to reach the middle tide zone more easily.

Figure 1: Study region with the 12 sampling spots of *Phragmatopoma caudata*. (S1) Portocel. (S2) Piraquê-Açú. (S3) Praia Grande. (S4) Manguinhos. (S5) Ponta de Tubarão. (S6) Iemanjá. (S7) Praia do Meio. (S8) Pedra da Sereia. (S9) Guarapari. (S10) Pontal de Ubu. (S11) Boca da Baleia. (S12) Itaipava Pier

2.3. Sample processing and microplastic separation

Methodological processes followed standard MP analysis in sediment that comprises density separation, sieving, and visual sorting (Hidalgo-Ruiz et al., 2012; Costa et al., 2021). The sample processing is illustrated in Figure 3 for a better understanding of the methodological processes used to analyze the samples of *P. caudata* colonies.

2.3.1. Analysis of the colony washing water and density separation

The colony blocks of *P. caudata* were thawed before processing, which took at least six hours to guarantee full defrosting depending on the weather of the day. We washed the blocks inside a bucket and filtered the water from the washing through two stainless steel geological sieves of 500 µm mesh and 90 µm mesh sizes. The remaining sediment on the sieves was transferred into two different glass beckers of 1L, one for each sieve material. The beckers were both filled with distilled water to suspend MP by density. The resulting heterogenic solution of sediment and water was agitated with a glass cane over two hours in intervals of 30 min to resuspend MPs particles. At most 30s after the last agitation, the supernatant water from this solution was filtered through a 90 µm mesh sieve. This short period between the last agitation and filtering avoids MPs' redecantation once they are less dense than the sediment and take a little longer to decant. The material retained in the sieve was transferred to a Petri dish covered with a filter paper of 80 g for further sorting.

2.3.2. Analysis of microplastic from *P. caudata* tissues

The first 30 full body individuals of *P. caudata* were sampled from the colony block and weighted together using a precision balance (accuracy 0.1g) to gauge the wet weight. Individuals were transferred into a falcon tube (50 ml) and then added 25 ml of KOH 10% to digest the tissues liberating the MPs. The falcon tubes were stored at 50º C over 48 hours to accelerate the digestion process. The solution in the falcon tubes was filtered through a 90 µm mesh sieve and transferred to a petri dish with an 80 g filter paper for further sorting.

2.3.3. Analysis of microplastic from the colony structure

After the washing and the *P.* caudata sampling, the colony block was fragmented and 150 ml of inner sediment was removed and placed in a 1L glass becker. The becker was filled with distilled water and agitated for two hours in intervals of 30 min. At most 30s after the last agitation, the supernatant water from this solution was filtered through a sieve of 90 µm mesh. The supernatant was filtered through a 90 µm mesh sieve and transferred to a petri dish with an 80 g filter paper.

After receiving the MPs processed samples, all petri dishes were maintained in a stove at 50°C to dry, once they cannot be stored wet due to mold growth risk. After completely drying, all dishes were stored for further analysis.

2.4. Microplastic sorting

To sort MPs particles we used a stereomicroscope Leica™ S8AP0 with an attached camera MC170 HD. A circle area was demarked in the petri dish to make the counting accurate (Figure 2). The filter paper was slightly humidified with distilled water to avoid the dry MPs to fly away from the petri dish, once they are extremely light when dry.

We used the definition of MP as plastic particles <5 mm, any particle bigger than that was considered mesoplastic as suggested by Andrady (2011). All MPs were sorted by color (Red, blue, orange, green, purple, black, yellow, and transparent) and type (filaments and fragments). Other plastic forms such as pellets, tangles, films, and strips were considered as 'others' in the counting and statistical analysis.

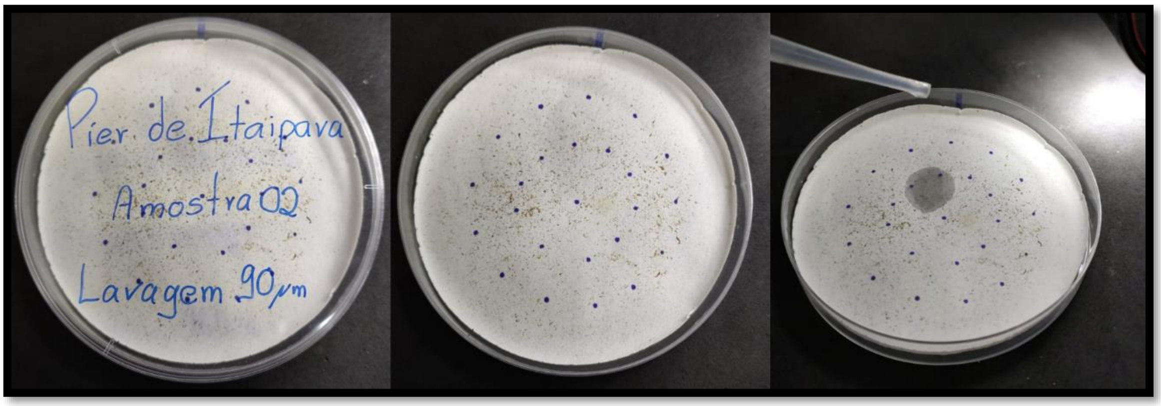

Figure 2: Image of one Petri dish showing the identification, the demarcation area, and the humidification.

2.5. Statistical analysis

All statistical analyses were performed using TIBCO Statistica 14.0 and graphics built in GraphPad Prism 9.3.0. Significant values followed $p < 0.05$. Normality and homoscedasticity were tested with Shapiro-Wilk and Levene respectively and, in case of abnormality, data were transformed using log10.

Kruskal-Wallis was used to test the difference in the number of filaments, fragments, and ‘others’ between the three regions, the sampling spots, and the matrixes. Multiple comparisons test was performed to detect where the pointed significant differences are. The differences are shown in the graphics using symbols (* and #). When difference symbols are not shown or there was no difference pointed by the multiple comparisons test.

The Spearman test was performed to see the correlation between the number of MPs found in the tissues of *P. caudata* and the wet weight. The Spearman test was also performed to test the relation between MPs available in the environment (Water) and MP incorporated in the colony structure and absorbed by *P. caudata* individuals.

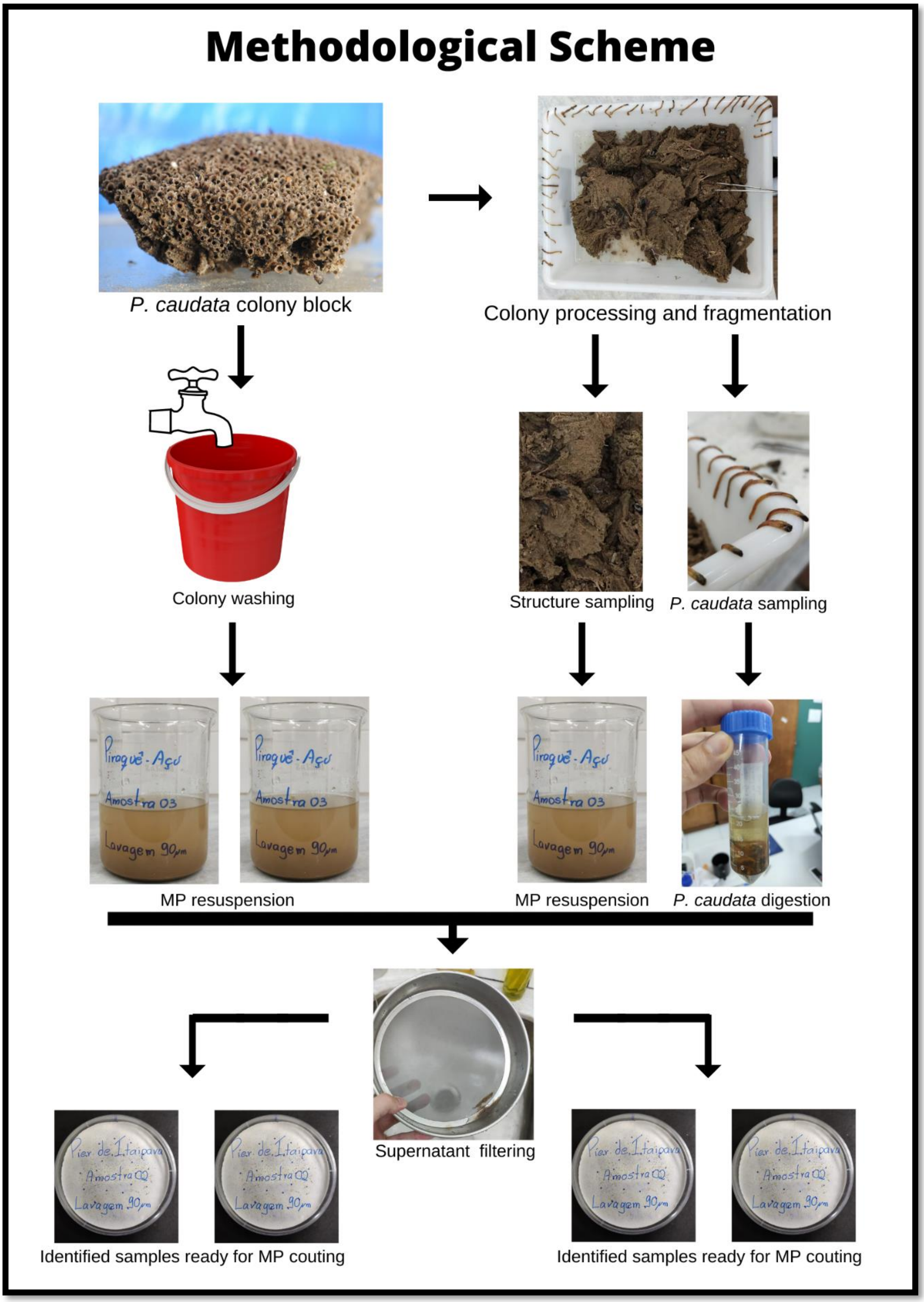

Figure 3: Illustration exemplifying the described methodological process of MP suspension and separation.

