



Anthropogenic microparticles accumulation in small-bodied seagrass meadows: The case of tropical estuarine species in Brazil

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ARTICLE INFO

Keywords:

Microplastic
Marine pollution
Tropical Atlantic
Sediment
Seagrass traits
Coastal ecosystem

ABSTRACT

Seagrass meadows have recently been highlighted as potential hotspots for microplastic and anthropogenic microparticles (APs). This study assessed AP accumulation in shallow sediments vegetated by small-bodied seagrass species (*Halodule wrightii*, *Halophila decipiens*, and *H. baillonii*) and in the adjacent unvegetated area in a tropical estuary on the East Coast of South America, Brazil, over the seasonal cycle. Anthropogenic microparticles were detected in 80 % of the samples, with a mean abundance of 142 ± 140 particles kg^{-1} dw ($N = 80$). Particles were predominantly blue (51 %), fiber (73 %), and smaller than 1 mm (80 %). We observed that seagrass sediments retained APs, although no significant variation was observed between seagrass and the unvegetated area, nor between the dry and rainy seasons. A positive correlation was found between sediment grain size and AP abundance. This study represents the first record of AP contamination in seagrasses from the Tropical Southwestern Atlantic bioregion.

1. Introduction

Anthropogenic particles are dispersed in every ecosystem worldwide, but the ocean is recognized as the primary reservoir for these contaminants (Barnes, 2002; Loganathan et al., 2023; Cózar et al., 2014; Law, 2017; González-Fernández et al., 2021; Finnegan et al., 2022). Due to their transboundary nature, synthetic (e.g., plastics) and semi-synthetic (e.g., cellulose fibers) items originating from anthropogenic use have been detected from surface waters to the deep sea and from the Arctic to the Antarctic Oceans (Cole et al., 2011; Cózar et al., 2014; Lima et al., 2015; Peeken et al., 2018; González-Fernández et al., 2021; Athey and Erdle, 2022). Even remote areas, such as oceanic islands, are vulnerable to this type of contamination (Rodríguez et al., 2020; Magalhães et al., 2022). The input of anthropogenic particles into marine ecosystems is driven by a range of factors, including river discharge, rainfall, wind, currents, coastal geomorphology, coastline shape, tidal

range, bathymetry, human population density, and solid waste management efficiency (Barnes et al., 2009; Li et al., 2016; Lebreton et al., 2017).

The anthropogenic material consist of plastic and other debris such as glass, cellulose, and metals originating from human activities, turning into marine debris when they reach the sea (Jambeck et al., 2015). When smaller than 5 mm, they are defined as anthropogenic microparticles (APs) (plastic and non-plastics of human origin) and, in the particular case of plastics, microplastics (MPs) (Barnes et al., 2009; Thompson et al., 2009; Frias and Nash, 2019). Primary MPs are manufactured at this size (GESAMP, 2015) for use as microbeads in cosmetics or as pellets that serve as raw materials to produce plastics materials (Laskar and Kumar, 2019). However, larger particles can suffer fragmentation caused by physical, chemical, or biological factors, such as the action of ultraviolet radiation, high temperatures, and mechanical stress from hydrodynamics, and, consequently undergo size and color modification

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<https://doi.org/10.1016/j.marpolbul.2024.116799>

Received 25 March 2024; Received in revised form 1 July 2024; Accepted 30 July 2024

Available online 23 August 2024

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from the original product, becoming secondary MPs (Sudhakar et al., 2007; Andrady, 2011; GESAMP, 2019; Zhang et al., 2021).

Recent studies in aquatic environments have shown that MP accumulate in coastal vegetated ecosystems such as mangroves and seagrasses, adhering to their canopies or being buried in their sediments (Remy et al., 2015; Yan et al., 2019; Jones et al., 2020; Huang et al., 2021; Cozzolino et al., 2020; Huang et al., 2020; Jones et al., 2020; Sánchez-Vidal et al., 2021; Navarrete-Fernandez et al., 2022). This accumulation can be explained by the fact that seagrass meadows act as natural filters in coastal waters, making them very efficient at retaining suspended particles from the water column as their canopy reduces hydrodynamics, resulting in higher sedimentation rates than in adjacent unvegetated ecosystems (Short and Short, 1984; Terrados and Duarte, 2000; Pinheiro et al., 2021). While reducing MP concentrations in the water column can be considered a potential ecosystem service (Sánchez-Vidal et al., 2021), it may also increase the chances of ingestion by marine fauna inhabiting seagrass ecosystems (Ouyang et al., 2022; Cozzolino et al., 2020).

Microplastic abundance studies in seagrass meadows are relatively new and scarce, with less than a decade of research (Goss et al., 2018; Li et al., 2023). Most studies focus on seagrass species in bioregions such as the Temperate North Atlantic, Mediterranean, Temperate North Pacific, Tropical Indo-Pacific, and Temperate Southern Oceans (bioregions defined as in Short et al., 2007), where meadows are primarily formed by large-bodied species like *Posidonia oceanica* and *Zostera marina*. For instance, the capture of MPs was observed in aegagropilae (balls formed by aggregates of seagrass fibers) and stranded remains of *P. oceanica* (Sánchez-Vidal et al., 2021). However, accumulation of APs in seagrass

sediments from the Tropical Atlantic bioregion, primarily formed by small-bodied species found in South America (*Halodule* spp. and *Halophila* spp.; Magalhães and Barros, 2017), has not yet been investigated. This deserves attention since the role of seagrasses in providing ecosystem services varies among species and bioregions (Nordlund et al., 2017). Differences in traits among seagrass species may drive the variation in the services, so it is necessary to investigate ecosystem services in a variety of sizes and forms, especially in bioregions that are underrepresented in seagrass research.

This study aimed to investigate contamination by anthropogenic microparticles (i.e., plastic and non-plastic particles <5 mm resulting from human activity) in seagrass meadows in the Southwest Tropical Atlantic bioregion. Here we tested the hypothesis that (I) seagrass meadows formed by small-bodied species are capable of accumulating a greater APs abundance in the sediment than unvegetated adjacent areas, and (II) the seasonal variability, driven by rainfall patterns, influences AP accumulation. To test these hypotheses, we focused on meadows within an estuarine ecosystem in the Southwestern Tropical Atlantic, composed of *Halodule wrightii*, *Halophila decipiens*, and *H. baillonii*, along with the adjacent bare sediment area.

