Microplastics pollution in green turtle in the Caribbean

Contaminación por microplásticos en tortuga verde en el Caribe

Pollution par les microplastiques chez les tortues vertes dans les Caraïbes

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ABSTRACT

The green sea turtle of the Atlantic *Chelonia mydas* is currently listed as endangered in Mexico, and plastics and micro plastics pollution is one of the most obvious threats. Knowledge of the contamination of green turtles remains largely incomplete in the Caribbean, particularly in Mexico, Yucatán Peninsula. The objective of this study was therefore to examine for the first time in Mexico Caribbean, the microplastic pollution in green turtles by via a non-invasive method, using and analyzing faeces. Sampling site was, isla Blanca area, a small stretch of the coast of Quintana Roo, this area receives a large amount of feces from marine turtles, when the green one is the dominant in this area. Abundance of micro plastics was determined using optical microscopy. The abundance of microplastics ranged from 49 ± 16.3 to 4 ± 1.7 microplastics per gram of each faeces. All of the particles were blue and transparent fibres. The use of scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX) made it possible to study the morphostructure of the fibres, showing signs of alteration. Their chemical composition was also examined and confirmed the microplastic nature of the fibres. Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy revealed the presence of nylon. Our results overall indicated a strong interaction between green sea turtles and marine microplastics off the coast of Quintana Roo.

KEYWORDS : microplastics, pollution, green turtle, Caribbean, non-invasive method

RESUMEN

La tortuga verde, *Chelonia mydas* se encuentra en peligro de extinción en México, y la contaminación por plásticos y micro plásticos es una amenaza adicional para sus poblaciones. Poca información se tiene sobre este tipo de contaminación en esta especie de tortugas, no existiendo información en general para el Caribe y menos aún para el Caribe mexicano, en la Península de Yucatán. Por lo tanto, el objetivo de este estudio fue examinar por primera vez en Quintana Roo, la contaminación por microplásticos en la tortuga verde a través de un método no invasivo, utilizando y analizando sus heces. Se muestreó el área de isla Blanca, que es una franja pequeña de la costa de Quintana Roo que recibe por efecto de las corrientes cantidades significativas de heces de tortugas marinas. La abundancia de microplásticos se determinó con microscopia óptica, y ésta osciló entre 49 ± 16.3 y 4 ± 1.7 microplásticos por gramo de heces. La mayoría de las partículas eran fibras azules y transparentes. El uso de microscopía electrónica de barrido (SEM) junto con espectroscopía de rayos X de energía dispersiva (EDX) permitió estudiar la morfoestructura de las fibras y analizar el desgaste de estas y su fracturación, así como su composición química elemental y la presencia de metales pesados, entre otros elementos. Con espectroscopia infrarroja por transformada de Fourier (FTIR) y la espectroscopia Raman se determinaron los tipos de polímeros, donde la mayor prevalencia fueron fibras de nylon. Los resultados de esta investigación, mostraron una fuerte interacción de contaminación marina por micro plásticos presentes en la tortuga marina verde, *Chelonia mydas.*

PALABRAS CLAVES: microplásticos, contaminación, tortuga verde, Caribe, método no invasivo

INTRODUCTION

Plastics, they are solid waste with a greater presence in daily life. Its production has increased exponentially in the last 20 years with a world annual production of 368 million tons. These polymers, when exposed to UV rays and mechanical wear due to the movement of waves, degrade and fragment from macroplastics (5–50 cm) to microplastic particles (<5 mm) and nanoplastics (≤ 0.1 μm). You can also find pearls from hygiene products and fibers from synthetic textiles. MP are emerging pollutants found in all ecosystems, from the poles to the tropics, and due to their ubiquity, they are potentially in all marine organisms (Wright et al 2013). Plastics in the marine environment absorb pollutants such as persistent organics, where almost half of their composition are hazardous chemical substances (Rochman 2013), causing alterations in gene expression (Imhof et al., 2017), alterations in development, fertility and oxidative stress (Jeong et al., 2017; Choi et al., 2018).