3. RESULTS

A total of 7005 particles of plastic were found distributed between 6668 filaments (95.19%), 108 fragments (1.54%), and 229 ‘others’ (3.27%) with significant difference between them ($H = 303.8$; $p < 0.05$) (Figure 4 and 5). The category ‘others’ comprises pellets, tangles, films, and stripes. We also found some mesoplastic particles (>5 mm) as suggested by Andrady (2015), but kept them out of the statistical analysis once they are not the focus of this study.

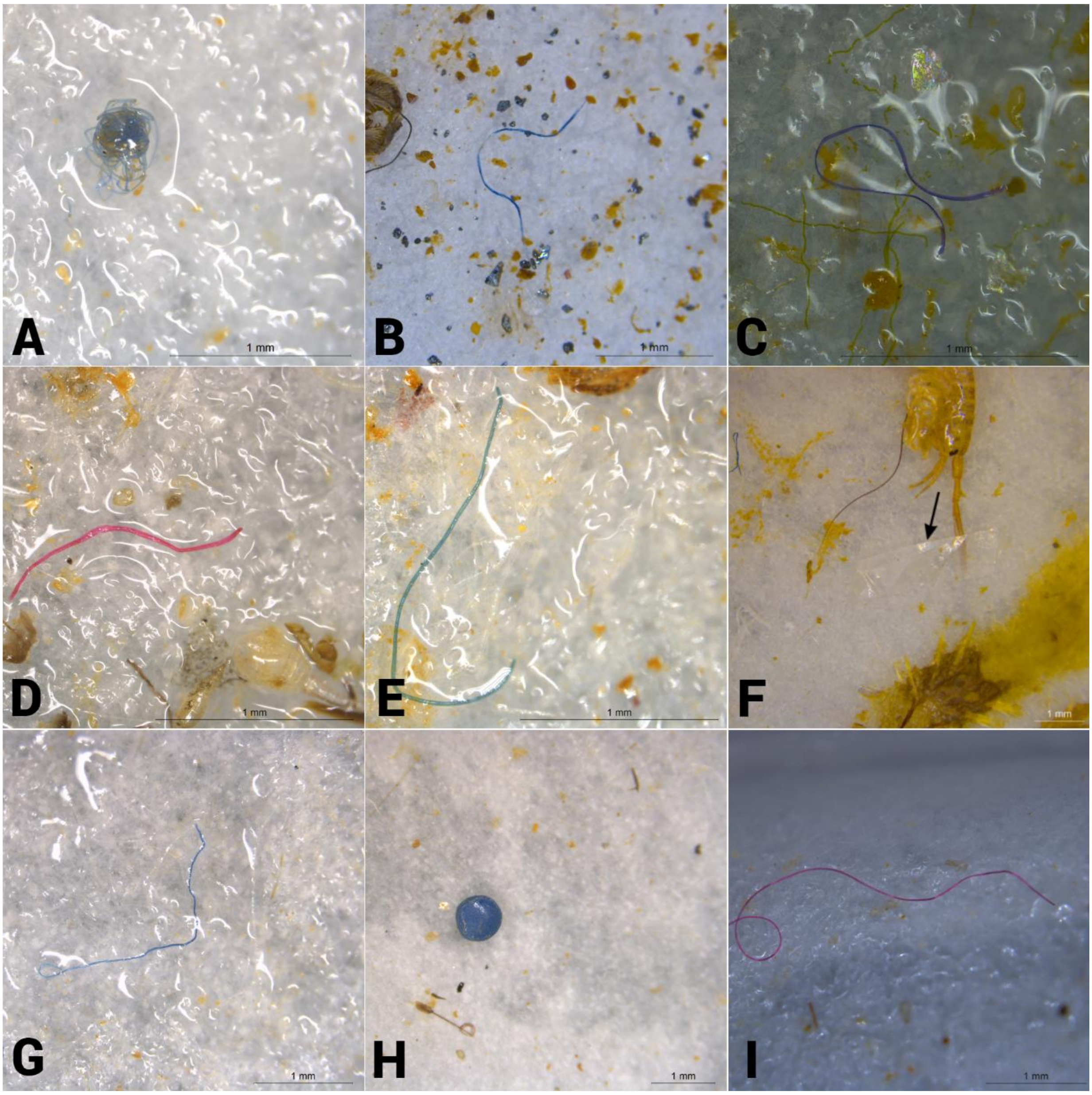

Figure 4: Microplastic particles found in the samples of this study. (A) A black tangle. (B) A blue filament next to a black filament. (C) A purple filament. (D) A red filament. (E) A green filament. (F) A stripe pointed by a black arrow and highlighted by its light reflection capability. (G) A blue filament. (H) A blue pellet, the only one found in all analyzed samples. (I) A red filament.

The Central region showed the highest numbers of MPs with 3961 plastic particles, followed by the North (1897) and South (1116) (Figure 5). The Kruskal-Wallis test pointed to a significant difference between the regions for filaments, fragments, and others (H = 8.46; H = 17.52; H = 14.34; $p < 0.05$), where the Central region showed the highest values for all MP types.

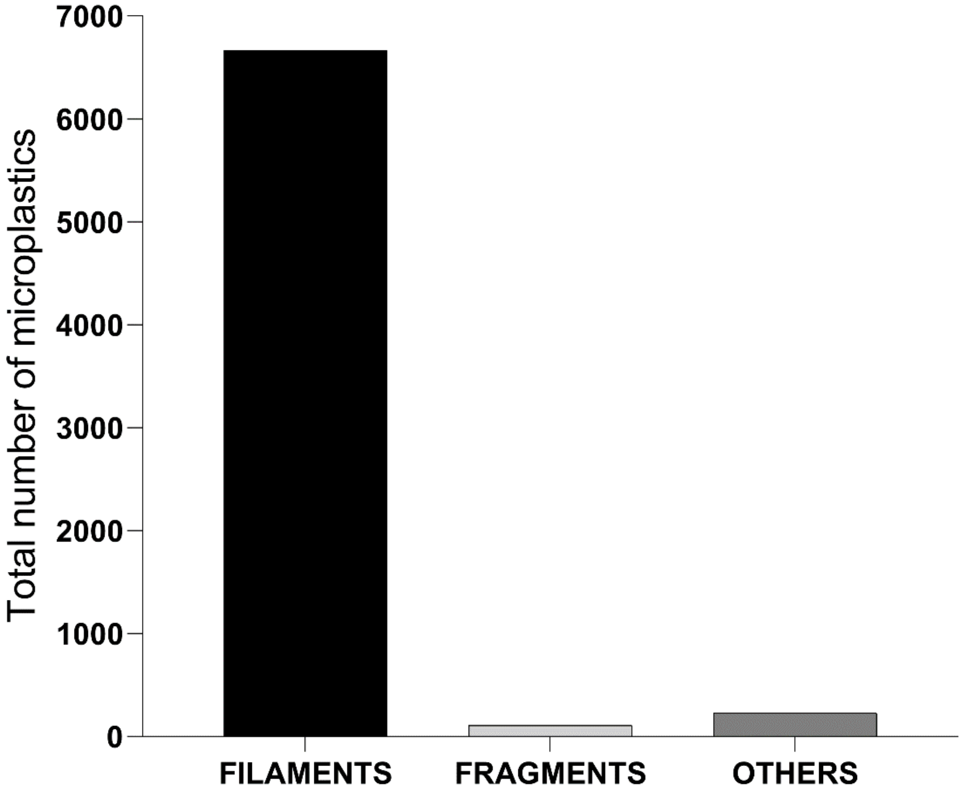

Figure 5: Total number of filaments, fragments, and ‘others’ found in this study.

There was significant difference between the colors of filaments (H = 491.53; $p < 0.05$) where the most frequent colors were black (2391), blue (2314), and red (1448) (Figure 6). Green and purple were the smallest numbers, and purple only appeared five times. Fragments were also different when compared by color (H = 130.61; $p < 0.05$) and showed a huge difference in the total number compared to filaments, where blue was the most frequent color (70) (Figure 7).