2. Materials and methods

2.1. Study area

The study was conducted in the Santa Cruz Channel Estuarine Complex (SCC) (7°41'09"S and 34°51'21"W) on the Northeast Brazilian coast (Fig. 1). This area is within the Environmental Protection Area

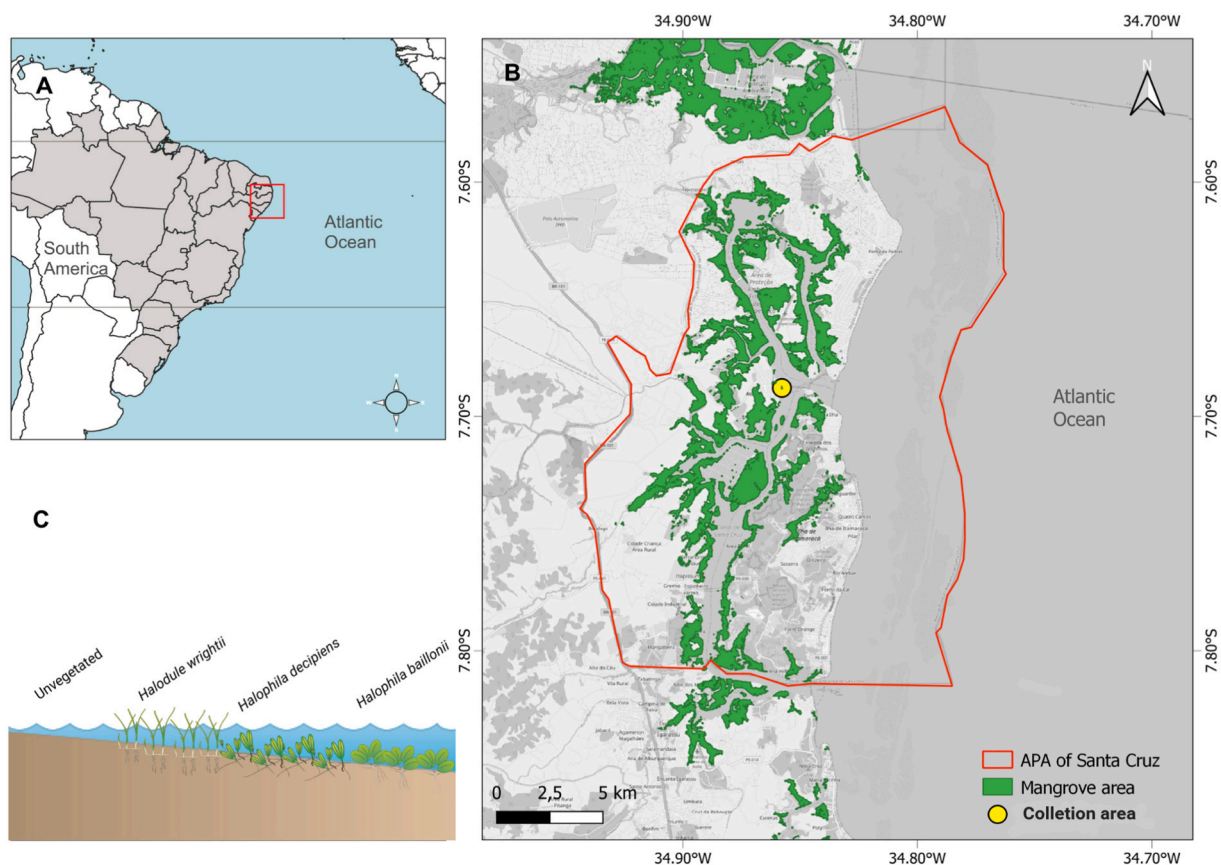


Fig. 1. (a) Study location on the East Coast of South America, Brazil (red square). (b) Collection area (yellow dot) in the Santa Cruz Channel Estuarine Complex (SCC), featuring the Environmental Protection Area (APA) of Santa Cruz (red polygon). (c) Zonation of the unvegetated and vegetated sampling areas, with the three seagrass species. Credits: APA and mangrove areas in (b): Natural Earth data, OpenStreetMap (OSM), and World Conservation Monitoring Centre (UNEP-WCMC) (version 3/2020); diagram in (c): IAN symbol - CC by 4.0. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(APA) of Santa Cruz, a Marine Protected Area. According to Brazilian legislation, human activities that promote the economy with proper management of the area, such as fish farming activities, are allowed (Decreto n° 32.488/, 2008; Moura, 2009) (Fig. 1). The APA shelters three estuarine areas and is a habitat/species management area established to protect endemic, rare, and endangered species (CPRH, 2008; Coutinho et al., 2018) as well as traditional communities that subsist on artisanal fishing and small-scale tourism (Coutinho et al., 2018; Niencheski et al., 2014). However, this area also hosts metallurgical, chemical, textile, automobile, sugarcane, food, paper, plastic production, paint, and cement industries (CPRH, 2008). The SCC encompasses 8000 ha of mangroves with mud and sandy soils, rich in organic matter in the floodplains (Moura, 2009), as well as seagrass meadows with three different species: *Halodule wrightii*, *Halophila decipiens*, and *H. baillonii* (Fig. 1c and Fig. 2).

The SCC has a tropical climate with a semidiurnal tide ranging from 1.1 to 2.2 m, and the maximum water depth in the main channel varies between 4 and 5 m (Coutinho et al., 2018; Medeiros and Kjerfve, 1993). During the field data collection period, the average water temperature was 29 ± 0.2 °C, the salinity 29 ± 0.8 and the average monthly rainfall for the rainy season (June to August 2021) was 109 ± 35 mm. During the dry season, the average monthly rainfall was 103 ± 52 mm (December to February 2022), while the water temperature and salinity remained stable throughout the sampling area at 32 ± 0 °C and 36 ± 0 , respectively (Table S1).

2.2. Sampling design

Sampling was carried out during the rainy (August 2021) and dry (February 2022) seasons at two sampling areas: one unvegetated (bare sand) and one vegetated area with three seagrass species. Seagrasses were distributed in the vegetated area in a zonation gradient, with *H. wrightii* in the intertidal area, followed by *H. decipiens* and *H. baillonii* on the edge of the subtidal area, with the seagrasses positioned slightly lower than the unvegetated area (Fig. 1c). For each species in the vegetated area (*H. wrightii*, *H. decipiens*, *H. baillonii*) and in the unvegetated area, during each season (dry and rainy), ten randomized sediment samples for AP analysis were collected, distributed in two plots (5 samples per plot), with a minimum distance of 50 m between plots and 1 m between samples (Fig. S1).