Figure 1. Green turtle *(Chelonia mydas*) feces sampling sites North of the Yucatan Peninsula, in the Mexican Caribbean. A) site of samples collected in the sea and B) site of samples collected on the beach

In the Caribbean, the presence of MP has been reported in various marine environments. On Colombian beaches, most of which are polyethylene and polypropylene (Acosta-Coley and Olivero-Verbel 2015). On the beaches of the Lesser Antilles, 261 ± 6 MP.kg^{-1} of sand is reported with a maximum of 620 ± 96 MP.kg^{-1}, where 95% were fibers (Bosker et al. 2018). In Mexico, on the beaches of Holbox Quintana Roo, abundance range was between 66.9 and 112.7 MP/m^2 was reported, dominating fragments of polyethylene, polypropylene, and polystyrene (Álvarez-Zeferino et al., 2020). In seagrass, *Thalassia testudinum* was found that 75% of its leaves present MP, mostly fibers (Goss et al. 2018). In marine organisms, using the mollusk Queen Conch (*Aliger gigas*) as an indicator in various Caribbean countries, the degree of contamination by micro plastics varied from 43 to 270 MP per individual^{-1}, with a predominance of fibers (Aranda et al., 2022).

Atlantic green sea turtle (*Chelonia mydas*) is present throughout the Caribbean around the continental coasts and oceanic islands. This species is the most affected by microplastic pollution among all species of sea turtles (Gall and Thompson 2015). This phenomenon is directly related to their use of the habitat and their food ecology. They carry out breeding migrations between feeding grounds and nesting areas, extending over thousands of km (Bass et al, 2006). They hatch on beaches in tropical and subtropical waters, and swim to pelagic habitat to spend the first year of their cycle life (Petry et al, 2021). Juveniles recruit in neritic areas, at seagrass beds or in surrounding coral reefs (Da Silva et al, 2015). Their omnivorous diet is essentially based on benthic consumption, based on soft-bodied animals as sponges. They are therefore likely to ingest the jellyfish-like plastic (Lagueux, 2021). Adults feed in these areas where their diet becomes herbivorous, and consists mainly of seagrass, *Thalassia testudinum* and *Halodule wrightii* (Bjorndal et al, 2005). They are all the more susceptible to micro plastic pollution due to their proximity to coastal centers (Petry et al, 2021). Particles can also become in their food, seagrass and macroalgae (Awabdi et al, 2013). The objective of this study was therefore to examine for the first time in Mexico Caribbean, the microplastic pollution in green turtles by via a non-invasive method, using and analyzing feces.

MATERIAL AND METHODS

Sampling sites. The feces of turtles were collected from two sites on Isla Blanca, Quintana Roo Mexico in the sea (Site 1) and at beach (Site 2) (Fig. 1A-B). Collection at the sea surface was carried out over an area 9 km long and 5 km wide. Seagrasses were present in the study area. These sites were chosen because there show a large aggregations of green turtles in the reef lagoon of Bahía de isla Mujeres, feeding on the seagrass *(Thalassia testudinum and Halodule wrightii)*. Currents and prevailing winds from the southeast carry the floating excrement from the lagoon to the beaches of Isla Blanca.

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Obtaining micro plastics: Three one-gram stool samples One gram of feces was weighed with a precision balance and it was placed using a spatula in a Falcon® tube. Three ml of hydrogen peroxide (30%) were poured into this same tube to destroy the organic matter and 7 ml of distilled water were added for 48 hours at room temperature to eliminate organic matter. Three replicates were prepared for each feces collected, in order to improve the robustness of the results. The 10 ml of the solution were analyzed under a stereoscopic microscope to count the micro plastics. A second digestion was carried out and microplastics were counted.

