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Occurrence of microplastics in bivalve molluscs *Anomalocardia flexuosa* captured in Pernambuco, Northeast Brazil

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ABSTRACT

Microplastics (MPs) are widely distributed in marine ecosystems, and their ubiquitous presence is raising concern, particularly about possible impacts on fisheries resources. In tropical regions, shellfish fisheries represent an essential source of income and subsistence for traditional communities, and adverse effects on these resources may have severe consequences on human health. In the present study, bivalve molluscs of the species *Anomalocardia flexuosa*, captured in the region of the Itapessoca estuary in Pernambuco, Brazil, were analysed. A total of 90% of the individuals presented MP particles in their tissue. We observed an average of 5.15 ± 3.80 MP particles per individual, and for each gram of soft tissue, 3.66 ± 2.59 MP particles were found. Our results showed that MPs are present in clams captured on the Pernambuco coast and that the species studied proved to be suitable for monitoring the levels of microplastic pollution.

1. Introduction

With the drastic growth in plastic production on the planet since the beginning of the 20th century, plastic pollution has become an environmental problem that grows and affects all environments. In the marine environment, due to a large amount of this material released into the oceans, plastic represents between 60 and 80% of all marine litter (Moore, 2008; Barnes et al., 2009; Gonçalves, 2016).

Microplastics (MPs) are defined as plastic particles with a size of <5 mm (Arthur et al., 2009; Dehaut et al., 2016). MPs can be divided into primary MPs, which are particles manufactured in microscopic size for use in industry (Neves et al., 2015; López-Monroy, 2019), or secondary MPs, which are particles caused by the fragmentation and degradation of larger plastic items (Auta et al., 2017; Regueira et al., 2019). Due to its small size (Wright et al., 2013) and the large amount of plastic waste discarded in marine environments, MPs have entered the entire food chain, occurring from zooplankton to large cetaceans (Regueira et al., 2019). The MPs' wide availability risks all animals' health, affecting these organisms in different ways, accumulating and causing changes mainly in the digestive and respiratory systems (Wright et al., 2013; Franzellitti et al., 2019). In humans, ingestion of MPs can cause chromosomal alterations, resulting in infertility, obesity and even the appearance of cancer (Sharma and Chatterjee, 2017).

Due to this problem, bivalve molluscs have been widely used as bioindicators of MPs pollution (van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015; Rochman et al., 2015; Regueira et al., 2019). Because they are naturally present in several coastal environments and are filter feeder animals, these bivalves can accumulate MP particles in their tissue, mainly associated with the digestive tract and gills. Studies carried out in Spain reported the presence of MPs in wild mussels (*Mytilus* spp.) (Regueira et al., 2019). In Peru, MPs were reported in the Peruvian scallop *Agropecten purpuratus*, sold in fish markets in Lima (De-La-torre et al., 2019). Also, in Uruguay, MPs were detected in the yellow clam *Amarilladesma mactroides* (Azambuja and Eguez, 2020).

In Brazil, the capture and cultivation of bivalve molluscs are done mainly by traditional communities on the coast. Among the prominent representatives of bivalve molluscs consumed in Brazil are the sururus (*Mytella* spp.), mussels (*Perna perna*), oysters (*Crassostrea* spp.), clams (*Anomalocardia flexuosa*) and scallops (*Euvola ziczac*) (ICMBIO, 2011).

On the northern coast of Pernambuco, in northeastern Brazil, the capture of bivalve molluscs, known as shellfishing, a primary source of income activity, is generally practised by women called shellfish gatherers (Barreira and Araújo, 2018). In these traditional fishing

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communities, seafood is part of their diet, and most of the animals captured are transported to Recife, the capital of Pernambuco, supplying the local market. This study aimed to evaluate the level of contamination of shellfish of the species *A. flexuosa* collected in the Itapessoca estuary in Goiana, Pernambuco, northeast Brazil.

2. Material and methods

2.1. Study area

The study area is located in the northern part of the coast of Pernambuco, more precisely in the estuary of Itapessoca river situated in the district of Ponta de Pedras in the municipality of Goiana (Fig. 1). In this estuarine complex, artisanal fishing activities are developed, such as shellfishing and the capture of fish and crustaceans, which sustain the families of traditional fishers communities in the area (de Moura et al., 2009). In the regions surrounding the estuary, economic interests, mainly in the aquaculture, real estate and tourism sectors, have increased the anthropogenic influence in the areas of the estuarine complex (Pelage et al., 2019). Despite being inserted in a Marine Protected Area (MPA) and of extreme importance for the regional fisheries and the traditional populations, there is a lack of policies to mitigate plastic pollution.

