

1 **Microplastic distribution in urban vs pristine mangroves: using marine sponges as bioindicators**
2 **of environmental pollution**

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17 **Highlights**

- 18 • Fibres were the only microplastic type in marine sponges from Isla del Carmen.
- 19 • Sponges exhibited MP concentrations from 556 to 5000 items kg⁻¹.
- 20 • Sponges bioaccumulate 17 times more MPs than seawater concentrations.
- 21 • Marine sponges act as a quantitative bioindicator of MPs to aquatic environments.
- 22 • Fishing, sewage and laundry activities were the main anthropogenic sources of MPs.

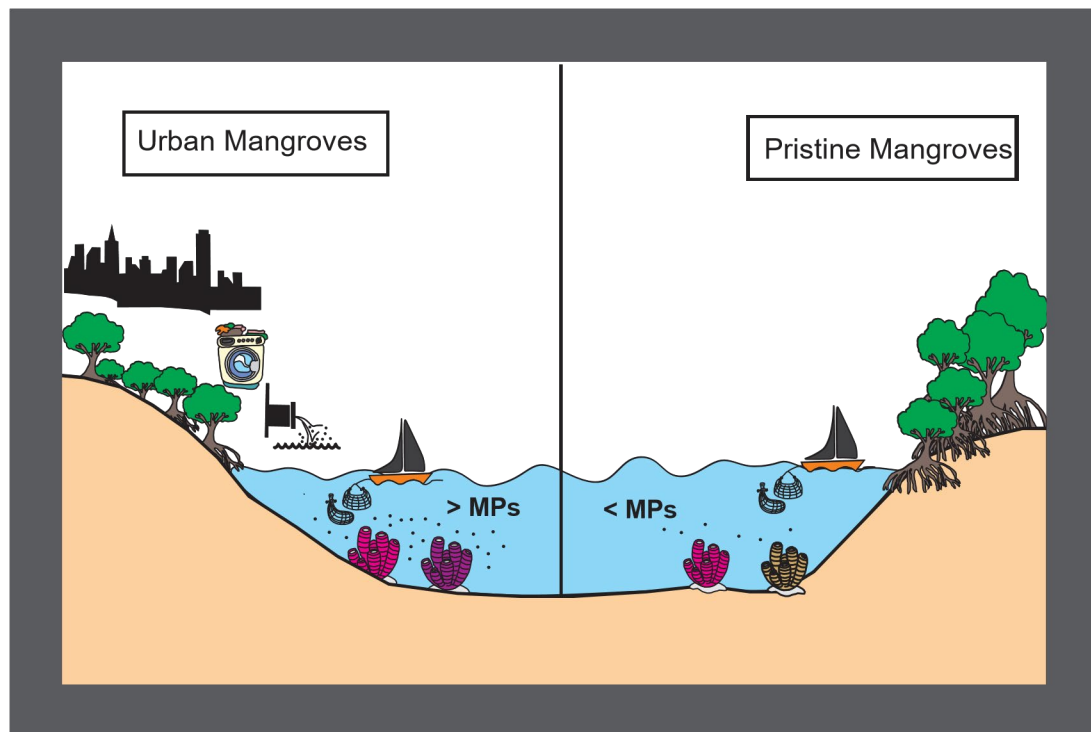
23 **Abstract**

24 Sessile benthic organisms are considered good bioindicators for monitoring environmental quality
25 of coastal ecosystems. However, these environments are impacted by new pollutants such as
26 microplastics (MPs), where there is limited information about organisms that can be used as reliable
27 bioindicators of these emerging contaminants. We evaluated MP concentrations in three
28 compartments: surface sediment, water and in three marine sponge species (*Haliclona*
29 *implexiformis*, *Halichondria melanadocia* and *Amorphinopsis atlantica*), to determine whether these
30 organisms accumulate MPs and reflect their possible sources. Results showed MPs in all three
31 compartments. Average concentrations ranged from 1861 to 3456 items kg⁻¹ of dry weight in marine
32 sponges, 130 to 287 items L⁻¹ in water and 6 to 11 items kg⁻¹ in sediment. The maximum MP
33 concentration was in the sponge *A. atlantica*, which registered 5000 items kg⁻¹ of dry weight, in
34 water was 670 items L⁻¹ and in sediment was 28 items kg⁻¹, these values were found in the disturbed
35 study area. The three sponge species exhibited MP bioaccumulation and showed significant
36 differences between disturbed and pristine sites (F= 11.2, p < 0.05), suggesting their use as
37 bioindicators of MP.

38 **Capsule abstract:** Bioaccumulation of marine sponges show how these organisms could be
39 considered as possible bioindicators to evaluate MP pollution in coastal ecosystems.