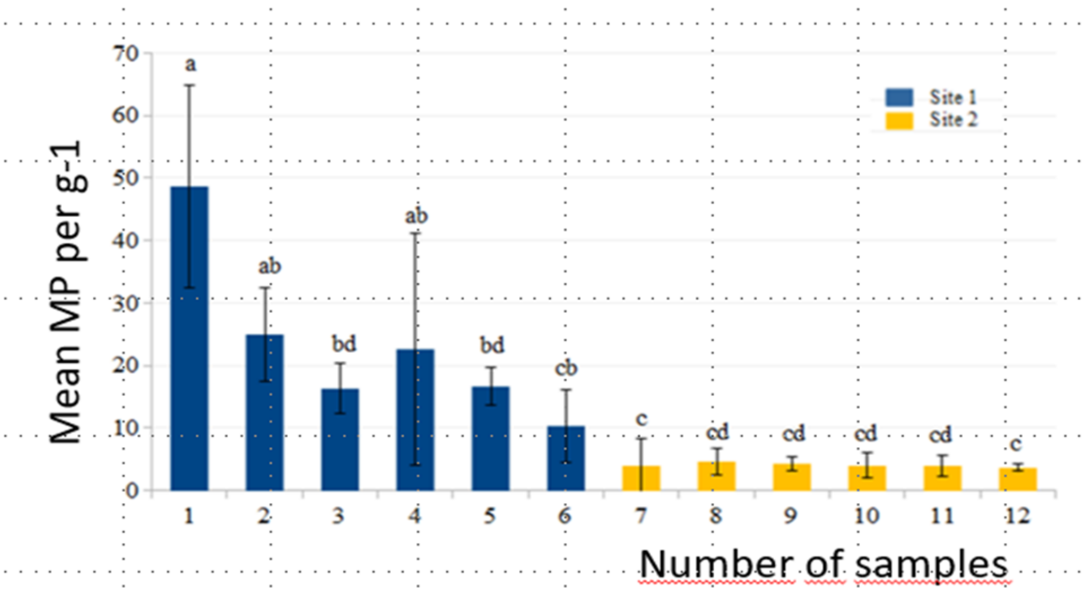

Figure 2. Average and SD of micro plastics per gram in feces samples of green turtle, Chelonia mydas at Isla blanca, Quintana Roo Mexico. Site 1 (bleu) corresponds to samples collected in the sea and site 2 (yellow) to samples collected in the beach.

Identification and quantification of Micro plastics. The abundance of MP was performed using a Leica stereoscope (Motic SMZ-171) at 40X magnification. Micro plastics (300-5000 µm, NOAA 2015) were counted and classified into fibers, films and spheres. Review of solution was realized in a glass Petri dish with a grid at the bottom. The analysis of 1 ml was carried out until the entire 10 ml solution was reviewed. To certify that all the micro particles are micro plastics and not calcareous/silica fragments, scanning microscopy and EDX analysis were used**.**

Scanning Electron Microscopy and EDXS Analysis. The MP particles were placed on a metallic copper support and metallized for 45 s with Gold-palladium (Quorum Q150R) for analysis in the scanning electron microscope (JEOL JSM-7600F). Elemental chemical composition detection was performed in the SEM at 20 kV in STEM. EDXS analysis was carried out on 15 random microfibers per month. The morpho structure of the microfibers and their fractures and level of wear, identifying and quantifying their elemental chemical composition.

Fourier transform infrared spectroscopy (IFTR) and Raman spectroscopy are used to identify the polymers that make up micro plastics (Thermo Scientific Nicolet 8700 FT-IR). The spectral range was 4000 to 400 cm-1 with an average of 200 scans at 6 cm-1 resolution. Raman spectra were collected using the InVia™ Raman Renishaw microscope (Wotton-under-Edge, Gloucestershire). A 633 nm laser was used at 50% power. The particles were examined in the spectral range of 400 to 3200 cm-1 with two accumulations, grid 1800, objective 50, with an exposure time of 10 s (Zumbardo-Bacelis, et al 2021).

Stadistical analysis. A nonparametric Kruskal-Wallis (KW) test was used to determine average number of microplastics. A nonparametric Mann-Whitney (W) test was used to determine the average number of microplastics per g differed significantly between sites (wilcox function.test of R).