Sediment samples (upper 10 cm layer) were collected using a cylindrical stainless-steel collector (5 cm diameter, 10 cm length), transferred to aluminum foil envelopes, and stored at -20 °C until further analysis. Replicated sediment samples (following the same design as previous samples) were also collected for granulometric analysis (10 samples for each area per season) using the same collector. Additionally, five replicated seagrass biomass samples were collected per plot and season with an acrylic collector (6 cm diameter, 10 cm depth), yielding 10 samples for each species per season (Fig. S1).

2.3. Characterization of seagrass meadow and sediment granulometry

Seagrass samples were washed in running water to remove sediments. From each sample, the ten longest leaves of *H. decipiens* and *H. baillonii* were measured (width and length, in cm) using a ruler (± 0.1 mm), and the *H. wrightii* leaves were measured using a stereomicroscope ($\times 10$ magnification). The shoots in each sample were counted for shoot density determination (m^2 shoots m^{-2}). The aboveground (AG, leaves) and belowground (BG, roots and rhizomes) portions of the plants were separated and dried in an oven at 50 °C until reaching a constant dry weight (dw) to estimate biomass (g dw m^{-2}).

For each sediment sample, a sub-sample of approximately 0.5 g of fresh weight (fw) was mixed with 0.15 mL of a sodium hexametaphosphate solution (~ 0.05 mol L^{-1}) for an hour to disperse the smallest sediment particles (Ward, 2020). The particle size analyses were performed using a laser scattering particle size distribution analyzer (Partica LA-950 V2, Horiba), which measured the water-dispersed particles with size ranging from 0.01 to 4000 μm (error of 5 % for particle diameter and size frequency). The granulometric fractions were sorted using the LA-950 Phi Scale Graph into very fine silt (3.91–6.57 μm), fine silt (7.81–13.14 μm), medium silt (15.63–26.28 μm), coarse silt (31.25–52.56 μm), very fine sand (62.5–105.11 μm) and fine sand (125–210.22 μm). Each fraction was expressed as a percentage of fresh weight (% fw) (Wentworth, 1922).

2.4. Extraction and characterization of anthropogenic microparticles

Anthropogenic particles in the sediment were extracted by density separation using a hypersaline solution, followed by chemical digestion and separation by filtration (Phuong et al., 2021). First, natural debris (e.g., shells, gravel, plant fragments) was carefully removed from the samples using tweezers. Then, the sediment was manually homogenized with a glass rod and subsequently oven-dried at 50 °C until a constant dry weight (dw).

A sub-sample of 20 g dw was transferred into 150 mL beakers and filled with hypersaline solution (NaCl , 352 g L^{-1}) to its maximum volume. The sediment in the solution was manually homogenized with a glass rod for 2.5 min, then covered with aluminum foil and left to settle for 24 h in a fume hood at room temperature. To remove possible materials of organic origin in suspension or adhered to the suspended particles, the supernatant (average 120 mL) above the decanted material was transferred with a glass pipette into a beaker and subjected to digestion with a sodium hydroxide solution (NaOH , 1 mol L^{-1}) (GESAMP, 2019; Phuong et al., 2021).

The digestion was performed in beakers covered with aluminum foil, with a supernatant to NaOH solution ratio of 2:3, for 24 h at 50 °C. Afterwards, the solution was filtered with GF/F Whatman fiberglass filters (47 mm diameter, 0.7 μm of pore size) with a vacuum system setup. The supernatant was subdivided to avoid filter clogging, using 2 to 3 filters per sample. Filters were placed in closed glass Petri dishes and



Fig. 2. Seagrass species inhabiting the Santa Cruz Channel Estuarine Complex (East Coast of South America, Brazil, in the Tropical Atlantic seagrass bioregion): (a) *Halodule wrightii*, (b) *Halophila decipiens* and (c) *Halophila baillonii*.

dried at room temperature.

Filters were visually examined under a stereoscopic microscope (Zeiss Stemi 508) at $\times 40$ or $\times 50$ magnification (detection limit of 20 μm) to identify possible APs based on the protocol of GESAMP (2019). Each potential AP particle was measured along its longest axis and classified, according to Hidalgo-Ruz et al. (2012) and Gündoğdu (2017), based on their shape (fiber, film, foam, fragment, and pellets) and color (black, blue, brown, green, red, transparent, and white). Then, AP particles ($< 5\text{ mm}$) were classified into 5 size classes: $< 0.1\text{ mm}$, 0.1 to 0.5 mm , 0.5 to 1 mm , 1 to 3 mm , and 3 to 5 mm .

The APs were counted in each sample, and AP abundance was expressed as the number of particles per kilogram of dry sediment (particles $\text{kg}^{-1}\text{ dw}$). The frequency of occurrence (%) of APs was expressed as the percentage of samples (in the sampling area and/or seasons) in which APs were detected.

2.5. Quality control and quality assurance

To mitigate contamination at the laboratory, precautionary measures listed in Hanvey et al. (2017) were adopted. Before the laboratory analysis, all workstations and equipment were cleaned with 70 % filtered ethanol (GF/F fiberglass, $0.7\text{ }\mu\text{m}$ of pore size) and cellulose paper towels. The glassware and utensils were washed with filtered distilled water and oven-dried at 50°C . Every person in the laboratory wore a 100 % cotton lab coat and latex gloves. Samples were mostly handled in a fume hood, and beakers or Petri dishes were always sealed with glass lids or aluminum foil to prevent airborne contamination.

The NaCl and NaOH solutions were prepared with filtered distilled water, stored in borosilicate containers, and covered with aluminum foil. A procedure blank was performed in parallel with each sample batch (5 samples per batch) to evaluate airborne contamination. This blank consisted of a beaker filled with 150 mL of NaOH solution and subjected to the same procedures as the samples. From the 16 blanks performed, five particles (2 blue films, 1 black fiber, 1 blue fragment and 1 red fragment) were detected. Whenever contamination was detected in the blanks, the characteristics (shape and color) of the given particles were subtracted from the corresponding batch.

2.6. Statistical analysis

The seagrass meadow characteristics (aboveground biomass, belowground biomass, leaf length, leaf width, and shoot density), sediment grain size, and APs abundance were presented as mean and standard deviation (SD). All data was tested for normality (Shapiro-Wilk test) and homoscedasticity assumptions (Fligner test). Data transformations (\log_{10} or square root) were performed when the parametric assumptions were not met. A two-way ANOVA was used when parametric assumptions were met, followed by a post hoc Tukey test. When the assumptions were not met, even after data transformation, the non-parametric Scheirer Ray Hare (SRH) tests were applied, followed by Dunn's post hoc test (with Bonferroni correction).