40 **Keywords:** Microplastics, Marine Sponges, Mangroves, Isla del Carmen, Gulf of Mexico. **Graphical**

41 **Abstract**



42

43 **Introduction**

44 Microplastics (MPs) are recognized, together with a range of new pollutants, as emergent
45 contaminants (Anderson et al., 2018; Yin et al., 2020). They can be defined as any synthetic material
46 with a specific density, polymeric matrix, colour, size less than 5 mm and undefined shape (fibres,
47 foams and microbeads) (NOAA, 2018; Stead et al., 2020 and Fred et al., 2020). This emergent
48 contaminant has recently been noted as a global concern, because of their potential environmental
49 impacts and wide distribution in marine and estuarine ecosystems (McEachern et al., 2019; Velez et
50 al., 2019 and Zhang et al., 2020). Urban wastewater, sewage discharge and agricultural runoff are
51 identified as point sources of MPs (Yeats et al., 2010; Li et al., 2020). Human activities such as fishing,
52 shipping, tourism, cosmetic and textile industries are also linked to plastic and MP contamination
53 and can be considered as sources of MP inputs to aquatic environments, either directly or indirectly
54 (Nuelle et al., 2014; Aliabad et al., 2019).

55 In tropical and subtropical coastal systems worldwide, mangroves are considered as natural
56 pollution filters (Maghsodian et al., 2021; Rezaei et al., 2021), because they are usually located close
57 to urban or industrial environments that dump their waste with little or no treatment. In general,
58 these ecosystems are recognized worldwide as natural environments that are exposed to a range of
59 pollutants including MPs, in part due to their role as a conduit between the terrestrial and marine
60 realms (Ward et al., 2016; Celis et al., 2020). Indeed, due to their role as nursery habitats for a range
61 of marine organisms, mangroves have been suggested as the first step in the pathway of MP
62 transference to higher levels of the trophic chain and begin a bioaccumulation and biomagnification
63 process along all the food webs.

64 Bioindicators are organisms that provide information about environmental quality where they occur
65 (Holt and Miller, 2011). They can reflect spatial and temporal variations in environmental conditions
66 due to changes in their diversity, abundances or for their capacity to accumulate pollutants (Zukal
67 et al., 2015; Bonanno and Vymazal, 2017). In general, these organisms should have some basic
68 properties such as natural abundance, wide geographical distribution, ease of identification, ease of
69 sampling and they must show a moderate tolerance to disturbance and stress (Carignan and Villard,
70 2002; Caro et al., 2010; Urban et al., 2012) that help to integrate environmental information. Marine
71 organisms that have been proposed as bioindicators of plastic debris are: seabirds (e.g., *Fulmarus*
72 *glacialis*, Linnaeus, 1761), loggerhead sea turtles (e.g., *Caretta caretta* Linnaeus, 1758), mussels (e.g.,
73 *Mytilus edulis*, Linnaeus, 1758), and other taxonomic groups such as fish, mammals, polychaetes,
74 bryozoans, holothurians and also bacterial communities (Bonanno and Orlando, 2018). In the case
75 of marine sponges (phylum Porifera), they have been proposed as bioindicators for heavy metals,
76 polycyclic aromatic hydrocarbons, and microbial pollution; due to their sessile condition, type of
77 feeding (by filtration), their high sensitivity to environmental changes and their relative abundance
78 in benthic ecosystems (Mahaut et al., 2013; Batista et al., 2014). However, the use of marine sponges
79 as bioindicators of MPs has hardly been investigated and there is limited information about marine
80 sponges that enable us to understand if these organisms are capable of reflecting the environmental
81 quality of aquatic ecosystems and if they could be used as bioindicators for MPs (Baird, 2016; Karlson
82 et al., 2017; Girard et al., 2020). Our hypothesis is that if they accumulate MPs in their body, MP
83 concentration is likely to be related to that recorded in the surrounding environment (e.g., water
84 column and surface sediments) where they occur.

85 Here we examine the accumulation of MPs in marine sponges, sediment and water from two
86 mangrove areas with different levels of human disturbance located within Laguna de Terminos, a
87 Natural Protected Area located in the southern Gulf of Mexico. The study evaluated whether the
88 concentration of MPs in these compartments varied spatially (between sites), and in the case of
89 sponges, between species. An assessment was also undertaken to assess whether sponges have the
90 potential to be used as bioindicators of MP pollution in mangrove areas.