The *A. flexuosa* were collected at coordinates 7°40′37″S 34°50′24″W in Barra de Catuama beach, in the Itapessoca River estuary (Fig. 2). The traditional communities' fishers captured the species during the low tide.

2.2. Sample collection and laboratory procedures

A total of 20 specimens of *A. flexuosa* were captured per month in October 2019, December 2019, and February 2020, totalling 60

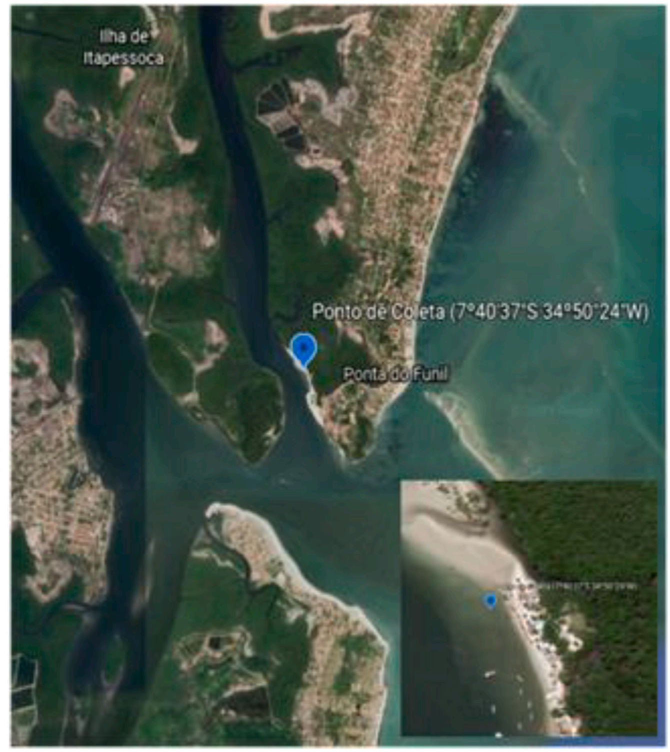

Fig. 2. Collect point in Barra de Catuama beach (Google Earth).