Seagrass meadow variables (seagrass characteristics described above) were compared among species (3 levels: *Halodule wrightii*, *Halophila decipiens*, and *H. baillonii*), seasons (2 levels: dry and rainy seasons), and their interactions. Sediment grain size was compared between seasons (2 levels: dry and rainy seasons), sampling areas (4 levels: *Halodule wrightii*, *Halophila decipiens*, *H. baillonii*, and unvegetated), and their interactions (species \times seasons).

Anthropogenic particle (AP) abundance was compared between sampling areas (2 levels: vegetated and unvegetated), seasons (2 levels: dry and rainy seasons), and their interactions. We also evaluated AP abundance variability among sampling areas including the 3 seagrass species (4 levels: *Halodule wrightii*, *Halophila decipiens*, *H. baillonii* and unvegetated), seasons (2 levels: dry and rainy seasons), and their interactions. AP characteristics (shape, color and size) were also compared between their respective factors and levels. A Spearman rank order

correlation test was run to determine the correlation between sediment grain size and AP abundance.

Results were reported with the “F value” for the ANOVA tests, “H” for Scheirer Ray Hare tests, and “p” for Spearman's correlation coefficient, along with their degree of freedom (df) and the associated p-value. A critical α level of 0.05 was used for all hypotheses tested. Data analysis was performed in the R programming language (version R.4.1.3, R Core Team, 2022) and RStudio software (version 4.1.3, 2022-03-10). The dataset generated for this study will be available at the Zenodo repository (<https://zenodo.org>) after publication of this articles.

3. Results

3.1. Characterization of the seagrass meadow and sediment granulometry

AG and BG biomass varied among species ($F_{2,54} = 11.4$, $p < 0.05$ and $H = 20.14$, $p < 0.05$, respectively), but only AG varied between seasons (AG: $F_{2,54} = 5.30$, $p < 0.05$ BG: $H = 2.05$, $\text{df} = 2$, $p > 0.05$) (Table S2). Overall, *H. wrightii* presented AG and BG biomasses more than two and three-fold higher, respectively, than *H. decipiens* and *H. baillonii* in both seasons (Table 1, Fig. S2).

Leaf length ($H = 39.36$, $p < 0.05$) and leaf width ($H = 38.30$, $p < 0.05$) varied among species, with *H. wrightii* having longer and narrower leaves than *H. decipiens* and *H. baillonii* (Table 1, Fig. S2). However, the leaf length and leaf width did not vary under the influence of the seasons (Table S2). Shoot density did not vary among species ($H = 0.36$, $p > 0.05$) or seasons ($H = 2.68$, $p > 0.05$), but their interaction ($H = 12.52$, $\text{df} = 2$, $p < 0.05$) indicated that *H. wrightii* had the highest density during the rainy season ($7253 \pm 5530\text{ m}^{-2}$) (Fig. S2, Table S2).

Overall, the unvegetated area had significantly finer sediments than the vegetated areas ($H = 23.22$, $p < 0.05$), and the variation in sediment grain size was significant between vegetated and unvegetated areas during the seasons ($H = 8.95$, $\text{df} = 3$, $p < 0.05$) (Table S2). The sediment of the vegetated areas was characterized by fine sand or fine silt, depending on species and season, while the sediment of the unvegetated area was fine silt for both seasons (Table 1, Fig. S3).

3.2. Anthropogenic microparticle abundance between vegetated and unvegetated areas, seagrass species, and seasons

A total of 227 APs was identified in the 80 sediment samples, with an average size of $0.59 \pm 0.84\text{ mm}$, occurring in 80 % of the samples with an average AP abundance of $142 \pm 140\text{ particles kg}^{-1}\text{ dw}$. The unvegetated areas showed the highest frequency of occurrence of APs in the samples (85 %), while *H. decipiens* showed the lowest (75 %) (Fig. 4d). There were no significant differences in AP abundance between the vegetated and unvegetated areas ($H = 0.00$, $p > 0.05$), and the interaction of season with areas ($H = 1.14$, $p > 0.05$). Among the seagrass species, there was also no significant difference ($H = 0.29$, $p > 0.05$), nor with the species and seasons interaction ($H = 2.21$, $p > 0.05$) (Fig. 3, Table S4). There was a weak positive correlation between sediment grain size and AP abundance ($\rho = 0.27$, $p < 0.05$).

3.3. Anthropogenic microparticle general characterization and variation with vegetated and unvegetated areas, seagrass species, and seasons

Considering the seagrass species (and the unvegetated control area), fibers were the most common AP shape, accounting for 73 % ($103.7 \pm 110.6\text{ particles kg}^{-1}\text{ dw}$) of the AP detected, followed by films (21 %, $30 \pm 62.5\text{ particles kg}^{-1}\text{ dw}$) and fragments (6 %, $8.1 \pm 24.3\text{ particles kg}^{-1}\text{ dw}$) (Table S3). Pellets and foams were not detected in the samples. The AP shape did not vary between vegetated and unvegetated areas ($H = 0.09$, $p > 0.05$), nor was there any interaction between areas and seasons (Table S4). AP shape did not vary among seagrass species ($H = 0.38$, $\text{df} = 3$, $p > 0.05$), nor with the species and season interaction (Table S4).

Blue was the most common color (51 % of the total of particles, 71.9

Table 1

Characterization of the seagrass meadows, sediment grain size, and anthropogenic microparticles found in seagrass meadows formed by three small-bodied species (*Halodule wrightii*, *Halophila decipiens*, and *Halophila baillonii*) and the adjacent unvegetated area, during the rainy and dry seasons, in the Santa Cruz Channel Estuarine Complex (East Coast of South America, Brazil, in the Tropical Atlantic seagrass bioregion). Values shown as mean \pm SD ($n = 10$).