91 **Study area**

92 Mangrove ecosystems from Isla del Carmen belong to a natural protected area known as Flora and
93 Fauna Protection Area Laguna de Terminos, which includes the second largest coastal lagoon
94 environment in Mexico (about 7050.16 km²) (INEGI, 2018) (Figure 1). Isla del Carmen is a barrier
95 island which 77% of its area is covered by mangrove forests, while the remaining 23% is covered by
96 the urban area of Ciudad del Carmen, which is the second most populated city (248,303 inhabitants)
97 in Campeche State (INEGI, 2018). The inner part of Isla del Carmen is characterized by shallow
98 seagrass meadows (dominated by a mix of *Thalassia testudinum* Banks ex Köning, 1805 and *Halodule*
99 *wrightii* Ascherson 1868) bordered by mangrove areas (*Rhizophora mangle*, *Avicennia germinans*,
100 *Laguncularia racemosa* and *Conocarpus erectus*). There, *Amorphinopsis atlantica* Carvalho, Hadju,
101 Mothes & van Soest, 2004, *Halichondria melanadocia* Laubenfels, 1936 and *Haliclona implexiformis*
102 (Hechtel, 1965) are among the most common sponge species in the area (Castellanos-Pérez et al.,
103 2020) (Figure 2). Although this is a natural protected area, artisanal fishing activities are permitted
104 throughout the year.

105 **Materials and methods**

106 **Field sampling**

107 In order to determine whether marine sponges reflect the environmental quality of mangrove
108 ecosystems, two sampling sites with a clear and contrasting anthropogenic influence were selected.
109 The disturbed area was identified as site “A” (18° 38′24.07” N, 91° 47′49.86” W), characterized by
110 mangroves impacted by untreated urban water discharges presence from Ciudad del Carmen as well
111 as wastewater from an adjacent slaughterhouse. The distance from the sampling point to the
112 shoreline was approximately 50 m and the depth was 1.1 m at high tide.

113 The undisturbed area is located 30 km away from area “A” and was identified as site “B” (18°
114 44′31.62” N, 91° 32′13.66”). This site is characterized by pristine mangroves, where the only human
115 activities are artisanal fishing and the distance from this sampling point to the shoreline was
116 approximately 50 m and the depth 1.45 m at high tide. At every site 3 sediment samples, 3 water
117 samples and 10 sponge individuals were taken. Surface sediment samples were taken using a
118 handheld aluminium corer. Water samples were obtained taking surface water with a gallon
119 container. Samples of two different sponge species were also collected (by hand while snorkelling)
120 at each site. At site A, five individuals of *H. implexiformis* and five of *A. atlantica* were collected, and
121 at site B, five *H. implexiformis* and five *H. melanadocia* individuals were collected (Figure 2),
122 representing the dominant species in both areas (Castellanos-Pérez et al., 2020). The collection
123 methods were stratified random point for each compartment and were undertaken in August 2019.

124

125 **Laboratory work**

126 Organic and inorganic samples were processed as follow: Sponge samples were cleaned for
127 epibionts and washed with distilled water to remove sediment and detritus on the surface. They
128 were then dried in an oven at 40°C for 7 days to obtain the dry weight. In each sponge individual,
129 three subsamples from 0.2 to 1.2 g were extracted with a 1.2 cm diameter aluminium corer to
130 examine MPs inside the sponge body. These subsamples were crushed in a pestle and mortar to
131 obtain a finely ground material. Before crushing these subsamples were observed under a Carl Zeiss
132 Stemi 305 stereomicroscope at 4x magnification to observe and remove any material (rocks, shells
133 or macroplastics) to facilitate the process. Each sponge subsample was introduced into a solution of
134 distilled water, hydrogen peroxide and ammonium hydroxide in equal parts for three days at 50°C
135 to digest the organic material. After this treatment the material was distributed in glass petri dishes
136 and put to dry in an oven for 3 days. Once the material was dried, it was observed under the
137 stereomicroscope. MPs were carefully removed using dissecting forceps, quantified by shape and
138 colour, and placed in flasks with distilled water for subsequent analysis. Sediment samples were
139 dried at 50°C for 72 h. MP separation from the sediment was undertaken by flotation using a
140 saturated solution of NaCl, where 100 g of dried sediment was shaken by a magnetic stirrer in 1 L of
141 saturated solution for 5 min and 2 min rest to let the sediment settle in the flask bottom. Later, using
142 a vacuum system the supernatant was filtered in 0.45 µm nitro cellulose filters (Millipore) and rinsed
143 with 250 mL of distillate water to avoid salt precipitation during the filter drying process. After this,
144 each filter was dried at 50°C for 24 h and it kept in glass petri dishes until MP assessment using the
145 stereomicroscope was undertaken. Finally, employing a vacuum system 100 mL of seawater was
146 filtered on 0.45 µm nitro cellulose Millipore and each filter was treated at the same way those used
147 for sediment sampling. Care was taken to avoid any potential environmental contamination
148 throughout the analysis and sample process. Simple precautions such as exhaustive rinsing with

149 distillate water were undertaken for all the materials and equipment. Glassware material was used
150 instead of plastic, wherever it was possible, and each sample was covered before and after analysis
151 to avoid atmospheric contamination.