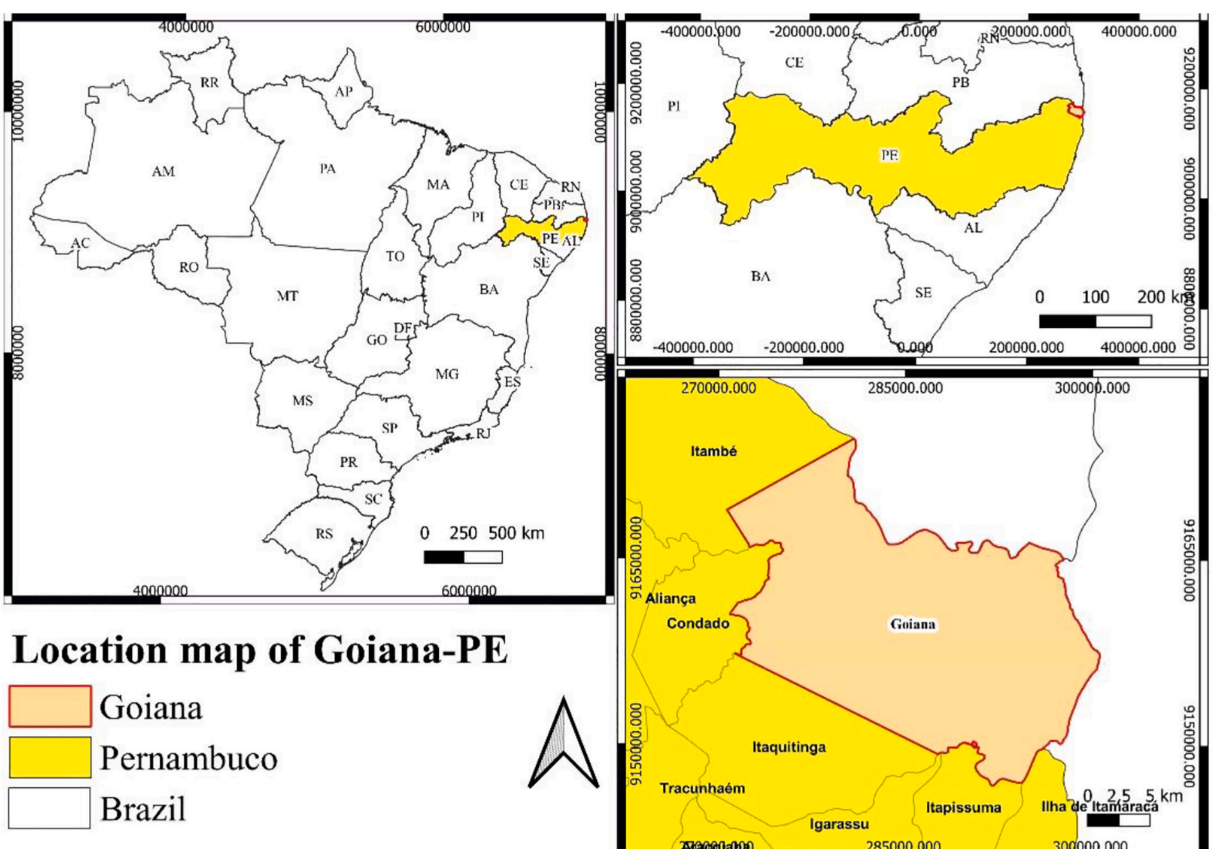

Fig. 1. Location map of Goiana in the state of Pernambuco, Brazil.

individuals collected. The sampling was conducted during the low tide in the spring tides. Then, individuals were stored in plastic bags and taken to the laboratory, where they remained frozen at a temperature of −20 °C until sample processing. In the laboratory, the biometry was carried out using a calliper with a precision of 0.002 mm, to obtain the shell length, which is the maximum distance on the anteroposterior axis (Lt, mm), and the shell height, which is the maximum distance on the dorsoventral axis (Sht, mm) (Oliveira et al., 2013). The total weight (Wt = Shell + Soft parts, g) and the weight of the soft parts (Swt, g) were measured on an analytical scale.

2.3. Contamination control

Before the microplastic extraction, several steps were taken to avoid airborne and cross-contamination, followed the procedures recommended by Justino et al. (2021). The operations included the use of disposable gloves and 100% cotton lab coats; also, all the steps were conducted in a reserved and cleaned room. The whole equipment used in the extraction was previously sanitised, and the analysis processes were carried out with the shortest possible time of exposure to air. Moreover, all the solutions used were filtered in a vacuum pump system equipped with laboratory glassware through a 1 μm Millipore glass fibre filter (Merck Millipore AP1504700).

A custom-made glass chamber the size of the glass fibre filters was used to prevent airborne contamination while observing the filters under the microscope. Additionally, blanks procedures were made for each battery of 10 samples to quantify possible contamination by fibres and dust in all processes. The blanks followed the same protocol applied to the samples to certify the absence of contaminants and minimise the quantification errors in the process, as recommended by Cole et al. (2014).

2.4. Microplastics quantification and identification

For the MPs extraction in the clams, an alkaline digestion protocol was performed, followed the recommendations by Dehaut et al. (2016). Individuals were dissected, and the soft parts of the molluscs underwent a digestion process using 10% KOH solution with incubation in an oven at 60 °C for 24 h. After this process, the samples were filtered through a glass fibre filter (1 μm pore size Merck Millipore AP1504700) using a vacuum pump equipped with laboratory glassware. Then, the filters were oven-dried and, finally, observed under an optical microscope to quantify MP particles. The filters were placed in a glass chamber to visually identify, count, and measure the MP particles present in each sample. For visual identification of particles, an optical microscope coupled with a digital camera, using 4× and 10× magnification lenses and, when necessary, a 40× lens was used to visualise the smaller particles better. Microplastic particles were characterised according to their morphology (Galgani et al., 2013).

Although the digestion protocol is a valuable tool to separate organic material and facilitate visual identification, it is not sufficient for identifying polymers, and since our samples were too small (<1 mm), polymer characterisation was not feasible. Due to this, we followed the method described by Ferreira et al. (2019) to confirm the plastic debris by drying the samples in an oven to check if their physical characteristics changed or not. All items with visual characteristics similar to plastic materials with vibrant colours or brightness, which did not change shape, did not wither upon loss of water and were not easily broken, were considered microplastics.