Season	Species	Seagrass					Sediment		Microparticles		
		Aboveground biomass (g dw m ⁻²)	Belowground biomass (g dw m ⁻²)	Leaf width (mm)	Leaf length (cm)	Shoot density (shoots m ⁻²)	Mean size (μ m)	Predominant type	Microparticle frequency of occurrence (%)	Microparticle abundance (particles kg ⁻¹ dw)	Mean Microparticle size (mm)
Rainy	<i>Halodule wrightii</i>	15.8 \pm 6.7	36.1 \pm 17.6	0.18 \pm 0.06	6.2 \pm 1.2	7253 \pm 5530	76.7 \pm 69.4	Fine silt	80	150 \pm 103	0.52 \pm 0.73
	<i>Halophila decipiens</i>	2.7 \pm 2.2	4.6 \pm 5.8	0.38 \pm 0.08	1.0 \pm 0.2	1755 \pm 1256	136.4 \pm 58.7	Fine sand	60	140 \pm 146	0.34 \pm 0.67
	<i>Halophila baillonii</i>	7.3 \pm 5.9	9.9 \pm 8.1	0.48 \pm 0.17	1.3 \pm 0.1	3012 \pm 2215	173.2 \pm 80.3	Fine sand	90	185 \pm 216	0.62 \pm 0.74
	Unvegetated	–	–	–	–	–	33.6 \pm 12.5	Fine silt	90	170 \pm 119	0.54 \pm 0.60
Dry	<i>Halodule wrightii</i>	10.1 \pm 7.7	19.8 \pm 17.6	0.09 \pm 0.05	9.8 \pm 2.3	1111 \pm 687	173.4 \pm 160.5	Fine sand	80	140 \pm 118	0.61 \pm 0.92
	<i>Halophila decipiens</i>	6.4 \pm 5.7	6.9 \pm 10.6	0.48 \pm 0.05	1.5 \pm 0.2	4065 \pm 4137	114.8 \pm 61.3	Fine silt and fine sand	90	145 \pm 121	0.80 \pm 0.95
	<i>Halophila baillonii</i>	4.1 \pm 3.7	7.6 \pm 7.2	0.44 \pm 0.08	1.2 \pm 0.3	3012 \pm 2929	108.1 \pm 100.1	Fine silt	70	115 \pm 142	0.72 \pm 0.96
	Unvegetated	–	–	–	–	–	46.6 \pm 45.7	Fine silt	80	90 \pm 97	0.80 \pm 1.3

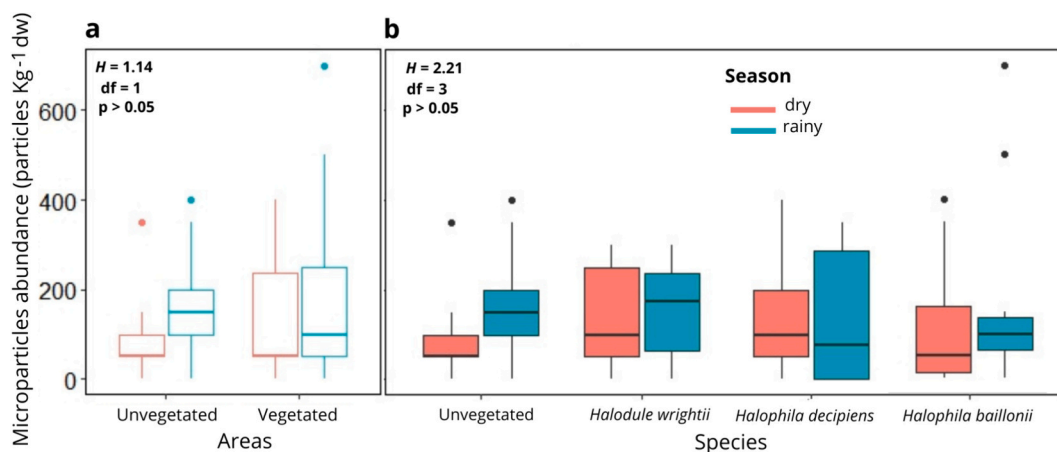


Fig. 3. Anthropogenic microparticle (AP) abundance (particles kg⁻¹ dw) over seasons for (a) vegetated and unvegetated areas, and (b) seagrass species in the Santa Cruz Channel Estuarine Complex (East Coast of South America, Brazil, in the Tropical Atlantic seagrass bioregion). The boxplot bars represent the median, the boxes the interquartile range (IQR), and whiskers extend to the maximum and minimum values within 1.5 times the IQR, with outliers shown as individual points beyond this range.

± 95.8 particles kg⁻¹ dw) of the microparticles, followed by black (28 %, 40.6 ± 54.8 particles kg⁻¹ dw), and transparent (10 %, 14.4 ± 30.8 particles kg⁻¹ dw). Brown, green, red, and white represented <11 % of the APs detected (Table S3). AP colors did not vary between vegetated and unvegetated areas ($H = 0.58$, $df = 1$, $p > 0.05$), nor was there any interaction between areas and seasons (Table S4). No variation was found among seagrass species ($H = 0.89$, $df = 3$, $p > 0.05$), nor with the species and season interaction (Table S4).

The longest dimension of APs had an average length of 0.59 ± 0.84 mm, ranging from 0.017 to 4.48 mm. The most prevalent size class was <0.1 mm (35 %, 52.5 ± 79 particles kg⁻¹ dw) (Table S3). The APs size did not vary between vegetated and unvegetated areas ($H = 0.11$, $df = 1$, $p > 0.05$), nor was there any interaction between areas and season (Table S4). Among seagrass species ($H = 0.3$, $p > 0.05$) and seasons ($H =$

0.9 , $p > 0.05$), the microparticle sizes were also not significantly different (Table S4).

4. Discussion

4.1. Anthropogenic microparticle accumulation in the sediment of small-bodied seagrass meadows and adjacent unvegetated areas

Our findings showed that the AP accumulation capacity in the seagrass meadows sediment formed by tropical small species does not differ from the adjacent unvegetated areas. Moreover, the AP abundance did not vary between the three study species (*Halodule wrightii*, *Halophila decipiens*, and *H. baillonii*), despite *H. wrightii* presenting AG and BG biomass more than two and three-fold higher than the other species.