152 **Microplastics identification**

153 In this study, all MPs were verified using the hot needle test. Although this test cannot be used to
154 identify MP polymer types such as polyethylene, propylene, and PET, it is acceptable as an
155 economical way to verify particles are MPs based on their response to a hot needle (Lusher et al.,
156 2017; Campbell et al., 2017; Silva et al., 2018; Kapp and Yeatman, 2018). Additionally, we used
157 photographs published in scientific journals as base data.

158 **Data analysis**

159 The normality and homoscedasticity of the data (MP concentrations in sponges, sediments, and
160 water) were examined using the Shapiro-Wilk and Levene's test, respectively. One-way Analysis of
161 Variance (ANOVA) were performed to determine whether there were significant inter-site variations
162 in MPs concentration and colour in the three marine compartments (sponges, sediment and water
163 column). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to assess
164 variations in MPs concentration between sponge species. These analyses were performed using
165 Statistica 6.0 software. To evaluate the marine sponges' ability to accumulate MPs with respect to
166 their availability in the environment, we used the bio-accumulation factor (BAF), which is defined as
167 the uptake of a contaminant by an organism from the abiotic environment (non-living chemical and
168 physical components of the environment that affect living organisms, for instance sunlight,
169 sediment, water and pollution) (Rand et al. 1995). Usually, the BAF for sponges is calculated as the
170 ratio between the chemical concentration (in this case MPs) recorded in sponges divided by MP
171 concentration in seawater or sediment (Negri et al., 2006; Venkateswara et al., 2009; Padovan et al.,
172 2012; Batista et al., 2014; Orani et al., 2018). For this work we used MP concentration in seawater
173 to obtain the BAF values. Values equal or higher than 1 are considered indicative of bioaccumulation.

174 **Results**

175 **Microplastic abundance in water, sediment and marine sponges in the pristine and disturbed** 176 **environments**

177
178 Results showed MP presence in the three compartments in both, the disturbed (site A) and pristine
179 (site B) environments (Figure 3). In seawater, the average abundance of MPs was 287 items L⁻¹ in
180 site A (ranged from 20 to 670 items L⁻¹) and 130 items L⁻¹ in site B (from 20 to 670 items L⁻¹). In
181 sediments, the average abundance was 11 items kg⁻¹ of DW in site A (6 to 28 items kg⁻¹) and 6 items
182 kg⁻¹ of DW in site B (from 4 to 10 items kg⁻¹). While in marine sponges the average abundances were
183 3456 items kg⁻¹ of DW in site A (from 2000 to 5000 kg⁻¹) and 1861 items kg⁻¹ in site B (from 556 to
184 4444 items kg⁻¹). In general, every compartment had the highest MP concentration in site A, but MP
185 average concentration in the seawater and sediments did not vary significantly between the sites
186 (F=0.58, p > 0.05, F=0.32, p > 0.05, respectively). Although, the differences between water and
187 sediment were not significant, there was almost a 2:1 ratio between the disturbed and pristine
188 environments that shows a clear spatial distinction in MP abundance.

189

190 **Microplastic kind, size and colour found in water, sediment and marine sponges**

191 In this work, only MP fibres were recorded in every compartment and studied site. Their average
192 sizes ranged from 2.3 to 6.2 mm and their colours were blue, red, black and white (Figure 4). In both
193 sites, we found blue and red fibres in water, sediment and sponges. White fibres were found only in
194 water; while black fibres were only identified in sediment and sponge samples. Blue fibres were
195 more abundant (between 40% and 74%) than red (15% and 30%) and black (11% and 30%) in the
196 sediment and sponges, respectively; while white fibres were more abundant (41%) than blue (39%)
197 and red (20%) fibres in water.

198 **Inter-site and inter-species variability of MPs concentration**

199 The results show the presence and bioaccumulation of MPs in the three studied sponge species and
200 inter-site differences between average concentrations. The average concentration of MPs in the
201 species *A. atlantica* and *H. implexiformis* were 3455 and 3457 MPs kg⁻¹ DW of sponge and their
202 ranges per species were from 2000 to 5000 and from 2500 to 4444, respectively in site A; while in
203 the species *H. melanadocia* and *H. implexiformis* concentrations were 2033 and 1688 MPs kg⁻¹ DW
204 and they varied from 556 to 4444 and from 909 to 2143, respectively in site B (Figure 5). MP
205 concentration in sponges did not vary between species (site A: F= 0.0001, P > 0.05; site B: F= 0.17,
206 P > 0.05) but did vary significantly between sampling sites (F= 11.2, p < 0.05). These results suggest
207 that the sponges *H. melanadocia* and *H. implexiformis* from site A exhibited significantly lower MPs
208 concentration than the species *A. atlantica* and *H. implexiformis* from site B. The average
209 bioaccumulation factor (BAF) of MPs in the species *A. atlantica* and *H. implexiformis* was 17 for both
210 species within site A. At site B the BAF was 10 and 8 in the species *H. melanadocia* and *H.*
211 *implexiformis* respectively. These results suggest that marine sponges can bioaccumulate MPs by a
212 factor of at least 8 compared to MP concentrations in the surrounding seawater.