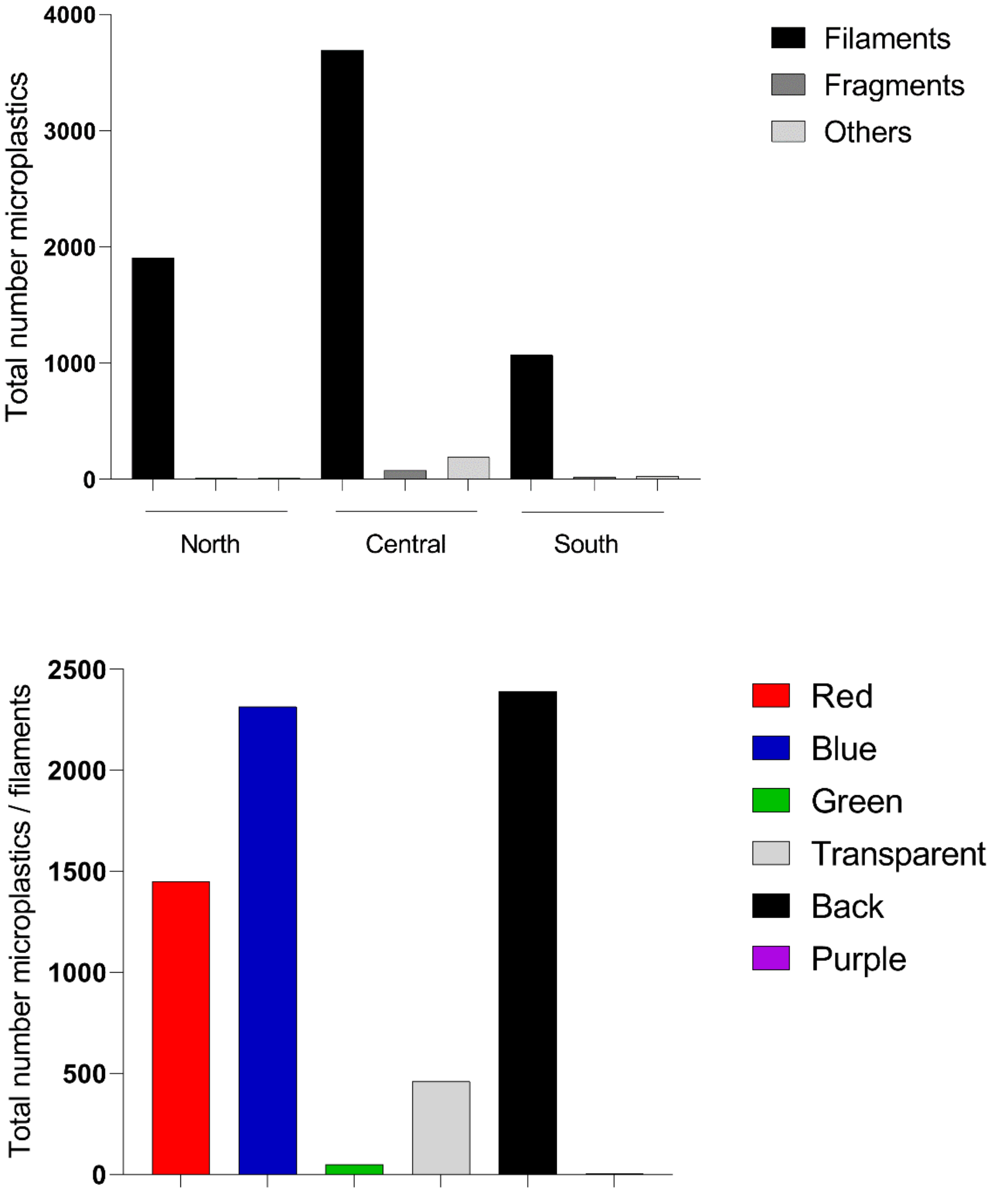

Figure 6: Total number of microplastics. (A) Total number of Filaments, Fragments, and ‘Others’ sorted by region. (B) Total number of filaments sorted by color.

There was significant difference between ‘others’ (H = 28.2; $p < 0.05$) and the most frequent shape was stripes (175), followed by tangles (34) and films (19). Only one pellet was found (Figure 7). Regarding the matrixes, samples from the colony washing water, respectively W90 and W500, showed the highest amount of MPs (3810 and 1574 respectively). Samples from the water and from *P. caudata* tissue showed the smallest numbers of MP (Figure 7). There was significant difference in the number of filaments and ‘others’ between the matrixes (H = 61.61; H = 10.62; $p < 0.05$) and no difference for fragments (H = 4.33; $p > 0.05$).

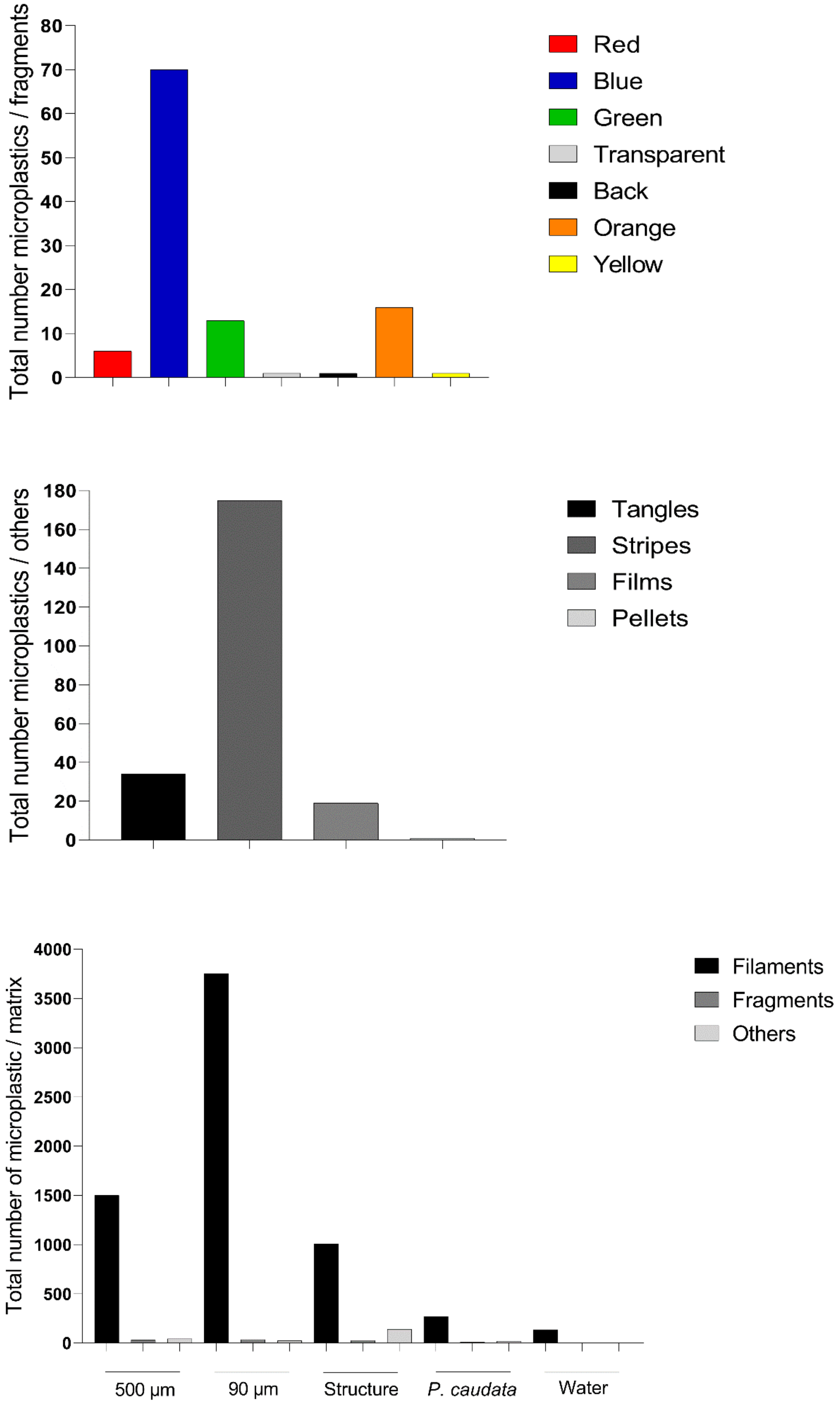

Figure 7: Total number of microplastic: (A) Total number of fragments sorted by color. (B) Total number of 'others' sorted by type. (B) Total number of microplastic sorted by matrix.

3.1. The North region

The North region comprises the following four sampling spots: Portocel (S1), Piraquê-Açú mouth (S2), Praia Grande (S3), and Manguinhos (S4). There was a significant difference between the shapes in the North (H = 109.07; $p < 0.05$) and from the total number of 1921 MPs, 1905 were filaments, 11 were fragments, and nine ‘others’ (Figure 8).

The Kruskal-Wallis test showed a significant difference between the colors of filaments (H = 165,27; $p < 0.05$), where the most frequent filament colors were blue (921) and black (729), followed by red (190), transparent (54), green (10), and purple (1) (Figure 9).

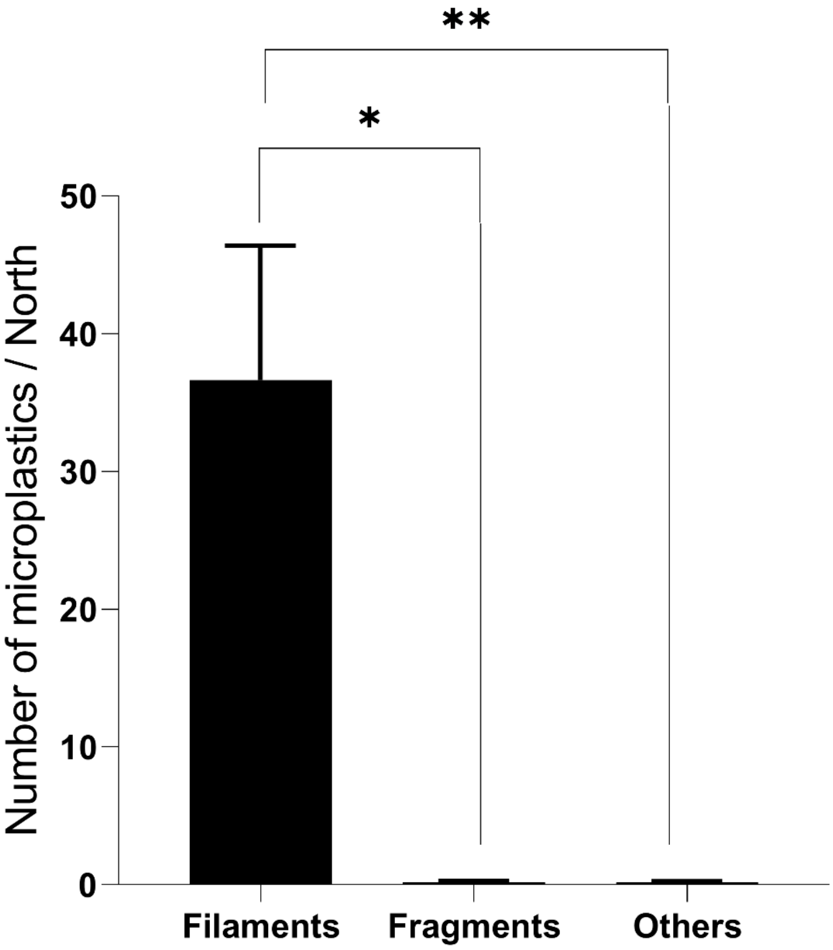

Figure 8: Mean and standard error of microplastics found in the North region sorted by its type: filaments, fragments, and ‘others’. (*)(**) Significant difference pointed by the Kruskal-Wallis and the multiple comparisons tests ($p < 0.05$).