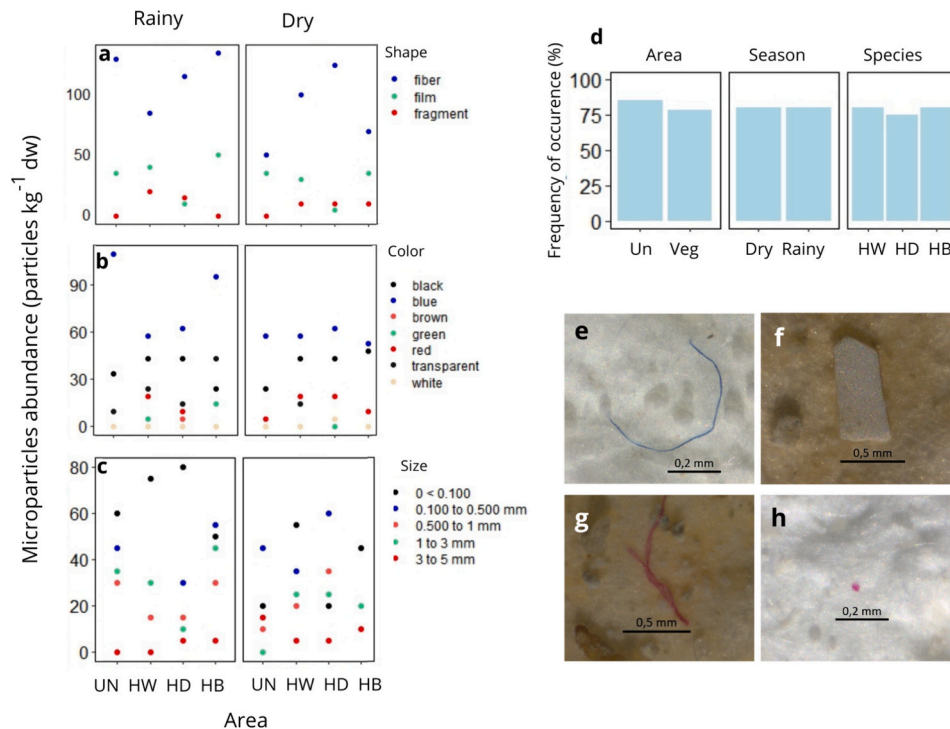


Fig. 4. Anthropogenic microparticle (AP) abundance regarding shape (a), color (b), and size (c) for unvegetated (UN - unvegetated) and vegetated areas (HW - *H. wrightii*, HD - *H. decipiens* and HB - *H. baillonii*). Frequency of occurrence (d) according to area (Un - unvegetated and Veg - vegetated), season (Dry and Rainy), and species in the Santa Cruz Channel Estuarine Complex (East Coast of South America, Brazil, in the Tropical Atlantic seagrass bioregion). Points in panels a, b and c represent the mean value of the AP abundance. AP particles detected in the samples: (e) blue fiber, (f) white fragment, (g) red fiber, and (h) red fragment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The average morphological characteristics observed (aboveground biomass, belowground biomass, leaf length and width) in the studied meadows were smaller than those previously reported for the Brazilian coast, as well as for other areas from the Tropical Atlantic and Temperate North Atlantic bioregions (Pangallo and Bell, 1988; Duarte and Chiscano, 1999; Den Hartog and Kuo, 2006; Short et al., 2007; Kruczynski and Fletcher, 2012; Magalhães et al., 2015). Therefore, seagrass dimensions and their characteristics are most likely a crucial factor for the lack of variability in AP accumulation in the coastal sediments between vegetated and unvegetated areas and among species. Additionally, sizes below the standard averages of the species may indicate an effect of anthropogenic impacts on meadow or of local environmental factors, such as hydrodynamics.

Indeed, the study area is notably characterized by chronic and acute mercury contamination. A substantial discharge of 22–35 metric tons of mercury-contaminated sediments occurred in the Botafogo River since 1965 due to activities associated with a chlor-alkali factory, and a major leakage event in the precipitation basin was documented in 1991 (Meyer et al., 1998; Albuquerque et al., 2019). Furthermore, elevated mercury concentrations have been identified in the local biota and human population (Nilson et al., 2001), highlighting that the small-bodied species might be sensitive to these anthropogenic impacts. Therefore, survey and long-term monitoring studies are essential in the study area.

The abundance of APs observed in this study in the vegetated and unvegetated areas was similar to that reported for other studies for small-bodied species with MP survey (*Halophila ovalis*, *Halodule uninervis*, *Zostera capensis*, *Z. noltei*, and *Ruppia megacarpa*) from other tropical and temperate bioregions (Tahir et al., 2019; Cozzolino et al., 2020; Boshoff et al., 2023; Wright et al., 2023). However, an exception to this accumulation pattern was observed by Huang et al. (2021), who found differences in MP abundance between meadows of *H. ovalis*, *Halophila beccarii*, and unvegetated areas in an estuary from the tropical bioregion in Southern China. In Huang et al. (2021), MP abundance was also

correlated with sediment grain size. The presence of dense mangrove forests, anthropogenic activities, and local hydrodynamics were highlighted as factors influencing the abundance of MP in the seagrass meadows (Huang et al., 2021).

Other studies with small-bodied species have also emphasized that MP accumulation in the sediment is contingent upon meadow characteristics and the life history of species (Huang et al., 2020; Wright et al., 2023). The studies conducted in meadows of larger-bodied species from different bioregions, such as *Posidonia oceanica* (Mediterranean), *Zostera marina* (Temperate) and *Enhalus acoroides* (Tropical), revealed a greater MP accumulation in vegetated sediments compared to adjacent unvegetated areas (Huang et al., 2020; Jones et al., 2020; Dahl et al., 2021; Navarrete-Fernandez et al., 2022) (Table 2). Considering that the magnitude of the ecosystem services provided by seagrass meadows varies according to species and bioregion (Nordlund et al., 2017), further studies should focus on covering unstudied seagrass species and evaluating whether seagrass traits and meadow complexity influence AP accumulation in different bioregions.

4.2. Accumulation of anthropogenic microparticles under the influence of environmental factors

Beyond the potential influence of seagrass characteristics on AP accumulation in the sediment, anthropogenic activities, and environmental factors such as seasonality, hydrodynamics, and sediment characteristics can influence accumulation capacity (Dahl et al., 2021; Menicagli et al., 2021; Navarrete-Fernandez et al., 2022; Cao et al., 2024). For large species like *Posidonia oceanica* in the Mediterranean, seasonal variability was observed in both vegetated sediments and the unvegetated areas, with accumulation also being correlated with different sediment depths and rainfall volume (Navarrete-Fernandez et al., 2022). However, seasonality did not influence either AP abundance or size in our samples. Similarly, MP abundance in the sediments

Table 2

Review of microplastic (MP) studies conducted in seagrass sediments. Adapted from [Unsworth et al. \(2021\)](#) with an update based on a literature review conducted in this study. * (Microparticle abundance).