213 **Discussion**

214 **Microplastic origin, sources and possible chemical composition**

215 The chemical composition of MPs is a good tool to identify the origin of these pollutants, because it
216 is possible to identify the type of plastic that is deposited in aquatic environments and infer what
217 was made with it. However, it is both costly and complicated, due to the nature of microplastics
218 (small), and chemical analysis is not the only way to undertake an evaluation of the source. Using
219 physical characteristics rather than chemical composition is a good and cheap approximation.
220 Plastics from different sources obtain different shapes when degraded by weathering conditions and
221 it is feasible to use MP shape to identify the origin and source. Borges et al. (2020) identified that
222 most fibres found in aquatic environments are related to fishing and laundry activities. Wang et al.
223 (2019) suggested that films are likely to be derived from plastic bags and agricultural films. Foams
224 have been suggested to be related to the breakdown of foam material such as expanding foam,
225 floating fishing gear and floating containers that are used for packaging and fisheries industries
226 (Zhou et al., 2020) and pellets and microbeads are related to cosmetic and washing products
227 (Andrady et al., 2017). Therefore, if MPs of different shapes are found in a specific environment, this
228 suggests that they come from different origins. Several studies worldwide have noted that urban
229 sewages and waste water treatment plants can be considered as an important source of MPs and
230 also have noted that fibres are the main shape for MPs from this source (Fendal et al., 2009; Browne

231 et al., 2011; Deng et al., 2020). This is because sewage systems usually receive most of the
232 wastewater generated by laundry activities in urban centres.

233 Our results suggest that MPs obtained in this work come from the same origins, because, we find
234 fibres as the only MP shape in both study sites. Both fishing activities and sewage outfall are well
235 known as environmental MP fibre sources. Therefore, it is suggested that these pollutants are likely
236 to come from the degradation of fishing nets, ropes, and laundry activities undertaken in Ciudad del
237 Carmen. Also, the sewage presence in the degraded site could explain why the average MP
238 abundances (in the three compartments) were almost double in the disturbed site compared to the
239 pristine site. Borges et al. (2020) identified Polyamide 6 + Polyamide 6.6, Polystyrene and nylon in
240 fibres collected in six fish species of the Campeche Bay (the same area as this current study). These
241 polymers are typically used in packages and packaging, twine, ropes, water sport items such as life
242 jackets, clothes, and fishing lines respectively. Chang et al. (2019) observed that fibres collected in
243 the Hong Kong coast were made of nylon, polystyrene, and polyethylene. Historically, the main
244 polymers in aquatic environments were found to be polyamides and polyester, which are common
245 materials in fishing activities (Pruter, 1987) and these polymers have been recorded in high
246 abundance in every aquatic environment (Wang et al., 2018). Therefore, it is highly likely that
247 plastics related to fisheries activities and clothing such as polyamides, nylon, polystyrene, and
248 polyester represent the chemical composition of the fibres found in this work.

249 **Microplastic bioavailability**

250 Microplastic bioavailability in organisms is related to physical characteristics such as size, shape and
251 colour. Usually, their bioavailability is due to their small size that allows them to be easily ingested
252 by aquatic organisms (Jovanovic et al., 2017). Their shape and colour are identified as factors that
253 produce a misunderstanding in aquatic predators about what they are eating (Wright et al., 2013;
254 Masia et al., 2019) as MPs can be readily mistaken for prey, particularly where biofouling has
255 occurred. Borges et al., (2020) noted MP presence in the gastrointestinal track of six commercial
256 marine fish species caught for human consumption along the coast of Campeche, Mexico. They
257 found fibres, fragments, and pellets were the main shapes of MPs. Borges et al. (2020), recorded
258 black, white, red, blue, orange, green and brown fibres and pellets. Their results highlighted
259 differences in the shape and colour of MPs found between demersal fish and pelagic species. These
260 differences showed evidence that the density of the MP material plays a key role in determining
261 their fate in marine fish habitats. Sun et al. (2017) evaluated the characteristics of MPs ingested by
262 different groups of zooplankton in natural conditions, including copepods, chaetognaths, jellyfish,
263 shrimps and fish larvae and the encounter rates between them. Their results highlighted that the
264 size of MPs ingested by zooplankton ranged from 125 μm to 167 μm . They identified fibres, particles,
265 and other types of MPs between the zooplankton groups. They also found that the proportion of
266 shapes of the MPs varied between zooplankton groups. For example, fibre proportions decreased
267 from copepods to fish larvae and the encounter rates of MPs/ zooplankton increased from copepods
268 to chaetognaths, jellyfish, shrimps and fish larvae. Although MP toxicity is related to the additives,
269 and the organic and inorganic pollutants adsorbed that can be transferred to aquatic organisms,
270 some works have noted that MP colour could provide clues about the capacity of these particles to
271 adsorb heavy metals and organic pollutants. Huang et al. (2020) have noted that MPs with dark
272 colours (black and blue) can better adsorb heavy metals and Frias et al. (2010) suggested that white
273 MPs contained less adsorbed persistent organic pollutants than MPs with other colours.