The individual's total weight was considered to obtain data on the number of particles present in each analysed individual. Also, to calculate the concentration of MPs in the soft parts, the weight of the soft parts (Swt) was taken into account, where the concentration of microplastics (Mc) in the animal was calculated as follows (Eq. (1)):

$$Microplastic\ concentration = \frac{\text{No of particles}}{\text{Soft parts weight}} \tag{1}$$

Additionally, we estimated the consumer exposure for each 1 kg of clam meat using the mean concentration of MP in the soft parts. Moreover, Spearman's correlation test was used to evaluate the relationship between MP detected and clams' biometry (soft parts and total weight g). The Spearman's rank correlation was done with the software R version 3.6.3 (R Core Team, 2020) and conducted considering a level of significance of 5%.

3. Results

A total of 309 MP particles were recovered from the 60 individuals analysed (90%). The number of particles found in the individuals ranged between 0 and 16 particles, and the average of particles in the specimens of *A. flexuosa* was 5.15 ± 3.80 MP particles per individual. MPs present in the soft parts (Pm) of the shellfish analysed ranged between 0 and 9.44 particles g^{-1} of Pm, and the average was 3.66 ± 2.59 particles g^{-1} of Pm. The mean size of MPs found in the clams ranged between 17 and 1057 μm. Regarding the types of MPs identified in the tissues, most were fragments 54%, followed by fibres 43% and pellets 3% (Figs. 3 and 4). Only one sample was contaminated with purple fibre from the six blank samples analysed. No microplastic particles were found in the other blank samples, with an average of 0.16 particles per filter.

The total weight of *A. flexuosa* specimens ranged between 4.32 g and 16.82 g, with a mean of 9.19 ± 2.90 g. The wet weight of the soft parts ranged between 0.83 g and 2.60 g, with an average weight of 1.44 ± 0.38 g. However, there is no relationship between the detected MP and the weight of soft parts and the total weight of clams (Spearman's rank correlation, $p > 0.05$). Therefore, we estimate that consumers are exposed to about 3660 MP particles for each kilogram of clam meat.

4. Discussion

This is the first study investigating and confirming that microplastics (MPs) contaminate the molluscs collected by shellfishing in the Itapessoca estuary and the first evaluated the *Anomalocardia flexuosa* in Brazil.

In the Mangue Seco region, which is close to the Itapessoca estuary in Goiana, it was reported that the total weight of individuals captured for commercialisation ranged between 0.03 g and 16.66 g, and the weight of the soft parts ranged from 0.01 g to 9.48 g (Souza, 2012), which coincides with the values found in our study, concluding that the individuals of *A. flexuosa* analysed had commercial size. However, we did not observe any correlation between the number of MPs detected and the biometry of clams, unlike a recent study on an Amazonian anemone, which noticed a correlation between the weight and the number of particles found in the gastrovascular cavity (Morais et al., 2020). However, these differences might be associated with the biology of the species. In northeast Brazil, no previous research has been carried out on the contamination of bivalve molluscs by MPs, which is the first record for the region. Despite the difficulty in comparing the values found in this study with other works, due to the difference between the species and analysis methods used, in Southeastern Brazil, a study carried out in the state of Rio de Janeiro, in Guanabara Bay, Birnstiel et al. (2019) identified an average of 31.2 ± 17.8 MP particles per individual of the wild and cultivated *Perna perna* mussel species, and an average of 4.12 particles g^{-1} of soft parts. In southern Brazil, Paraná, Vieira et al. (2021) carried out a study to detect the presence of MP particles in the hepatopancreas of *Crassostrea gasar* oysters, where they found an average of 9.6 MP particles for 150 mg of analysed hepatopancreas. With the standardisation of analysis methods and the use of the same species, it may become possible to monitor the pollution levels by MPs throughout the Brazilian coast.