RESULTS

Abundance of micro plastics. All of the samples of feces analyzed contained microplastics (Fig.2). All the feces samples presented MP, both those collected at sea and on the beach. The samples collected in the sea present a higher amount of MP. The highest abundance was 49 ± 16.3 $MP.g^{-1}$. The samples of feces collected on beach presented a lower abundance of MP, in an average of 4 ± 1.7$MP.g^{-1}$. The Kruskal Wallis test showed that the abundance of microplastics between samples was significantly different (KW= 148.42, $p < 2.2e-16$). According to the post-hoc test, the microplastic abundances of samples 1, 2 and 4 were significantly different from those of all the feces of site 2 ($p<0.05$). Mean number of microplastics in site 1 was significantly different between feces. Nevertheless, no significant difference was noticeable between samples of site 2 (Fig. 2).

Microplatics forms. Hundred percent of the microplastics were fibers. Transparent and blue colors are dominant ones. All feces from site 1 had at least all four colors; blue, transparent, black and red). Green microfibers are rare with 3.23%.

Morfo structure of micro plastics. Images acquired by a SEM showed fiber and details in figure 3. A rough

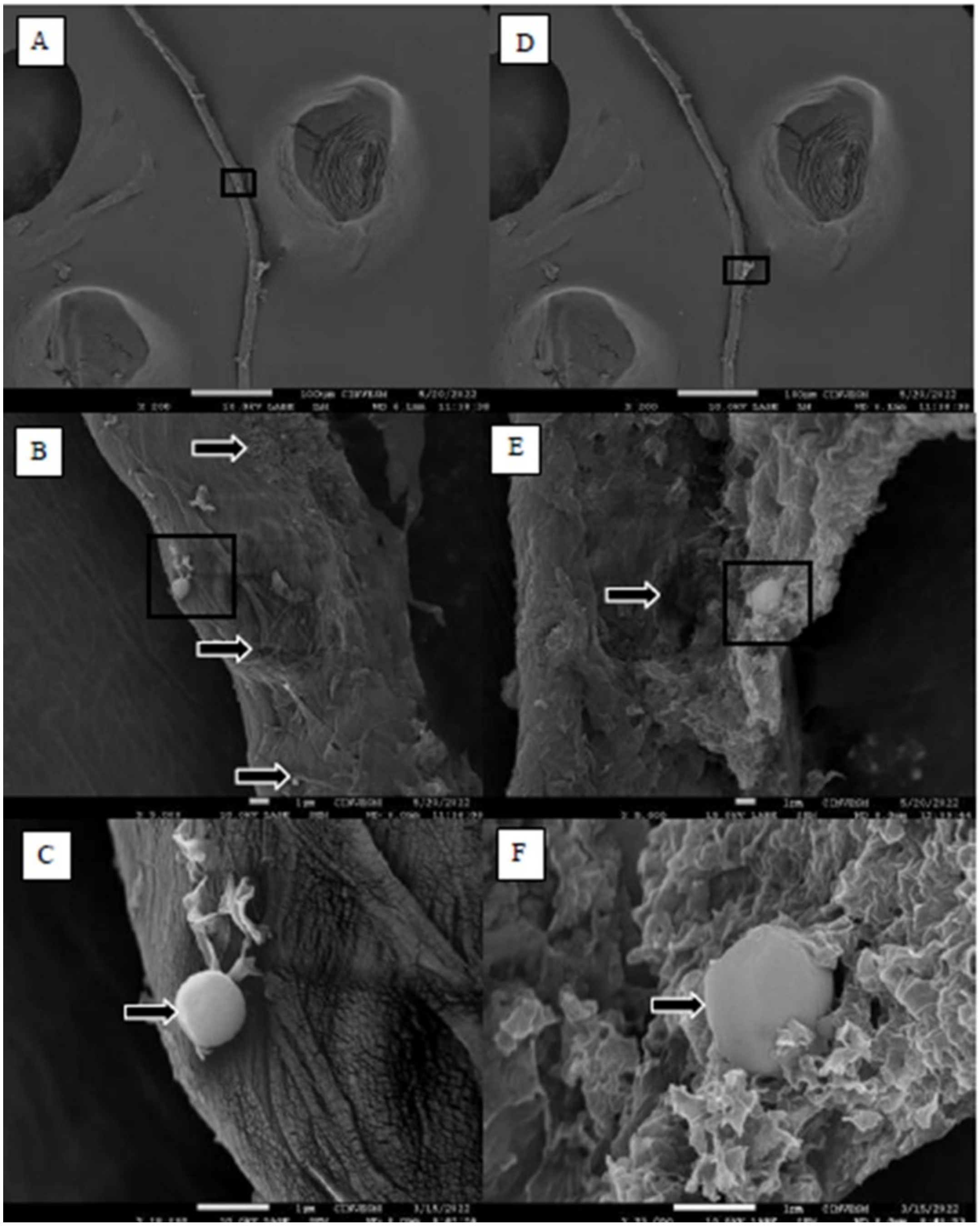

Figure 3. Images acquired by a SEM A) of a rough and rough fiber (200 x), B) fractured area showed by an arrow. The black box shows a structure adhered to the microfiber of micro fibers and calcareous structure indicated by arrow at the bottom of the photo (5000 x). C) A spherical structure adhered to microfiber (18 000x). D) The black box shows a structure adhered (200 x). E) Detail of the area presenting fractures in the micro fiber (5000 x). F) Detail of structure spherical indicated by arrow.

and rough fiber (A) and fractured area (B). it was observed various structures adhered to the microfiber of micro fibers and calcareous structure (C) and a spherical structure adhered to microfiber. Detail of the area presenting fractures in the micro fiber (E) and Detail of structure spherical (F)

Analysis Raman and Infrared. For a blue fiber and a black fiber (Figs.4A and 4D), the spectra infrared has highlighted well-defined absorption bands corresponding to those of a polymer: Nylon (Figs.4B and 4E). According to the FTIR spectrum of the fiber blue, Nylon (or polyam-