274 Our data recorded MP fibres in water, sediment and benthic organisms. These results showed the
275 presence of MPs in all three compartments, within both urban/degraded and pristine mangrove
276 ecosystems of Isla del Carmen and highlighted MP bioaccumulation in organisms. Although we
277 expected to find the same shapes and colours in marine sponges, we noted that white fibres were
278 not present in sediment or sponge samples (Figure 6). This suggests that white fibres could remain
279 in the water column because they are made of a less dense material or they are consumed by fish
280 that prevent settling of this material to the sediment and being filtered by marine sponges. Borges
281 et al. (2020) noted that demersal fish collected close to the coast of Campeche Bay ingested more
282 fibres than pelagic species and the main MPs collected in all fish species analysed were white. They
283 attributed these differences to the fact that demersal fish inhabits in sandy bottoms covered by
284 *Thalassia testudinum* where fibre MPs are apparently more abundant than fragments. The Borges
285 et al. (2020) study was conducted in the same region as our study sites and this removal from the
286 water column, may in part explain why we did not find white MPs in marine sponges. Moreover, in
287 this work we did not find MPs smaller than 2 mm which would suggest bioaccumulation in
288 zooplankton as these organisms bioaccumulate MPs < 200 µm. However, sponge predators such as
289 polychaetes, crabs, fish and turtles (Pawlik, 1983; Padilla et al., 2010) are likely to bioaccumulate
290 and biomagnify MPs through the trophic chain.

291 **Feasibility of using sponges as bio-indicators of MPs pollution in coastal ecosystems**

292 Marine sponges could be considered good bioindicators of MPs because they are efficient filter
293 feeders (Reiswig, 1971; Riisgård et al., 1993). Their physiology lets them filter between 100 to 1200
294 mL per hour and per gram of dry weight through their pores and channels (Vogel, 1977). This fact
295 makes them suitable organisms to detect MPs suspended in the water column. Also, they are
296 geographically well distributed, spread along many coastal ecosystems such as coral and algal reefs,
297 seagrasses and mangroves (Batista et al., 2014). Although, there is a lack of information about using
298 marine sponges as MP bioindicators, some works have noted that these organisms can capture
299 microparticles in their bodies. Recently, Girard et al. (2021) proposed sponges as bioindicators for
300 microparticulate pollutants, where MPs were included. They identified 34 different particle types
301 such as polystyrene, particulate cotton, titanium dioxide and blue pigmented particles. Their results
302 showed that marine sponges can incorporate a variety of microparticles inside their skeletal fibres,
303 the ectosome or both depending on the particle size and sponge species. These results suggested
304 that the fluctuation in material ratios found inside the sponges' tissues was a response to the spatial
305 variation of surrounding microparticles. Modica et al. (2020) used a sponge collection from the
306 Cantabrian Sea Maritime museum to detect synthetic textile microfibers in sponges collected 20
307 years ago in the North-east Atlantic Ocean. They analyzed 170 samples that belonged to 34 sponge
308 families. Their results showed that specimens of all the sponge families examined (n=34 families)
309 were able to retain MPs. They also found that the presence of MPs on the sponge samples were not
310 related to sampling depth (as these particles were present along the whole sampling depth range,
311 from 1 to 23.5 m) nor the ecotope where they were collected (e.g., *Laminaria* spp., rocky wall, rocky
312 shade, cave and sand).