Author	Extraction method	Site/system	Bioregion (Short et al., 2007)	Species	N	Average MP abundance (particles kg ⁻¹ dw)	Dominant shape	Dominant color	Dominant size
Tahir et al., 2019	ZnBr	Indonesia/island	Tropical Indo-Pacific	<i>Halophila ovalis</i> , <i>Cymodocea rotundata</i> , <i>Enhalus acoroides</i> , <i>Thalassia hemprichii</i> , <i>Syringodium isoetifolium</i> and <i>Halodule uninervis</i>	81	131 ± 100	fiber (84 %)	blue (35 %)	–
Huang et al., 2020	ZnCl ₂	China/estuary	Tropical Indo-Pacific	<i>E. acoroides</i>	12 (3 per habitat)	197 ± 16 780 ± 147	fiber (58 ± 16 %)	blue (40 ± 11 %)	< 250 µm
Jones et al., 2020	NaCl	Scotland/coastal	Temperate North Atlantic	<i>Zostera marina</i>	25	300 ± 30 (20)	fiber (> 69 %)	blue (> 45 %)	mean = 0.95 mm ± 0.05 SE
Jeyasanta et al., 2020	NaI	India/island	Tropical Indo-Pacific	–	36	203 ± 75	fiber (45 %)	transparent (29 %)	<1 mm (58 %)
Cuzzolino et al., 2020	NaCl	Portugal/coastal lagoon	Temperate North Atlantic	<i>C. nodosa</i> , <i>Z. marina</i> and <i>Zostera noltei</i>	40 (10 per site)	Intertidal: 18 ± 15 (0–11), subtidal: 35 ± 27 (1–21)	fiber (87.4 %)	blue (45 %)	<1 mm (58 %)
Huang et al., 2021	ZnCl ₂	China/coastal	Tropical Indo-Pacific	<i>Zostera japonica</i> , <i>H. ovalis</i> and <i>Halophila beccarii</i>	–	Site1: 230.49, site2: 192.45, site3: 43.60	fiber	blue	125–250 µm
Kreitsberg et al., 2021	NaCl	Estonia/coastal	Temperate North Atlantic	<i>Z. marina</i>	29	248 (total particles)	fiber (97 ± 5 %)	blue (79 ± 15 %)	<1 mm (mean = 1.351 µm)
Unsworth et al., 2021	ZnCl	England and Wales/coastal	Temperate North Atlantic	<i>Z. marina</i> and <i>Z. noltii</i>	80 (40 per site)	215 ± 163	fiber (92 %)	blue	–
Cheng et al., 2021	ZnCl ₂	USA/estuary	Temperate North Atlantic	<i>Z. marina</i>	25	116 ± 21	–	–	–
Dahl et al., 2021	NaI	Spain/coastal and island	Mediterranean	<i>Posidonia oceanica</i>	–	–	–	–	–
Zhao et al., 2022	ZnCl ₂	China/coastal	Tropical Indo-Pacific	<i>Z. marina</i>	–	Huiquan bay: 440 ± 39, Sanggou bay: 208 ± 33, Shuang Dao bay: 238 ± 31.2, Changdao island 159 ± 18	–	–	–
Navarrete-Fernandez et al., 2022	NaCl	Mediterranean Sea/coastal	Mediterranean	<i>P. oceanica</i>	18	–	–	–	1 and 2.5 mm (56 %)
Boshoff et al., 2023		South African/estuary	Temperate Southern Oceans	<i>Zostera capensis</i>	72	91 ± 85	fragment	–	–
Wright et al., 2023	Mixture of H ₂ O ₂ (30 % v/v) and ferrous sulfate catalyst	Western Australia/estuary	Temperate Southern Oceans	<i>H. ovalis</i> and <i>Ruppia megacarpa</i>	72	1000 ± 100.37	fiber (70 %)	clear	1.23–50 µm
Present study	NaCl	Brazil/estuary	Tropical Atlantic	<i>Halodule wrightii</i> , <i>Halophila baillonii</i> and <i>H. decipiens</i>	80	142 ± 140*	fiber (73 %)	blue (51 %)	< 1 mm (80 %)

vegetated by small-bodied species meadows was not influenced by the monsoon season in a subtropical region ([Huang et al., 2021](#)). Thus, the seasonal influence of AP accumulation in seagrass meadows is likely linked to species characteristics or other environmental factors.

Interestingly, sediment grain size was positively correlated with AP abundance in our study, although no variations were observed among species, areas or seasons. MP abundance in seagrass sediments was also correlated with grain size in a Tropical Indo-Pacific estuary ([Huang et al., 2021](#)) but in this case, MP abundance was negatively correlated with grain size. The observed opposite correlation in this study reveals

the need for broader studies to understand the distribution, transport, accumulation, and even resuspension of APs and their relation to sediment characteristics. Overall, it is imperative to explore the factors driving APs accumulation, including the meadow complexity, sediment characteristics, seasonality, the coastal and estuarine dynamics, as well as their interaction with local fauna. Such investigations, preferably in the medium and long term, are essential to enhance our comprehension of microparticle accumulation in seagrass meadows ([Chubarenko et al., 2018](#); [Pinheiro et al., 2021](#); [Shiravani et al., 2023](#); [Zhu et al., 2024](#)).

Local environmental factors can also play an important role in AP

accumulation of seagrass meadows. For instance, Dahl et al. (2021) observed an increase in MP abundance over a 100-year sediment profile, mainly due to the establishment of intensive agricultural activities in the coastal zone, which largely used plastics for greenhouse crops. Although Marine Protected Areas are assumed to have fewer anthropic impacts, they are also vulnerable to AP contamination (examples of studies carried out in Northeast Brazilian estuaries: Lima et al., 2015; Ferreira et al., 2019; Justino et al., 2021; Bruzaca et al., 2022; and in other regions: Abessa et al., 2018; Cheng et al., 2021; De la Torre et al., 2023; Nunes et al., 2023). Our study showed that, despite being within a Marine Protected Area, seagrass meadows presented AP contamination at similar levels than recorded in non-protected areas (Tahir et al., 2019; Unsworth et al., 2021; Boshoff et al., 2023). Therefore, APs are easily transported in the aquatic ecosystems and are detected in considerable abundances regardless of the conservation status of the area (Nunes et al., 2023), further exacerbating the problem of anthropogenic contamination.

Rivers are an important environmental factor for transporting APs to the coastal areas (Yan et al., 2019; Lebreton et al., 2018), which can favor higher anthropogenic waste deposition in seagrasses (Cheng et al., 2021; De Smit et al., 2021; Unsworth et al., 2021). However, AP accumulation in seagrass meadows may not be necessarily related to local factors, since APs can be dispersed in coastal areas from long-range ocean currents (Cole et al., 2011; Cózar et al., 2014). Concerning the SCC system, the primary freshwater inflow originates from the northern part of the area, where samples were collected. During the dry season, marine water masses enter the estuary, mainly through the northern entrance of the SCC (Medeiros et al., 2001). Jones et al. (2020) observed that *Z. marina* meadows accumulated MPs in a protected area (Scottish Natural Heritage), where MPs in the environment were linked to the strong influence of tidal currents that transport them.