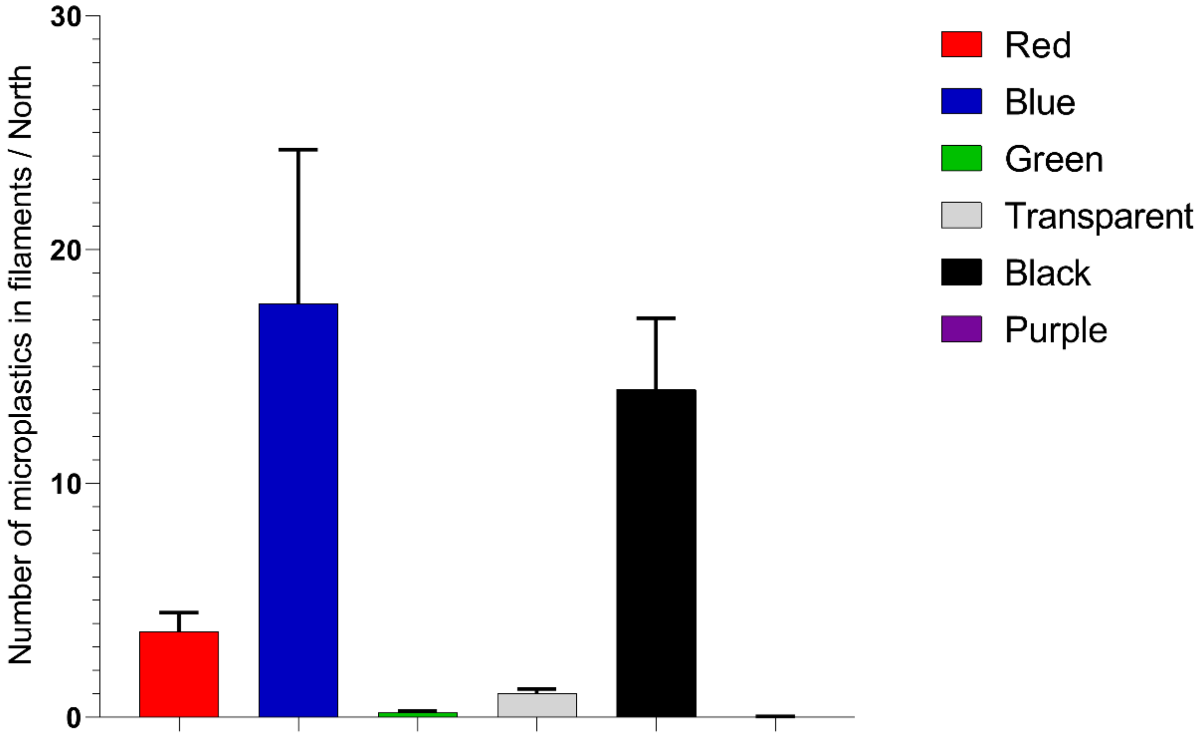

Figure 9: Mean and standard error of filaments sorted by color found in the North region.

Among the sampling spots, S2 showed the highest levels of MPs filaments (1281), a huge difference from the other spots (Figure 10), with significant difference between them (H = 11.64; p < 0.05). S1 had the smallest numbers (154) while S3 and S4 showed a similar level of MPs (252 and 212, respectively). The colony samples from S4 did not have any individuals of *P. caudata*, so they did not have data from digested tissues.

The North region showed the smallest number of fragments (16) and the most frequent fragment color was blue (7). S1 showed the highest values for ‘others’ (6), followed by S2 (2), and S3 (1). Both fragments and ‘others’ did not show significance between the sampling spots (H = 6.21; H = 3.92; p > 0.05). S4 did not show any tangle, stripe, film, or pellet (Figure 10).

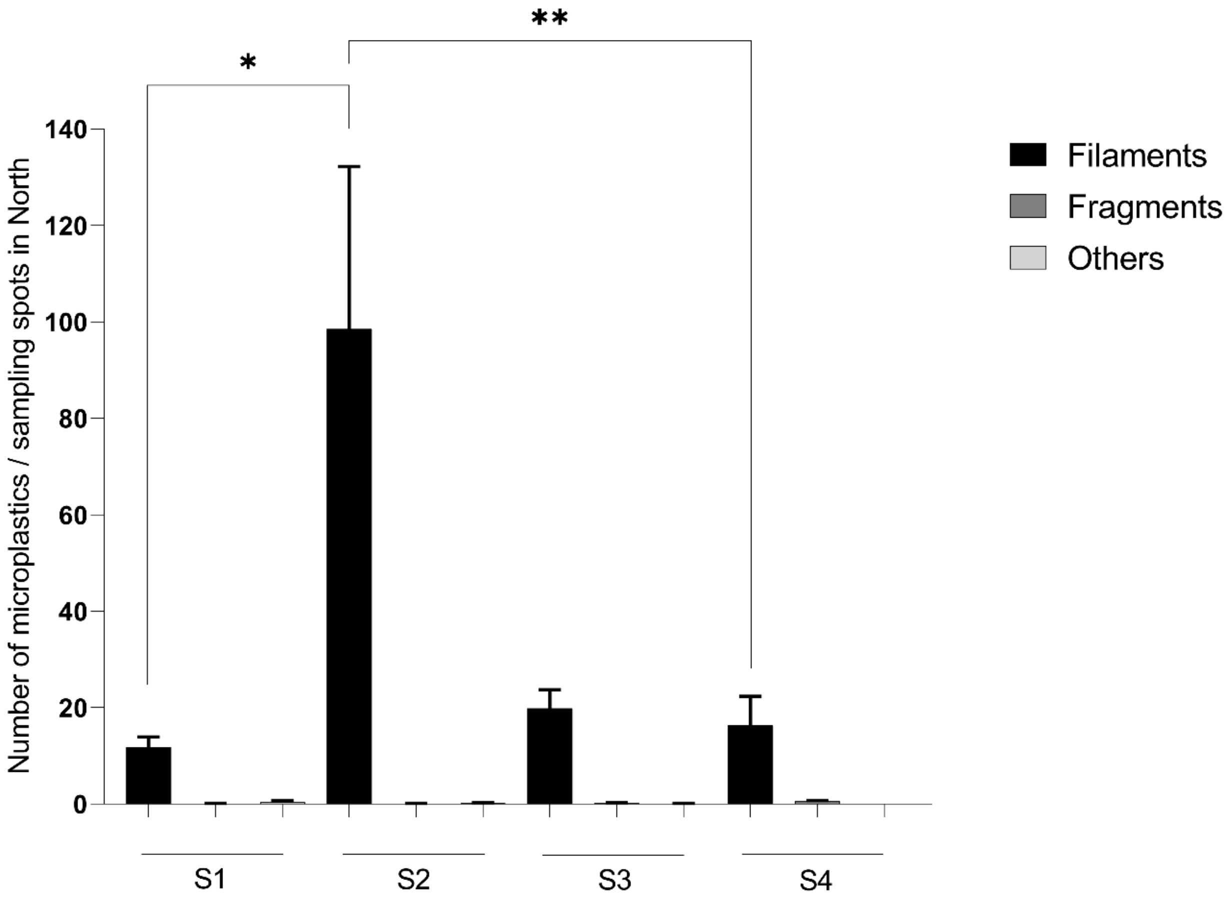

Figure 10: Mean and standard error of microplastics found in the North region sorted by their type in each sampling spot. (S1) Portocel. (S2) Piraquê-Açú. (S3) Praia Grande. (S4) Manguinhos. (*)(**) Significant difference pointed by the Kruskal-Wallis and multiple

3.2. The Central region

The Central region comprises the following four sampling spots: Ponta de Tubarão (S5), Iemanjá (S6), Praia do Meio (S7), and Pedra da Sereia (S8). Of the total amount of 3961 MPs, 3691 are filaments, 78 are fragments, and 192 ‘others’ with significant difference between them (H = 90.16; p< 0.05) (Figure 11).

There was significance between the colors of filaments (H = 167.77; p < 0.05) where the most frequent colors were black (1208), blue (1089), and red (1015), followed by transparent (350), and green (29) (Figure 11). There were no purple filaments in the Central area.

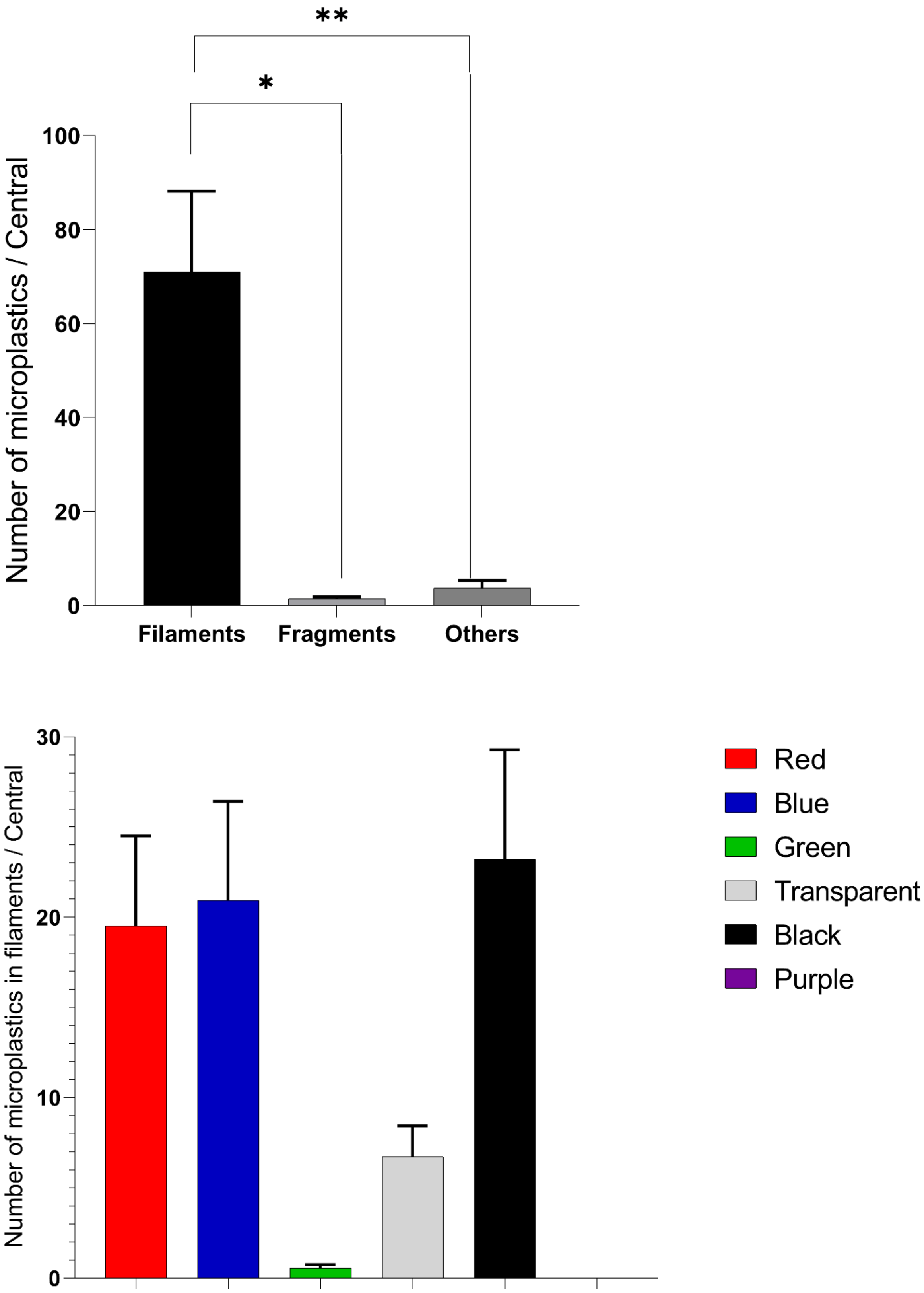

Figure 11: Mean and standard error of microplastic in the Central region. (A) Distribution of microplastic sorted by type in the Central. (*)(**) Significant values showed by Kruskal-Wallis and multiple comparison tests. (B) Total of filaments sorted by color in the Central region.