313 Regarding the bio-accumulation factor (BAF), as we expected, the three sponge species showed
314 values higher than 1 and confirm the ability of these sponge species to bioaccumulate MPs from the
315 two contrasting mangrove environments examined. The higher BAF values recorded in the sponges
316 from the disturbed site could be due to the fact that the organisms had higher MP concentrations

317 than sponges in the pristine site. Regardless of the fact that MP concentration in seawater was not
318 recorded as statically significant between the two sites. Also, the inter-site variation found in MPs
319 concentration in sponges suggests that marine sponges can reflect spatial variations of MPs from
320 places with different pollution level as has been documented in the mussel *Mytilus edulis* from the
321 North Sea (Karlsson et al., 2017). There were no significant differences in the average concentration
322 of MPs among the sponge species studied, thus, these results suggest that these three species
323 exhibit a similar capacity to accumulate MPs in their tissues. A search for information available on
324 MPs in sponges was conducted to compare the concentrations reported in this study with other
325 species collected from the natural environment. Data were only found for the sponge *Hymeniacidon*
326 *perlevis* (Montagu, 1814) from Lake Veere, Netherlands (Karlsson et al., 2017) and the
327 concentrations reported for this species (25000-57000 particles kg⁻¹ DW of sponge) were much
328 higher than those recorded in the sponge species of our study. This lack of information about MP
329 concentrations on marine sponges highlights the importance of our study.

330 **Conclusion**

331 The results of this study provide evidence that the three sponge species have the capacity to
332 incorporate MPs, and concentrations of this contaminant are reflected by spatial variations in the
333 degree of exposure to potential sources. Furthermore, because they are common, relatively
334 abundant and inhabit accessible areas for monitoring, we consider that their use as bioindicators in
335 integrated monitoring programs for these coastal environments along their distribution range would
336 be feasible and low cost. In addition, given the scarce knowledge about the accumulation of MPs in
337 sponges, it is recommended to continue examining this capacity (as potential bioindicators of MPs)
338 in a wider range of sponge species and from different types of environments.

339 **Acknowledgements**

340 OCH and MARS are CONACyT research fellows commissioned to the Universidad Nacional Autónoma
341 de México (project No. 345) and Universidad Autónoma de Carmen (project No. 1205). We thank to
342 Mario Alejandro Gomez-Ponce, Andres Reda-Deara and Hernan Alvarez-Guillen for their technical
343 assistance with field samplings.

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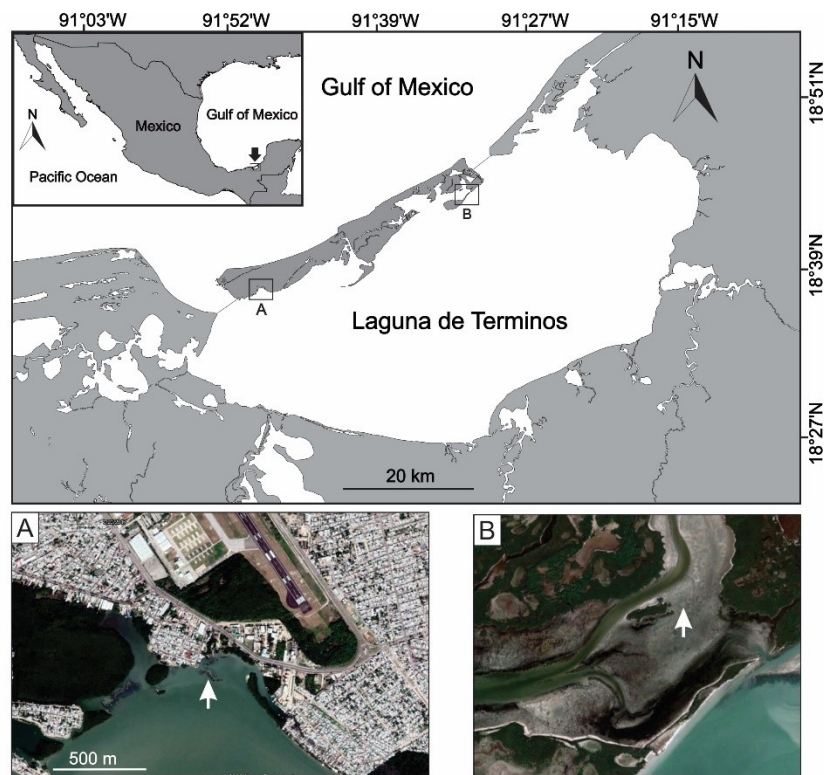
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558 Figure 1 Study area and sampling location

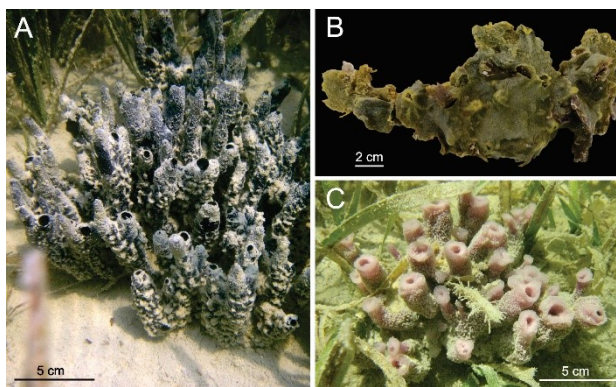


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Note: Sampling places identified as follow: (A): Disturbed area (El rastro), (B): Undisturbed area (La deseada).