Another consideration for future studies on AP accumulation in seagrass meadows is the contamination of the associated biota. The accumulation of APs in seagrass meadows can serve as vector for MP contamination through the direct or indirect ingestion of particles by pelagic and benthic fauna that use this ecosystem as feeding and nursery grounds (Ferreira et al., 2019; Jeyasanta et al., 2020; Li et al., 2021; Ouyang et al., 2022).

4.3. Characteristics of anthropogenic microparticles

Plastic materials generally have a long lifespan, and biotic and abiotic processes (e.g., enzymatic oxidation, exothermic oxidation, hydrolysis) are linked to changes in the characteristics of these particles, causing alterations in their physicochemical properties, appearance, texture and, even mechanical properties (Zhang et al., 2021). These characteristics can also indicate the contamination source, such as agricultural and aquaculture activities (Boshoff et al., 2023; Huang et al., 2020). The transport and distribution of plastics in the aquatic environment are also influenced by particle characteristics, potentially facilitated by their density, shape, and size (Chubarenko et al., 2016; Kowalski et al., 2016; Martí et al., 2020).

Blue fibers of <1 mm were the most common AP in this study. This dominance is most likely associated with anthropogenic activities within the SCC, where artisanal fishing is widely practiced by the local community (making it the most productive fishing center in the state of Pernambuco), and the discharge of untreated domestic sewage, industrial effluents, shrimp aquaculture, and local tourism (Quinamo, 2006; Coutinho et al., 2018). Additionally, the lack of adequate solid waste treatment, a common issue in low-income areas, reinforces the observed levels of local contamination (Jambeck et al., 2015; Zhu et al., 2024). Blue fibers are commonly associated with domestic laundry and fishing activity (Crawford and Quinn, 2017; Cesa et al., 2019; De Falco et al., 2020; Zhang et al., 2021). These characteristics are consistent with finding from other studies on MPs in seagrass meadows (Tahir et al., 2019; Cozzolino et al., 2020; Jones et al., 2020; Huang et al., 2020,

2021; Kreitsberg et al., 2021; Unsworth et al., 2021).

Colors can also the duration that APs have been in the marine environment (GESAMP, 2019; Martí et al., 2020). For example, the dominant blue color in our study may indicate that MPs have spent a shorter time in the environment, as the particle colors have remained stable. Over time, particles may undergo changes in appearance, color, and texture, such as becoming dull, yellowing, losing their sheen (Zhang et al., 2021).

4.4. Studies on anthropogenic pollution in seagrass meadows

Studies on the accumulation of microplastics, marine debris, or APs in seagrass meadows remains limited (Goss et al., 2018). Only 15 studies have investigated this topic, primarily focusing on the Tropical Indo-Pacific, Temperate North Atlantic and Mediterranean bioregions (Table 2). Additionally, 25 % of the 72 recognized seagrass species have been studied regarding their capability to accumulate APs in sediment (Short et al., 2011; Li et al., 2023; Tahir et al., 2019) (Table 2). To our knowledge, this is the first study in the Tropical Atlantic bioregion to investigate AP accumulation in *Halodule wrightii*, *Halophila decipiens* and *H. baillonii*.

4.5. Limitations

The primary objective of this study was to investigate the role of small tropical seagrasses in the accumulation of anthropogenic microparticles, focusing on microplastics. However, some limitations must be recognized regarding the methodology. First, the particles were only visually identified under the microscope, and polymer composition analysis was not conducted, preventing the classification of the found particles as microplastics. Therefore, the term “anthropogenic microparticles” was used for this study. Second, we recognize that additional sampling effort across multiple seasonal cycles would have enhanced the robustness of our data and results. Further research should focus on medium- and long-term monitoring of anthropogenic microparticle abundance to investigate temporal and spacial variability.

5. Conclusions

This study represents the first investigation of anthropogenic microparticle accumulation in seagrass meadows in the Tropical Atlantic bioregion. Our findings demonstrate that APs are pervasive even within Marine Protected Areas. The particles were dominantly blue fibers smaller than 1 mm, and their characteristics were similar between vegetated and unvegetated areas. The small-bodied species assessed here accumulated APs in a comparable abundance to adjacent unvegetated areas. Moreover, seasonal influences on AP abundance were not significant. The apparent lack of influence from studied factors may stem from the susceptibility of small-bodied seagrass species to anthropogenic contamination at the study site, potentially limiting their capacity to capture AP. Nonetheless, this finding remains inconclusive, as similar patterns have been observed in studies involving larger seagrass species.

Identification of the drivers of AP accumulation in seagrass meadows should involve broader assessment of seagrass species with different plant traits and meadow complexities, alongside consideration of local environmental factors. Standardized methods for sediment sampling, AP extraction, and identification are crucial to facilitate more accurate comparisons across studies. Furthermore, future medium- and long-term research should explore the potential impacts of AP accumulation on seagrass meadows and associated biota.

CRedit authorship contribution statement

Ana Maria da Costa de Souza: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal

analysis, Data curation, Conceptualization. **Guilherme Vitor Batista Ferreira:** Writing – review & editing, Validation, Methodology, Investigation. **Carmen Barrena de los Santos:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Flávia Lucena Frédou:** Resources, Methodology. **Karine Matos Magalhães:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they are not aware of any competing financial interests or personal relationships that could influence the results of the work reported in this article.

Data availability

Data will be made available on request.

Acknowledgments

AMCS is grateful to the National Council for Scientific and Technological Development (CNPq) for granting a master's scholarship (Proc. 88887.635401/2021-00). We thank the LMI TAPIOCA CAPES/COFECUB program (88881.142689/2017-01) and the Serviço Geológico do Brasil (SGB)/Companhia de Pesquisa de Recursos Minerais (CPRM). KMM thanks the Research in movement project (23082.024313/2015-81) of the Federal Rural University of Pernambuco (UFRPE). GF thanks FACEPE (Fundação de Amparo à Ciência e Tecnologia de Pernambuco) and FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro) for providing research grants (BFP-0107-5.06/21 and E-26/200.098/2024, respectively). FF thanks CNPq (305014/2016-1). CBDLS received Portuguese national funds from FCT - Foundation for Science and Technology through projects UIDB/04326/2020 (DOI:10.54499/UIDB/04326/2020), UIDP/04326/2020 (DOI:10.54499/UIDP/04326/2020), LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020) and 2020.03825. CEECIND (DOI:10.54499/2020.03825.CEECIND/CP1597/CT0005).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2024.116799>.

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