In addition to monitoring the level of pollution, we can also estimate

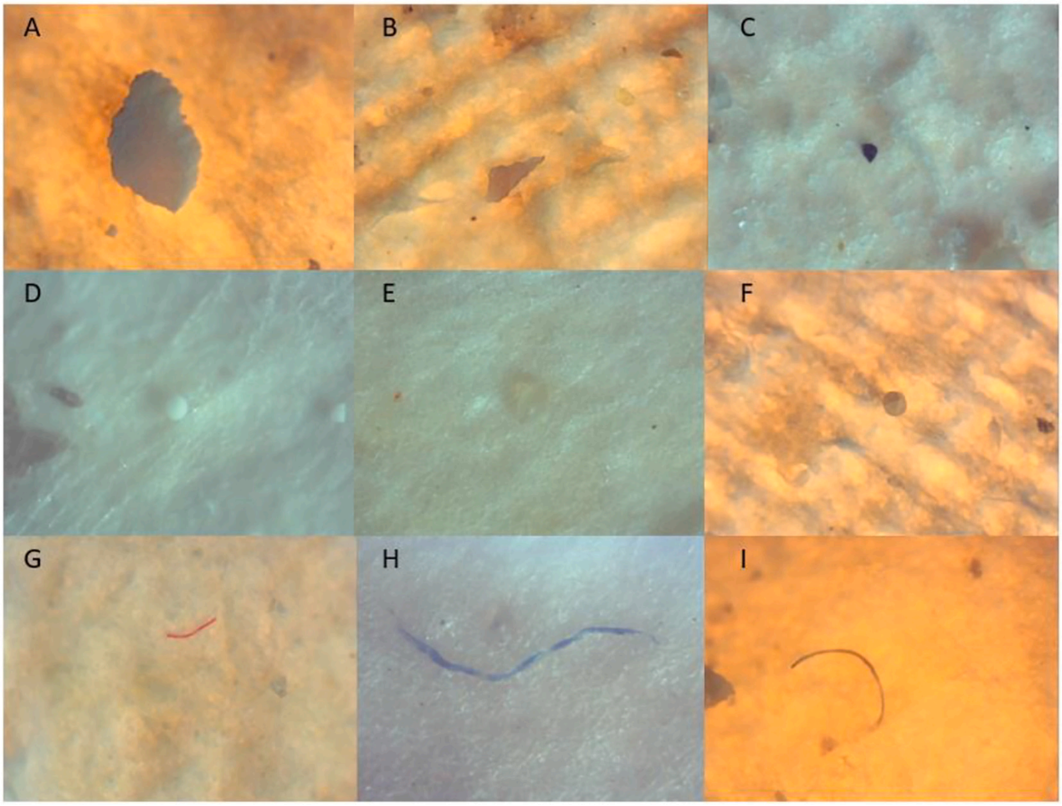

Fig. 3. The types of microplastic particles found in individuals of *A. flexuosa*. A–C fragments, D–F pellets and G-I fibres.

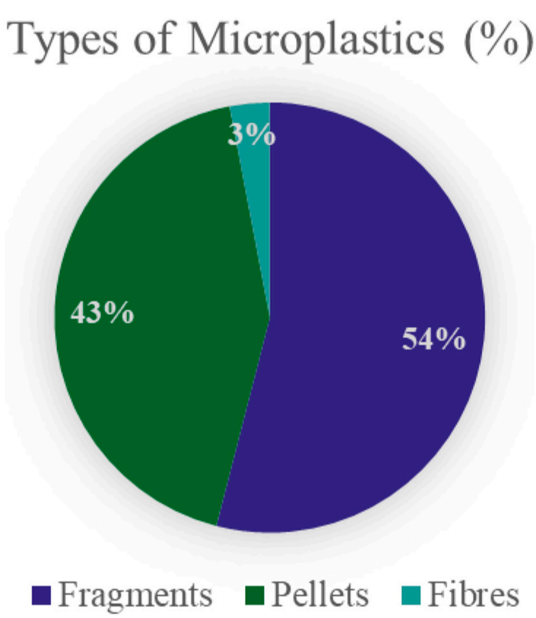

Fig. 4. The proportion of microplastic types found in the *Anomalocardia flexuosa* individuals.

the level of exposure to MP particles by consumers of *A. flexuosa* in the vicinity of the Itapessoca estuary in Goiana since the captured animals are mainly consumed by traditional fishers communities and sold in the fish markets both in Goiana and in neighbouring cities. According to the research by Silva-Cavalcanti and Costa (2009), between 10 and 17 kg of clam meat are captured per day; with the data found in our study, it is estimated that for each kilogram of *A. flexuosa* meat caught in the studied region, consumers of this meat may be exposed to around 3660 MP particles.