561 Figure 2 Sponge species



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Note: Sponge species used in this work and identified as follow: **A:** *H. melanadocia*, **B:** *A. atlantica* and **C:** *H. implexiformis*.

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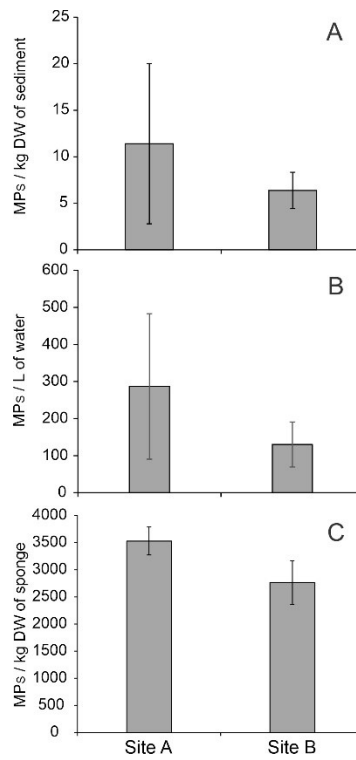
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569 Figure 3 Average microplastic concentration per compartment

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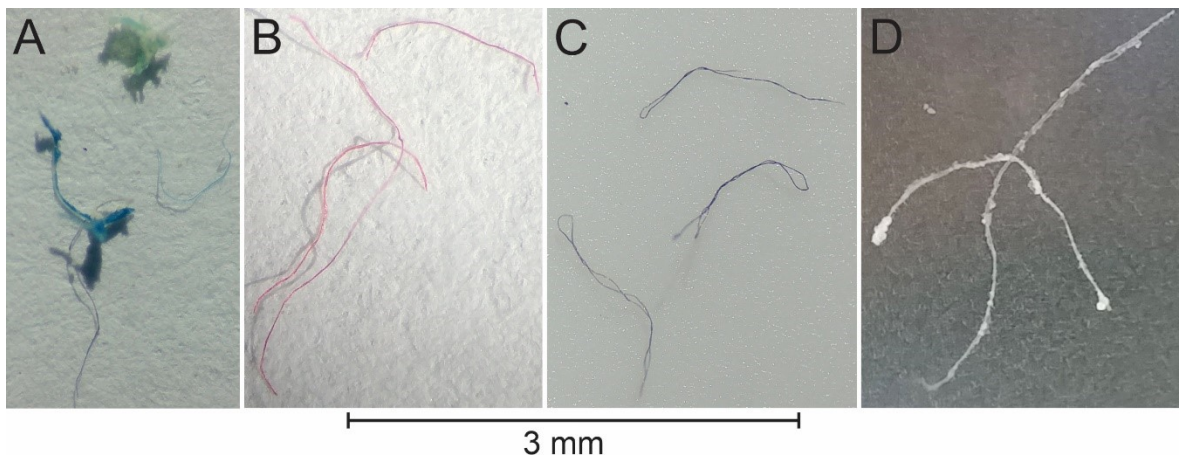
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Note: Sampling places identified as follow: (A): Disturbed area, (B): Undisturbed area and DW means dry weight.

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Figure 4 Microplastics fibers found in water, sediment and sponges



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Note: Type of MPs and colours identified in the three compartments. A: Blue fibres, B: Red fibres, C: Black fibres and D: White fibres.

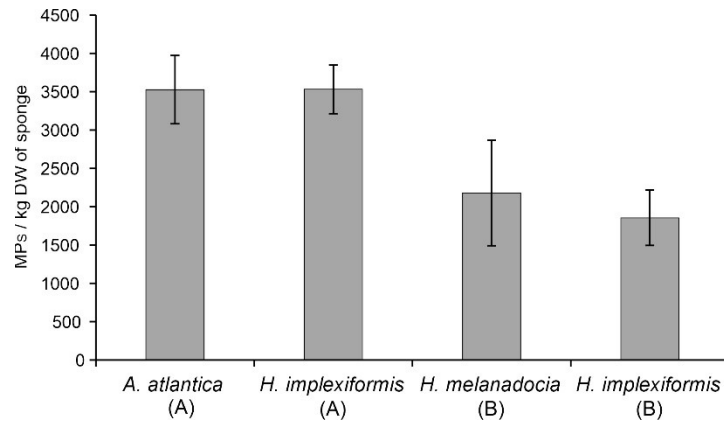
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Figure 5 Microplastic concentration between species



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Note: Sampling places identified as follow: (A): Disturbed area (El rastro), (B): Undisturbed area (La deseada)

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