Most sampling spots in the Central region showed high values of filaments, except S6. There was a significant difference in the number of filaments between sampling spots ($H = 10.18$; $p < 0.05$) where the highest numbers of filaments were found in S5 (1830) (Figure 12).

The Central region showed the most diversity in fragments and there was a significant difference in their number between the sampling spots ($H = 11.77$; $p < 0.05$) whereas S5 showed the highest values both for different color of fragments and in number. This also happens with the ‘others’ distribution in the Central region ($H = 11.47$; $p < 0.05$), however, the number of stripes presented in S5 is notably higher than in the other sampling spots (Figure 12).

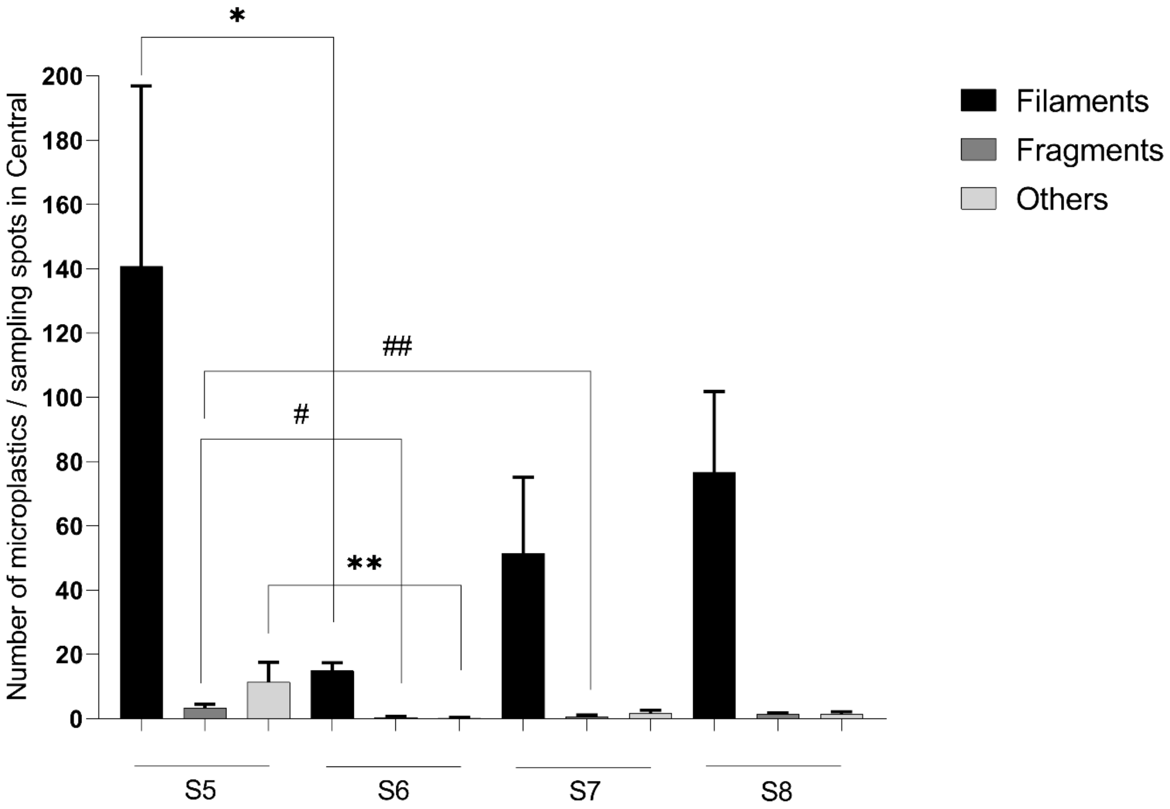

Figure 12: Mean and standard error of microplastic sorted by type in each sampling spot on the Central region. (S5) Ponta de Tubarão. (S6) Iemanjá. (S7) Praia do Meio. (S8) Pedra da Sereia. Significant difference showed by the Kruskal-Wallis and multiple comparison tests ($p < 0.05$) between filaments (*)(**) and between fragments (#)(##).

3.3. The South region

The South region comprises the following sampling spots: Itaipava Pier (S9), Pontal de Ubu (S10), Boca da Baleia (S11), and Guarapari (S12). Of the total amount of 1117 microplastic particles found in the South, 1072 were filaments, 19 were fragments, and 28 were 'others' (Figure 13) with significant difference between them (H = 114.91; p < 0.05).

There was a significant difference between the colors of filaments (H = 170.53; p < 0.05) where the most frequent colors were black (454), blue (304), and red (243). We also found 56 transparent filaments, 11 green, and only 4 purple (Figure 14).

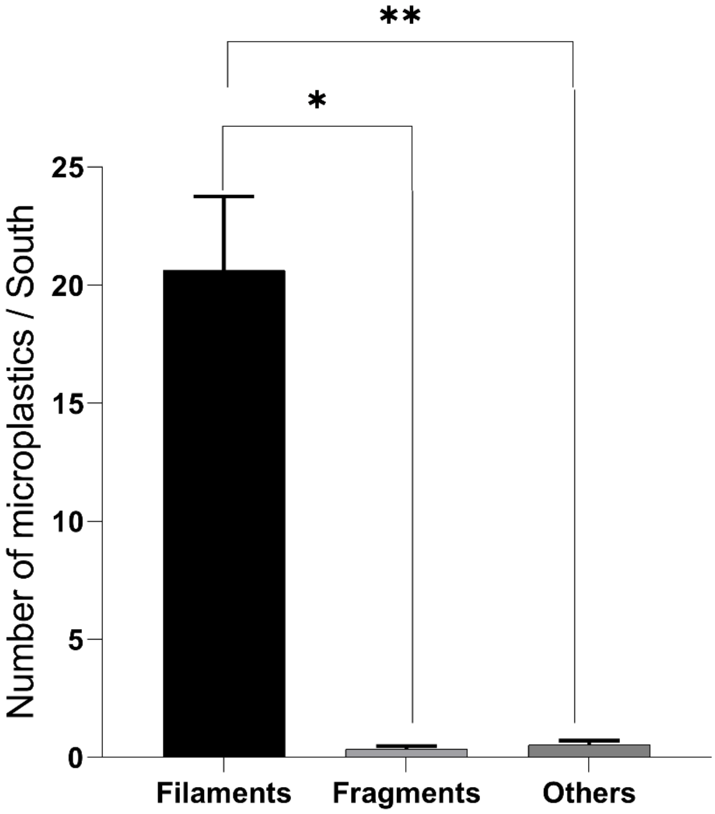

Figure 13: Mean and standard error of microplastic found in the South region sorted by their type. (*)(**) Significant difference showed by the Kruskal-Wallis and multiple comparison tests (p < 0.05).

The distribution of filaments in the South region was not discrepant as in the other regions. The numbers of filaments found in the sampling spots were S10 (353), S12 (299), S11, and S9 (150) (Figure 14).

S9 and S12 showed the highest numbers for the distribution of fragments and 'others' (17 and 11 respectively). Blue fragments appeared in all sampling spots of the South region. There was no significant difference in the numbers of filaments, fragments, and 'others' between the sampling spots (H = 5.55; H = 4.66; H = 3.5; p > 0.05). One pellet was found on S11.