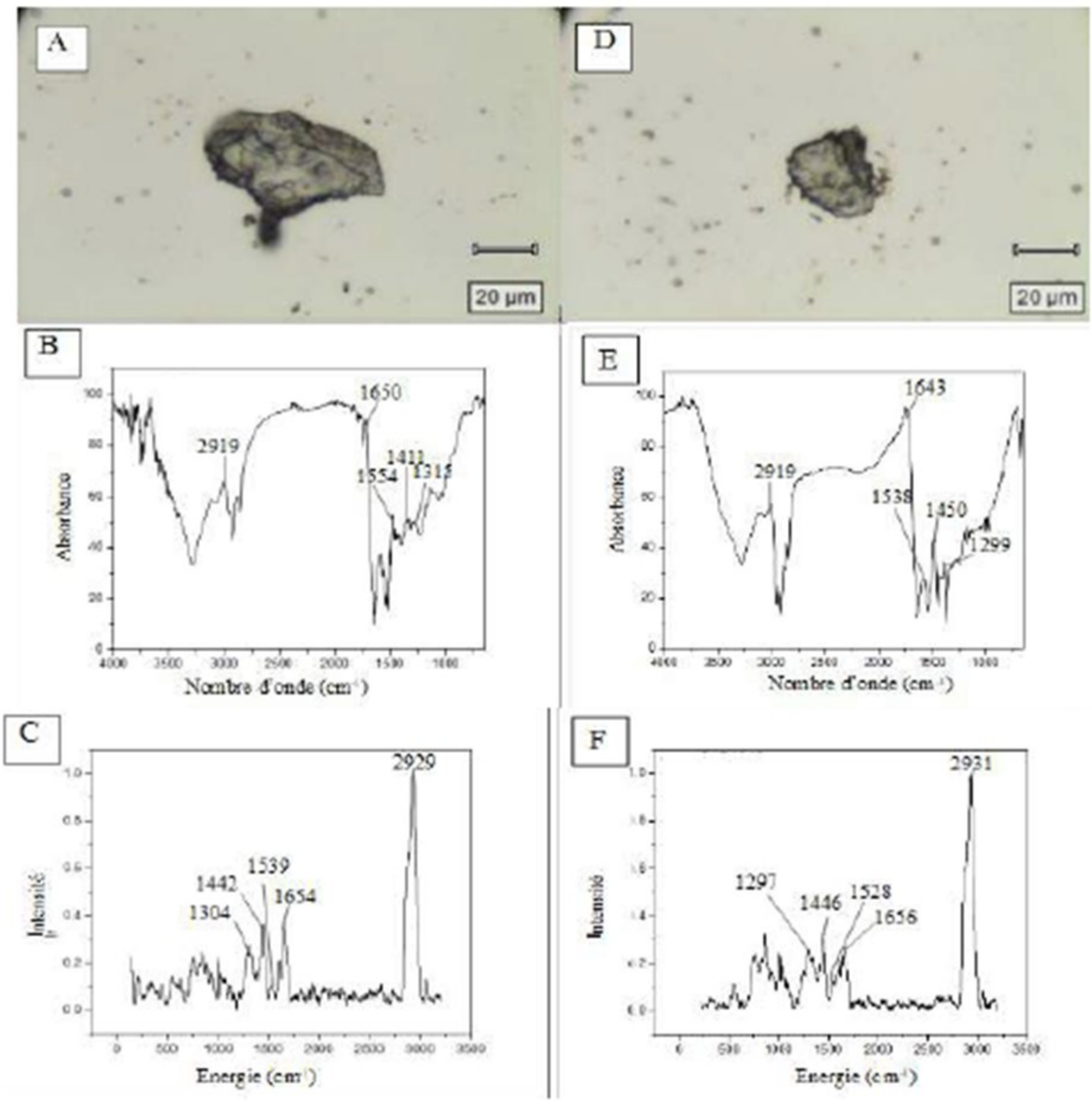

Figure 4. A) Photograph of a blue fiber (x50 Raman), B) Infrared spectrum and C) spectrum Raman showing absorption peaks (cm^{-1}) associated with nylon D) Photograph of a dark fiber (x50), E) Infrared spectrum and F) Raman spectrum showing absorption peaks (cm^{-1}) associated with the nylon.

ide) was characterized by the strong bands of Amide I, Amide II and of Amide III at 1650 cm^{-1}, 1554 cm^{-1} and 1241 cm^{-1} respectively (Fig. 4B). Spectra Raman confirmed the presence of Nylon in both fibers (Figs.4C and 4F).

DISCUSSION

This study on the microplastic pollution of green sea turtles Chelonia mydas, was the first to be carried out in the Caribbean of Mexico, also carried out with a non-invasive technique. Microplastics can stay in the last part of the intestine for more than 40 days before being defecated at sea (Biagi et al, 2021). The majority of studies focusing on microplastic pollution in turtles have been carried out using the upper part of the gastric contents of dead animals (Biagi et al, 2021).

According to a study conducted on 2 green turtles in Cairns (Australia), an average of 3.5 microplastics per individual was reported (Caron et al, 2018). In this same region, another study estimated an average of 11 particles per animal, based on the analysis of the intestinal system of 7 green sea turtles (Duncan et al, 2019). In the Atlantic Ocean, an examination of the intestines of 10 turtles demonstrated an abundance of 5 MP per organism (Duncan et al, 2019). the results of our research seem to be superior to these studies. The Yucatán