Researchers worldwide have been using bivalve molluscs as sentinels for MP pollution (Bom and Sá, 2021). In Spain, Regueira et al. (2019) reported that in mussels of the genus *Mytilus*, the number of MPs ranged between 0 and 14 particles per individual, and the mean of particles found was 3.05 ± 2.67 particles per individual. It was also possible to compare the pollution patterns in two different areas, Ria de Vigo and the Cantabrian Sea in northern Spain. Bråte et al. (2018), off the coast of Norway, reported that *Mytilus* spp. analysed had between 0 and 6 particles per individual, with an average of 1.5 particles per individual, and stated that the values found could not be compared with other studies

Table 1
Concentrations of microplastics in bivalve molluscs reported worldwide digested with 10% KOH solution.

Geographical area	Species	The concentration of microplastic (MP g^{-1} Pm)	Amount of microplastics per individual (MP ind^{-1})	Reference
France	*Mytilus* spp.	0.23 ± 0.20	0.60 ± 0.56	Phuong et al. (2018)
Norway	*Mytilus* spp.	0.97	1.5	Bråte et al. (2018)
Spain (Ria de Vigo)	*Mytilus* spp.	1.59 ± 1.28	2.19 ± 1.57	Regueira et al. (2019)
Spain (Cantabrian Sea)	*Mytilus* spp.	2.55 ± 2.80	2.81 ± 2.80	Regueira et al. (2019)
Peru (Lima)	*Agropecten purpuratus*	0.13 ± 0.03	2.25 ± 0.54	De-La-torre et al. (2019)
Brazil (Goiana-PE)	*A. flexuosa*	3.66 ± 2.59	5.15 ± 3.80	Present Study

due to differences in the analysis methods. However, comparing different areas may reveal different pollution patterns along the coast (Table 1). The values found in the present study, using the digestion method with a 10% KOH solution, indicate that, like other species of bivalve molluscs, *A. flexuosa* is a suitable species for monitoring pollution by MPs, as it is distributed widely along the coast of Brazil (Belém et al., 2013).

In the present study, only one of the blank samples was contaminated, with an average of 0.16 microplastic particles per filter. This value was lower than those found by Catarino et al. (2018), Bråte et al. (2018) and Regueira et al. (2019). Therefore, using the analysis chamber under a microscope and pre-filtering the solutions used to wash the samples may have helped reduce contamination during sample handling and analysis.

In addition to the quantitative study, the qualitative analysis of MP particles can serve as a reference when identifying the polluting sources of coastal environments. At the collection point in the Itapessoca estuary, the fragments were the most abundant particle type (54%), unlike the study carried out in Rio de Janeiro with mussels in Guanabara Bay (Vieira et al., 2021) and other studies in the world, where fibres were the most abundant (Davidson and Dudas, 2016; McGoran et al., 2017; Li et al., 2019; Zhu et al., 2020). According to the literature, the most urbanised regions tend to have a more significant amount of fibres due to the discharge of domestic sewage (Gago et al., 2018; Macieira et al., 2021). Other significant sources are fishing tackle abandoned in the environment and even devices for cultivating bivalve molluscs such as beds, ropes and lanterns (Gago et al., 2018; Regueira et al., 2019). In the region close to the Itapessoca estuary, the most common economic activities are artisanal fishing, aquaculture and tourism (de Moura et al., 2009), which contributed to the availability of MPs and other contaminants. The differences in the types of MPs found might be associated with the sources of contaminants in the area and also the differences in the feeding habit of organisms (Justino et al., 2021). Clams are an essential source of energy for the estuarine and coastal community, and the health of these organisms may reflect in problems for the entire food web.

5. Conclusion

In our study, we can conclude that there are MPs in the tissues of the bivalves captured in the Itapessoca estuary, northeast Brazil, which is of great concern as shellfishing represents an essential source of income and subsistence for traditional communities in the region. Moreover, we confirm that using the bivalve mollusc of the species *A. flexuosa* as a sentinel proved to be promising in monitoring pollution levels by MP off the coast of Brazil. Also, using an analysis chamber, we can help reduce the number of contaminating particles when analysing the fibreglass filters. Further studies should be carried out to examine the impact of MPs on other estuarine species, which are essential for the survival of traditional communities.

CRediT authorship contribution statement

David N.A. Bruzaca: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing – original draft. **Anne K.S. Justino:** Methodology, Validation, Formal analysis, Writing – review & editing. **Géssica C.P. Mota:** Writing – review & editing. **Gelcirene A. Costa:** Writing – review & editing. **Flávia Lucena-Frédou:** Resources, Writing – review & editing, Funding acquisition. **Alfredo O. Gálvez:** Project administration, Supervision, Resources, Writing – original draft, Funding acquisition.

Declaration of competing interest

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

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