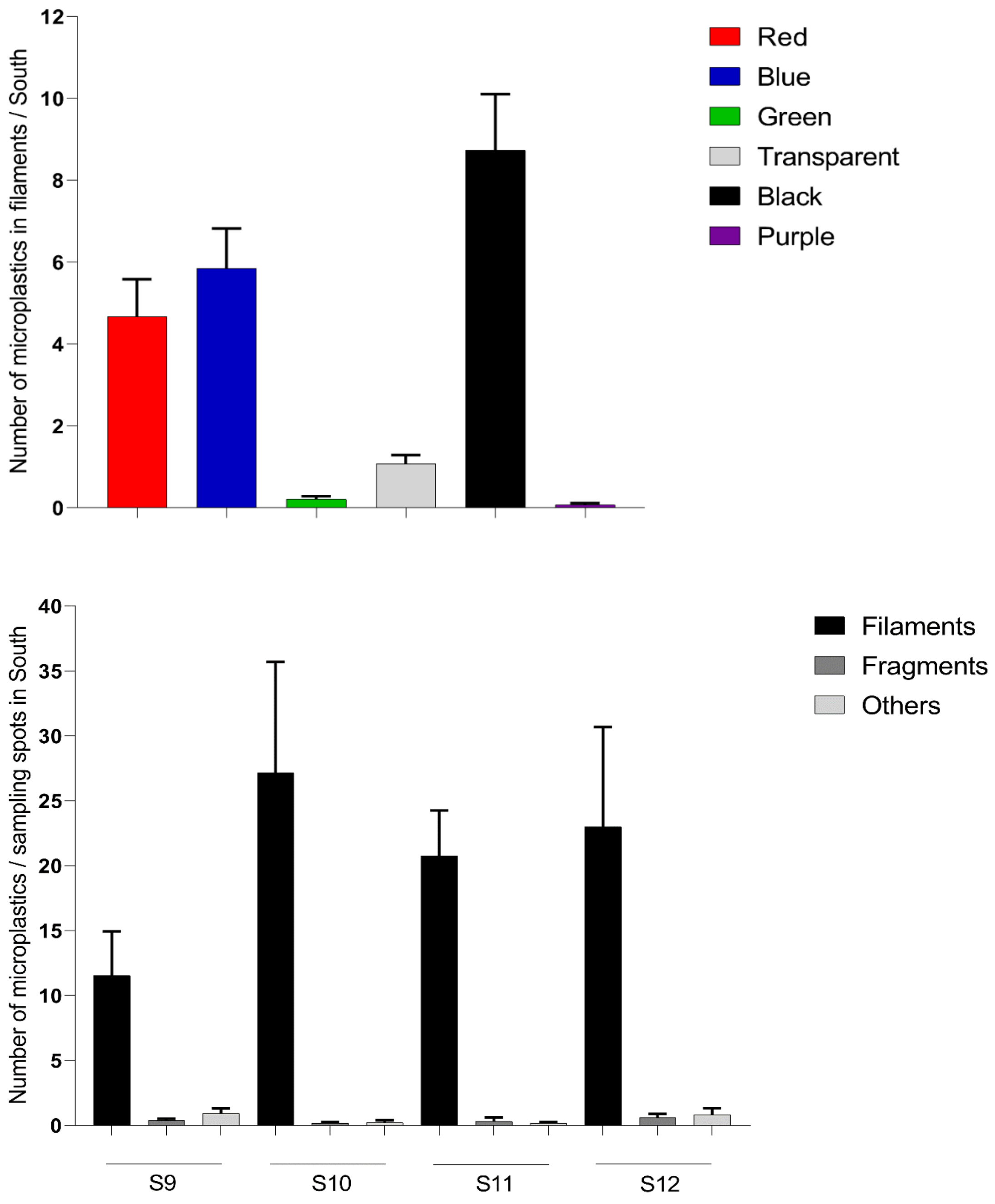

Figure 14: Microplastics in the South region (A) Mean and standard error of filaments found in the South region sorted by their color. (B) Mean and standard error of the microplastic found in each sampling spot of the South region sorted by their type. (S9) Guarapari. (S10) Pontal de Ubu. (S11) Boca da Baleia. (S12) Itaipava Pier.

3.4. General overview

The Kruskal-Wallis test showed a significant difference when comparing the three types of MP (filaments, fragments, and ‘others’) between all sampling spots (H = 34.09; H = 38.98; H = 33.57; $p < 0.05$).

The highest numbers of MP, for all three shape categories, were found in S5, which is also the highest value for MP in the Central region. The highest values of MP in the North and South regions were found in S2 and S10 respectively (Figure 17).

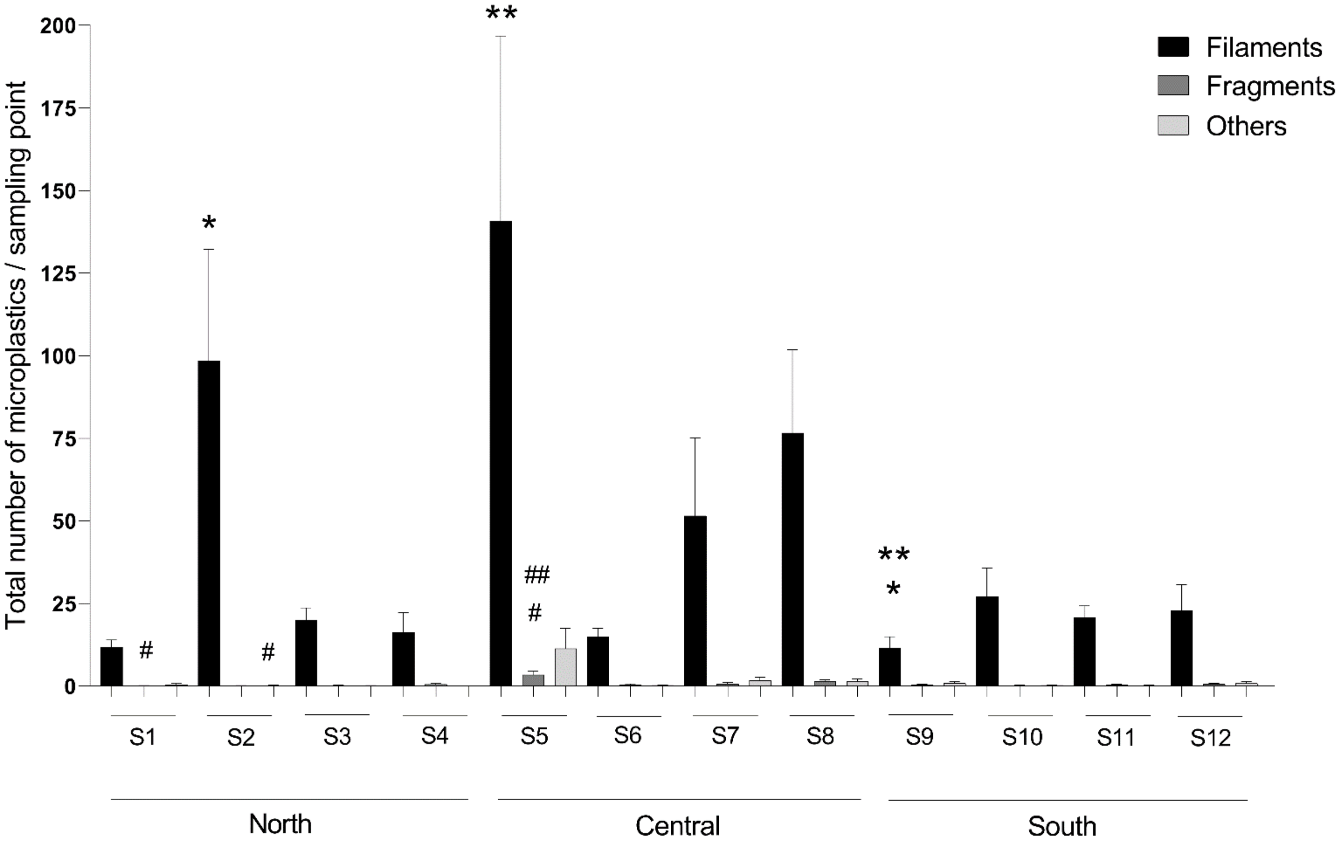

Figure 15: Mean and standard error of microplastics in all 12 sampling spots of the three regions sorted by their type: filament, fragment, and 'other'. (S1) Portocel. (S2) Piraquê-Açú. (S3) Praia Grande. (S4) Manguinhos. (S5) Ponta de Tubarão. (S6) Iemanjá. (S7) Praia do Meio. (S8) Pedra da Sereia. (S9) Guarapari. (S10) Pontal de Ubu. (S11) Boca da Baleia. (S12) Itaipava Pier. Significant difference showed by the Kruskal-Wallis and multiple comparison tests ($p < 0.05$) between filaments (*)(**) and between fragments (#)(##).

3.5. Correlation between wet weight and microplastic

The total wet weight of the *P. caudata* individual's pool can be seen in Table 2. The highest weights were found in the Central region, followed by the South and the North. The heavier individuals were found in S5, S6, S9, and S3.

There was a positive relationship between the wet weight and the total number of MP found in the digested tissues of *P. caudata* (Figure 16). The Spearman test showed that this positive relation was significant ($p < 0.05$; $r = 0.61$).

Table 2: Wet weight of the *Phragmatopoma caudata* collected for MP analysis from each sampling spot.

North		Central		South	
Sampling spot	**Wet weight (g)**	**Sampling spot**	**Wet weight (g)**	**Sampling spot**	**Wet weight (g)**
S1	1.3	S5	3.5	S9	2.5
	1.2		2.6		2.8
	0.8		2.2		2.3
S2	1.4	S6	2.6	S10	1.2
	0.6		1.8		1.0
	0.8		4.0		1.2
S3	2.2	S7	2.8	S11	1.2
	2.0		1.6		1.6
	1.8		1.6		1.8
S4	0	S8	2.6	S12	2.8
	0		2.1		2.4
	0		1.2		2.2

The Spearman test did not show significant relation when comparing MPs available in the environment (Water) with MPs incorporated in the structure (W500, W90, and Structure) and absorbed/adsorbed by *P. caudata* individuals.

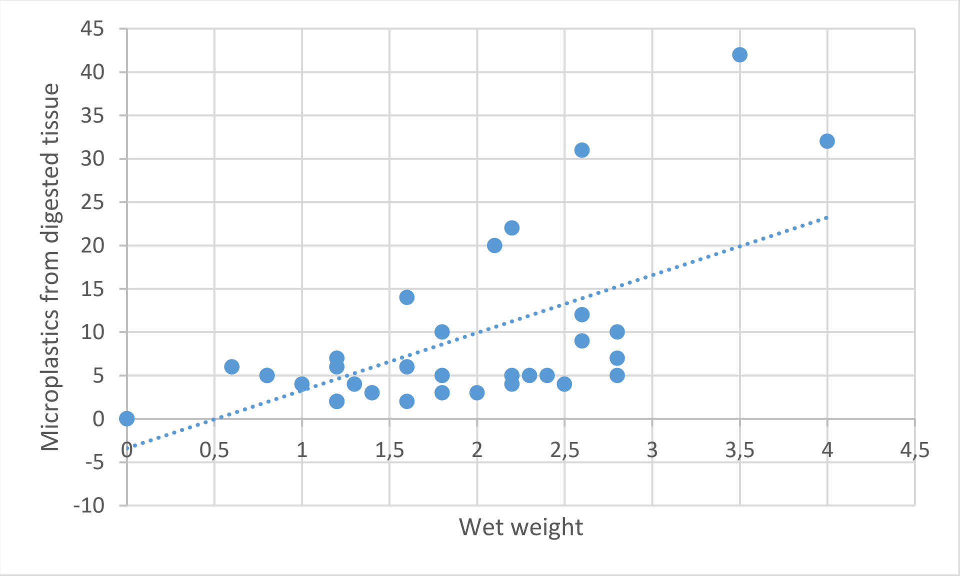

Figure 16: Dispersion analysis with grid line between the total number of microplastics from the digested tissues of *P. caudata* (y) and the wet weight (x).