current and the Loop current extend off the coast of Quintana from south to north with flow and speeds high. With the presence of large eddies that can reach up to 300 km in diameter and 1000 m in depth (Cruz-Salas et al. 2022). Therefore, it is likely that these marine currents deposit a large part of the MP at the level of Isla Blanca. This site is also a place of passage for fishing boats from Isla Contoy. It could therefore be more affected by MP pollution from the fragmentation of fishing nets and lines.

Green sea turtles could ingest large amounts of particles due to their herbivorous diet, as microplastics accumulate extensively on the marine vegetation they consume (Sinaei et al, 2021). All of the microplastics in the feces were fibers. Others authors also reported a predominance of fibers, extracted from the system green sea turtle gut (Caron et al, 2018; Pietroluongo, 2018; López Martínez et al, 2021). No domestic wastewater treatment system exists in eastern Yucatán Peninsula. This untreated water is discharged directly into the marine environment, which could explain the prevalence of type microplastics fiber from clothes washing (Krekeler et al, 2007). In addition, the use of nets fishing in the area contributes to the increase in the proportion of fibers compared to other types of microplastics (Anbumani and Kakkar, 2018). Further studies using our non-invasive method on a larger number of samples could provide a better understanding of the variations in microplastic contamination of green sea turtles in the Caribbean region.

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