4. DISCUSSION

All sampling spots were contaminated with MP particles, thus the analyzed beaches are 100% polluted with plastic. The number of filaments notably overcomes the total of fragments and 'others', pointing to a possible difference in the frequency of these types in the water column. P. caudata is known to select the size of the sediment debris with their ciliated tentacles (Dubois et al., 2005; Fournier et al., 2010), this selection varies according to the size of the individuals (Multer & Milliman, 1967; Gram, 1968) and this could influence the type and size of MP trapped in the colony structure and the particles mistaken as food. Also, the different weights of the plastic particles as well as the different densities of the distinct chemical structures of this debris can interfere with the particle selection process during colony construction, resulting in the difference between the types of microplastic in each spot.

The most frequent filament colors were black, blue, and red. This high rate of blue and black has already been reported in the literature (Cago et al., 2018). The prevalence of blue filaments is mostly related to fishnets and other fishery materials (Zhu et al., 2019) and probably because blue is a popular color around the world (Cago et al., 2018). A high rate of black fiber has also been reported (Cago et al., 2018; Cincinelli et al., 2021) and this could be probably due to the high usability of black in all areas such as the clothing market and industry. The other light-colored fibers transparent, red, and green are commonly mistaken as food by marine organisms, although, red was the least color found in *P. caudata* digested tissues, which could point to a possible selectivity of plastic debris by this species. Recording color in MP studies can generate a comparison data bank for future investigations relating the presence of different colors of plastic to possible changes in the surrounding environment, allowing government institutions to better built conservation and cleaning projects.

Red filaments are also easily mistaken with some microalgae during visual sorting, and therefore, their number is probably bigger than the environment's reality. This also happens with transparent filaments which can be mistaken for filter paper fiber and microalgae. Although the hot needle technique presented by De White (2014) is effective to differentiate organic materials from plastic ones, it wasn't performed in all samples once it would take a long period to test all the uncertain particles among the 7005 MPs.

The difference between the ‘others’ is mainly due to S5 in the Central region where the number of tangles, stripes, and films are respectively 76.47%, 66.28%, and 31.58% of the total found in this study. Tangles originate from long filaments or several filaments that got entangled, which could have happed during sample processing. Films and stripes are bigger shapeless plastic particles and therefore may have a smaller number due to particle selection in the colony construction process.

The number of MPs found in the washing waters was the highest amongst the analyzed matrixes. The number of MPs from W90 was 3.2 times bigger than in Structure, 12.6 times bigger than in *P. caudata* digested tissues, and 26 times bigger than in the Water. The number of MPs from W500 was 1.3 times bigger than in the Structure, 5.2 times bigger than in *P. caudata* tissues, and 11 times bigger than in the Water. The washing water from the colony, both W500, and W90, are the most informative matrixes for MP analysis using *P. caudata*, once they produce a high amount of MP data to compare, making statistical analysis more accurate. The structure is also a good indicator, however, a higher amount of inner sediment could show better results. The same relation of MP rate between tube structures and tissues of polychaetes, where the number of MP in the tubes was higher than in the tissues, was found by Knutsen et al., (2020). Data produced from digested tissues and the environment water were less informative, once they have a very small number of MP particles and this could be due to the small number of *P. caudata* individuals sampled and the lack of water sample replicates. MP accumulation depends on the type, shape, color (Missawi et al., 2020; Pequeno et al., 2021), and also on feeding habits (bour et al., 2018; Lourenço et al., 2017), suggesting, according to Pires et al. (2022), that non-selective polychaetes are more likely to accumulate MPs. This would explain why we found a number of MP so small in the digested tissues.

The high level of MP in the W90 and W500 matrixes could be due to two main factors. (1) Colony samples slightly crash and fragment during transportation from the sampling spots to the laboratory, this liberates sediment from the colony structure, therefore liberating MP that would be cough during the washing process. (2) The colony sample is tridimensional and thus the washing process washes also the inner faces of the sampled structure, making the washing matrixes not representative of only available microplastic around the colony, once the withdrawal process includes the sawing of the colony, fragmenting the inner portion.

4.1. The North region

The North region comprises the four sampling spots of S1, S2, S3, and S4. This region showed the middle number for all three types of MP (Filaments, fragments, and ‘others’), evidencing a smaller plastic pollution rate in the North when compared to the Central region. Despite the small rate of MP pollution, S2 has a great number of MP, representing alone 66.55% of all plastic particles found in the North, which is the cause of this region placing second in MP pollution rate.

S1 is characterized as a reflective coast with a large sand strip, the presence of a river mouth (Riacho river), and an artificial rocky shore (Portocel’s northern Pier) (Albino et al., 2006). The *P. caudata* colony structure was formed by a thin layer of tubes placed under the rocks of the Portocel Pier. The beach hydrodynamics was calm with little wave energy. Anthropic occupation in the region is not intense despite the presence of a large port complex. These factors together could influence the small number of MP found in Portocel.

S2 is characterized as an exposed beach, with a narrow sand strip, intermediate (between reflective and dissipative), the presence of laterite rocks, and a large river mouth (Piraquê-Açú River) (Albino et al., 2006). The Piraquê-Açú River basin is composed of the Piraquê-Açú and the Piraquê-Mirim Rivers with an area of 448 km² and a flow rate of 14.5 m³/s (Barroso et al., 2012; Leite, 2012). Rivers and other freshwater bodies can transport plastic debris disposed of on their shorelines into the ocean (Lee et al., 2013; Lechner et al., 2014; Lebreton et al., 2017; Eo et al., 2018), this means microplastic transportation and possibly formation by the river flow physical impacts. Thus, this continuous disposal of fresh water probably comes with a high amount of MPs gathered in the extension of the Piraquê-Açú basin and all its effluents. During low tide, dunes are formed in the mouth of the basin, a hydrodynamic structure that may help trap microplastics on the nearest beaches. These factors may be contributing to the high numbers of MP found on this sampling spot. The main difference between Portocel and Piraquê-Açú spots is the size of the rivers, the Riacho River is much smaller than the Piraquê-Açú basin.

S3 is a sheltered and dissipative beach with a large sand strip (Albino et al., 2006). Placed in the northern of Fundão city, S3 is inserted in a high populated area compared to S2 and S1. The beach also receives visitors, who sometimes do some events on the sand strip, producing a significant amount of garbage, usually plastic materials. Being a sheltered beach, S3 is protected and, therefore, away from the open sea. Masiá et al., (2019) observed that the density of MP on sheltered beaches is lower than on beaches near the open sea. This can explain the difference in the number of MP between the sampling spots in the North.

S4 is an exposed and intermediate/dissipative beach with the presence of laterite rocks and a narrow sand strip (Albino et al., 2006). The S4 beach is located inside a touristic neighborhood in Serra city, the region is known for its restaurants and has a high movement of people. Although being an exposed beach, the three replicate samples were empty of *P. caudata* individuals suggesting two major hypotheses. (1) The natural bench of *Phragmatopoma caudata* was dead and (2) Even after the death of the *P. caudata* the colony structure still works as a trap for the MPs already incorporated in the tubes, preventing them from returning into the water column, and as a home for several other organisms once the colony lasts for an uncertain time. This could explain why S4 had fewer microplastics than S3, even being an exposed beach.

4.2. The Central region

The Central region comprises the four sampling spots of S5, S6, S7, and S8. This region showed the highest numbers of MP found in this study comprising alone 56.54% of all 7005 particles, evidencing the highest rate of MP pollution in the analyzed areas. However, among the four sampling spots in the Central region, S5 represents 46.2% of plastic debris. The number of black filaments overcame the number of blue filaments and this only happened in this region. Also, the number of red filaments was considerably high when compared to the other regions (1015 in the Central against 190 and 243 in the North and South, respectively).

S5 is characterized as a dissipative sheltered beach with a large sand strip and frontal dunes (Albino et al., 2006). The sampling spot is located on the premises of the Tubarão port complex, under the management of Vale S.A, an international mining company. The Tubarão complex has an area of 14 km², was considered the largest iron ore port in the world (Vale S.A., 2016), and has a big impact on the economy and environment of Vitória, the capital of Espírito Santo state, where it is located. On 28 September 2018, an Environmental Commitment Term (TCA) was signed between Vale S.A. and the State government aiming to protect and regrow the sandbank in

Camburi beach, including Ponta de Tubarão (Termo de Compromisso Ambiental n° 035/2018).

The TCA predicts the installation of protection fences, planting of native flora, and the recovery of the northern part of the beach (S5) from iron ore debris disposed of in the region by the mining company in the 70s (Vale S.A., n.d.). The recovery project foresees the replacement of the superior layer of sediment (50 cm deep) with new clean sand material. Although the project predicts the protective removal of native big fauna like birds, amphibians, and rodents, it did not talk about the impact on the small fauna such as mollusks, polychaetes, and crustaceans, abundant in the area. The work on the recovery process removed all the mangrove trees on the beach, expanding the sand strip and leaving it exposed to beach waves. This removal process disturbs the bottom sediment liberating plastic particles and other debris accommodated at the bottom of the beach. Nevertheless, during sampling, it was possible to see hundreds of dead *Aplysia* in the sand due to bottom sediment perturbation, evidencing the scale of environmental disturbance of these projects directly on the beach. Also, next to Ponta de Tubarão, there is the mouth of the Camburi River, a small freshwater body that flows freely into the beach. The set of these factors, linked with the high rate of ship routes due to the port's presence, can be the cause of this spot being the highest rate of MP pollution found in this study. Due to the disposal of iron ore in S5 in the 70s, iron ore dust in the sediment of the beach is ubiquitous. These iron particles made the sorting of MP, especially black fragments, very difficult, once they can make MP identification inaccurate for being too similar to some plastic particles. Thus, it is pertinent to point out that all samples from S5 were also contaminated with iron ore particles besides MP.

S6 is a semi-exposed intermediate beach with a large sand strip, an artificial rocky shore (Iemanjá Pier), and a river mouth (the Passagem Channel). The Passagem Channel is part of the estuarine complex of the Santa Maria da Vitória River, connecting Vitória Bay with the Espírito Santo Bay (Miranda et al., 2002). Through its course, the Passagem Channel receives effluents like sewage from some districts in the surroundings (Castro, 2001) alongside garbage incorrectly disposed of directly into the channel's waters. Also, the Channel has a strong fishery activity holding some marinas and ship maintenance ports (Costa et al., 2017). The Iemanjá Pier receives an intense anthropic circulation, with a lot of fishers, bathers, and tourists, especially on some Umbanda celebration dates. Although the presence of the Passagem Channel certainly contributes to the number of MPs in Espírito Santo Bay, it is unknown the reasons that made Iemanjá have the smallest number of MPs in the Central area. The colony samples were collected on the left side of the Pier, opposite the Channel, once the Channel side does not have *P. caudata* structures (probably due to the salinity difference coming with the estuarine waters from Santa Maria da Vitória River), and this could be a reason for the small number of plastic.

S7 is a sheltered beach with an artificial rocky shore and intense ship circulation (Albino et al., 2006). The beach is located under the Terceira Ponte, a bridge that connects the two cities of Vitória and Vila Velha, and is probably the busiest road in the metropolitan region. The beach receives visitors and also has severe ship traffic activity. The most concerning factor in S7 is the mouth of enormous sewage coming from Vila Velha, the Costa Channel that runs through 23 different districts in the city (Gonçalves & Devens, 2017). These factors could contribute to the high levels of MPs found in S7.

S8 is characterized as an intermediate, exposed beach with a large sand strip and frontal dunes (Albino et al., 2006). The sampling spot is located in a highly anthropic area which makes it a very busy beach that receives a lot of visitors. This anthropic circulation contributes to plastic and other kinds of trash disposing of. As an exposed beach, the currents of the open sea that runs through the area could be bringing MP particles which would also contribute to the high number of microplastics. Also, S8 is the only exposed beach analyzed in the Central region, and according to Masiá et al. (2019), exposed beaches tend to present more MPs than sheltered ones. Despite S5 being the most polluted sampling spot, its particularities, as seen, contributes to this result and therefore S8 has a considerably high rate of MP.

4.3. The South region

The South region comprises the four sampling spots of S9, S10, S11, and S12. This region showed the least variable number of MPs, the smallest sampling spot had 150 MPs (S9) and the highest 353 (S10). Thus, the South region is the least polluted area among all three analyzed regions. Nevertheless, S11 and S10 are considered safe spots for some chemical contamination like Tributyltin (TBT), once there is a low anthropic impact directly on these beaches such as the presence of ports and cities in the surroundings (Costa et al., 2017; França et al., 2020).

S9 is a reflective/intermediate, exposed beach with a large sand strip (Albino et al., 2006). The beach is located inside the new portion of Guarapari City and therefore is inserted in a highly-populated area. Guarapari city is known to receive a massive number of visitors, whether from the Espírito Santo State or from other Federal States, such as Minas Gerais, the northern portion of Rio de Janeiro, and some cities in southern Bahia. Although this may be the most anthropic-affected sampling spot in the South region, it yet showed the smallest number of MP, even as an exposed beach, and we couldn’t find a hypothesis for this situation.

S10 is characterized as an intermediate, exposed beach with a large sand strip and a natural rocky shore completely exposed to the open sea (Albino et al., 2006). The rocky shore in S10 is located 410 m away from the nearest road and there is an extremely low anthropic occupation in the surroundings. The results found show that microplastics do not behave as chemical pollutants, and thus chemical-clean sampling spots are not free of plastic particles.

S11 is a dissipative/intermediate, exposed beach with lateritic rocks and a large sand strip (Albino et al., 2006). Although the beach receives visitors, S11 is distant from residential agglomerates and has a small traffic of cars and boats/ships. There is no particularities in S11 that would explain the small number of MPs other than isolation from direct anthropic impact.

S12 is characterized as a dissipative/intermediate, exposed beach with a large sand strip (Albino et al., 2006). Although the beach is exposed, the samples were collected inside the relatively new pier built on the central beach of Itaipava city, turning the local sampled sheltered from direct wave impact. The area has a high anthropic occupation and, due to Pier’s presence, also ship traffic, which could be contributing to the presence of MPs in the area.

4.4. General Overview

According to the Development Plan for the Espírito Santo State (ES2030, 2013), the State is divided into ten micro-regions (MR). Three of them cover the 12 sampling spots contemplated in this study: the MR1 (Comprising the Central region and

Guarapari sampling spot), the MR4 (Comprising the South region), and the MR7 (Comprising the North region). The Development Plan showed the population growth between 1960 and 2010 (the last demographic census) where MR1 had a population growth of 7.8 times with a rate of 0.72 people/km^2 in 2010, while MR4 and MR7 presented only 2.2 (0.05 people/km^2) and 2.77 (0.04) times respectively. This evidences the similarity between the North and the South, despite the contrast created by Piraquê-Açú in the North.

The Brazilian Institute of Geography and Statistics (IBGE) estimated, based on the 2010 demographic census, the population of all cities in Brazil for 2021 (IBGE, 2022). This data supports the previous information from the ES2030 Plan (Table 2). The estimated population for all cities where this study has sampling spots also shows a similarity between the South and North region and a notably higher population/km^2 in the Central region.

Despite the similarity in the demographic data, the North region overcomes the South in the total number of MP only due to S2. The data produced in this study also shows that high anthropic areas tend to present high levels of MP, as seen in the Central region.

Table 2: Estimated population and city area data for 2021 based on the 2010 demographic census (IBGE, 2021) in order with population/Km^2 from the higher (Vitória) to the smaller (Itapemirim). In orange the Central region, in blue the North, and in yellow the South.

Cities	**Population**	**Area (Km^2)**	**Pop./Km^2**	**Sampling Spots**
Vitória	369,534	97.123	3.80	Ponta de Tubarão, Iemanjá, and Praia do Meio
Vila Velha	508,655	210.225	2.41	Pedra da Sereia
Serra	536,765	547.631	0.90	Manguinhos e Praia Grande
Guarapari	128,504	589.825	0.21	Guarapari
Aracruz	104,942	1420.285	0.07	Piraquê-Açú Mouth and Portocel
Anchieta	30,285	409.691	0.07	Pontal de Ubu and Boca da Baleia
Itapemirim	34,957	550.710	0.06	Itaipava Pier

The correlation between MPs in the digested tissues and the wet weight of the *P. caudata* showed a positive relation and the Spearman test showed that this relation was significant. This evidences that the bigger the individuals more microplastic they can ingest. Hamzah et al. (2021) tested the correlation between body width and ingested microplastic for *Namalycastis sp.*, an estuarine polychaete, and found a similar dispersion, which could be a pattern for polychaetes MP ingestion. However, it is important to note the difference between absorption, plastic particles ingested by organisms, and adsorption, plastic particles attached to the organism's body, and the *P. caudata* individuals weren't washed implying that MPs found in the digested tissues are a combination of absorbed and adsorbed particles. The R_s value for the Spearman relation between MP and wet weight was 0.61, suggesting that the correlation between them is moderate, reinforcing the need to rise the sampled number of individuals to better test this hypothesis.

The presence of pollutants such as tributyltin (TBT) have also been reported for all Espírito Santo shoreline since 2006, two year before the global TBT banning. This pollutant is present in the environment of almost all Espírito Santo coast, with intense impact observed near port complexes and marinas such as the metropolitan region in the Central area (Otegui et al., 2019). The presence of these contaminants in the waters, alongside Mp's capability of carrying chemicals through the food chain, and even the intense fishery activity that can be seen in several locations of the State, reinforces the alarming situation of MP contamination.

Data found in this study corroborates with Costa et al. (2021). They evaluated MP in *P. caudatal* tissues and tubes and found MP in all analyzed matrixes. Their most frequent filament color was also blue, although black filaments were massively smaller than the ones found in this study, it evidences that MP pollution is a continuous threat. The methodology used in their study was slightly different regarding the visual sorting and filtration, preventing statistical analysis, showing the urgent need to standardize MP methodological analysis. Costa et al. (2021) also found a very small number of pellets, suggesting their rareness in the environment. Also, the predominant color of fragments they found was blue, the same found in here pointing to two hypothesis (1) blue fragments are indeed the most frequent in the marine environment or (2) there is some accurate color selectivity mechanism during grain selection in *P. caudata*.

Effects of MP particles in polychaetes metabolism has been reported (Gusmão et al., 2016; Missawi et al., 2020; van Cauwenberghe et al., 2015). However, direct effects of MP ingestion in the metabolism of *P. caudata* is yet unknown, thus this study shows an alarming situation by evidences the massive presence of MP in the colony structure and tissues of these polychaetes. The same predominance of filaments were not seen for the polychaete *Marphysa sanguinea*, that showed a preference for plastic fragments (Pequeno et al., 2021), evidencing the diversity in the interactions with MP even inside the same group.

5. CONCLUSION

Microplastic pollution is indeed ubiquitous and therefore a great threat to modern society as a whole, especially intertidal coast zones due to its extremely sensible environments. It is still unknown the full impacts of MP in these environments, but the very presence of these particles in the structure of *P. caudata* colonies and tissues threats these microhabitats.

Filaments are more frequent in the marine environment than fragments and other types of microplastic. The most frequent colors are blue, black, and red, and this seems to repeat in several MP studies. Pellets are also rare, suggesting that, even though primary microplastic is massively produced in our society and at the same rate introduced in the environment, industrial pellets suffer from degradation or they end up somewhere else than the coastal zones.

The extensive bank of *P. caudata* colonies is a trustable, effective, and low-cost indicator of MP presence and abundance in coastal areas. However, it is crucial to keep investigating the mechanisms of particle selectivity in these species to understand the pattern of microplastic absorption, adsorption, and incorporation into colony structure. Also, it is unknown how much time this species takes to incorporate MP from the water column and therefore unknown how much the data from *P. caudata* colonies reflect the present time. Nevertheless, this logic applies in reverse, where colonies can trap MP for a long period, possibly reflecting the MP pollution through time, and preventing these plastic debris to return into the food chain.

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