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1 Microplastics pollution along the Lebanese coast (Eastern Mediterranean Basin):
2 Occurrence in surface water, sediments and biota samples
3

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8

9 The Mediterranean Sea is the largest semi-enclosed sea and one of the worst affected regional seas with
10 sub-basin scale heterogeneity in plastics concentration. Few studies on microplastics (MPs) pollution
11 have been conducted in the Eastern part of the Mediterranean basin. This study aims to evaluate, for the
12 first time, the MPs pollution of the Lebanese coast (Levantine Basin) as well as the most common
13 polymers found, and to assess the potential role of coastal landfills in this pollution. Two important
14 seafood species that are wholly consumed by the Lebanese community: the European anchovy *Engraulis*
15 *encrasicolus* and the spiny oysters *Spondylus spinosus* were sampled in three different sites englobing
16 the littoral (Tripoli, Beirut and Sidon). Sea water and sediment samples were also collected from the
17 same sites. Results showed different patterns of MPs concentration in the analyzed matrices: Sidon
18 water sample was highly contaminated in MPs (6.7 MPs/m³) whilst Tripoli had the highest MPs in
19 sediments (4.68 MPs/g). The occurrence of MPs in the biota was high (83.4% and 86.3% in anchovies and
20 spiny oysters, respectively). Both anchovies and oysters from Beirut region had the highest ingested
21 MPs/ individual (2.9 ± 1.9 and 8.3 ± 4.4 MPs/individual, respectively). This study is the first that
22 investigated microplastics ingestion by *Spondylus spinosus* while indicating the most common polymers
23 found in the three matrices (water, sediments and biota) in the Eastern Mediterranean. These results
24 highlighted the high MPs pollution found in the Levantine Basin in comparison to other Western
25 Mediterranean regions. In addition, the obtained results indicate a potential contribution of coastal
26 landfills to this pollution.

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28 **Keywords:** Microplastics, Levantine Basin, coastal landfill, filter-feeders, micro-Raman

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30

31 **1. Introduction**

32 The world plastic production is continuously increasing, reaching 350 million tons in 2017
33 (PlasticsEurope, 2018). Among the produced plastics, only 6–26% are recycled. Landfills receive
34 21–42% of wastes, whereas the rest are wildly released into the environment due to
35 mismanagement through a variety of pathways (Ellen MacArthur Foundation, 2016; O'Connor et
36 al., 2016). Most of the manufactured plastics will eventually end up in the ocean. By the year
37 2025, about 250 million tons of wastes are expected to enter the ocean water (Jambeck et al.,
38 2015) leading to their accumulation in the marine environment (Thompson et al., 2009; UNEP,
39 2014). Plastic pollution in the marine environment is becoming a problem of global concern.
40 These plastics are capable of transforming into dangerous small particles known as microplastics
41 (MPs) existing as particles of a size below 5 mm ubiquitously available in the aquatic
42 environments (Thompson et al., 2004; Andrady, 2011). They are either intentionally produced in
43 small sizes such as polystyrene beads for example, or they are the resultant of macroplastics
44 degradation (whether by Photochemical reaction, or mechanical actions.).

45 The Mediterranean Sea is prone to several anthropic pressures and has recently been described
46 as one of the most affected marine environments with regards to marine litter and microplastics
47 (Lebreton et al., 2012; Cincinelli et al., 2019). Mainly owing to the semi-enclosed situation and
48 limited outflow of surface waters, a densely populated coastline and intensive fishing, shipping,
49 touristic and industrial activities, substantial amounts of marine litter are accumulating in the
50 Mediterranean Basin. Also, most studies on microplastics have been conducted in the
51 northwestern Mediterranean Sea (e.g. Collignon et al., 2014; Fossi et al., 2016; Ruiz-Orejón et
52 al., 2016; Schmidt et al., 2018; Lefebvre et al., 2019). Results showed that their distribution and
53 composition are not homogeneous and geographical differences exist between Mediterranean
54 sub-basins (Suaria et al., 2016; Cincinelli et al., 2019). Such heterogeneity in MPs distribution

55 and of its concentration levels are likely reflecting a complex interplay between pollution
56 sources, hydrodynamic features such as currents, up and down-welling, gyres, fronts and the
57 shape of the coastline (Suaria et al., 2016; Schmidt et al., 2018; Cincinelli et al., 2019). In
58 addition, the absence of a standardized sampling protocol for the identification and
59 quantification levels of MPs could also be responsible for this heterogeneity in results.

60 Plastics in general, and microplastics specifically, are prone to be ingested by a wide range of
61 organisms (Lusher et al., 2016) and about one hundred species in the Mediterranean have been
62 affected either via ingestion or entanglement (Deudero and Alomar, 2015). Microplastics were
63 already found in several Mediterranean fish species such as *Boops boops*, *Sardina pilchardus*,
64 *Mullus barbatus* and *Merluccius merluccius* (Giani et al., 2019; Bellas et al., 2016; Rios-Fuster et
65 al., 2019) and in wild mussels (*Mytilus galloprovincialis*) caught off the Adriatic Sea and central
66 Tyrrhenian Sea (Gomiero et al., 2019; Renzi et al., 2018a).

67 Although it is suggested that it is the most polluted region, few studies have been conducted in
68 the Eastern part of the Mediterranean Basin (Van der Hal et al., 2017; Güven et al., 2017;
69 Gündoğdu and Çevik, 2017). In the Levantine basin, the Lebanese coast is highly affected by
70 numerous anthropogenic pressures such as harbor activities, fishing, domestic and industrial
71 wastewater discharges (generally without prior treatment). Waste production in Lebanon has
72 increased significantly during the last decade and the country has experienced waste crises. The
73 regulated dumping sites could no longer manage the tremendous quantity of municipal garbage
74 generated and, therefore, numerous unregulated and open dumping sites were created
75 throughout the country mainly along the coast (Khalil et al., 2018). The average composition of
76 municipal solid waste generated shows an important percentage of plastics (15%) which is two
77 to three times higher than that found in Sweden, France and the United States (El-Hoz, 2019).
78 Though the processes may be slow, landfills are affected by UV radiation and mechanical
79 abrasions leading to the fragmentation of plastics to microplastics, and small plastic debris
80 would find their way into the aquatic environment via leachates discharges (Alimi et al., 2018).

81 Despite these concerns, there have been no studies specifically examining the occurrence and
82 abundance of microplastics along the Lebanese coastal environment. The aim of this study was
83 to investigate the quantity and types of microplastics found in three matrices (sea water,

84 sediments and marine biota) along the Lebanese coast. All suspected MPs were characterized
85 by size, color and identified according to their polymer nature using micro-Raman spectroscopy.
86 Fish and bivalves are known to ingest microplastics (Collard et al., 2015; Phuong et al., 2018;
87 Kazour et al., 2019), making them a potential vector of toxic chemicals through food chains and
88 into human diets. In the present study we analyzed two marine species that are wholly
89 consumed by the Lebanese community: one pelagic fish species: the European anchovy,
90 *engraulis encrasicolus* (Linnaeus, 1758) and a bivalve species, the spiny oyster, *Spondylus*
91 *spinosus* (Schreibers, 1793).

92

93 **2. Materials and Methods**

94 **2.1. Study and sampling area**

95 Lebanon is located in the Eastern Mediterranean (Levantine Basin) with a coastal length of 220
96 km (Figure 1). Three sampling sites were chosen along the coast according to their degree of
97 anthropogenic pressures making them pollution hotspots (Shaban, 2008). The first site, Tripoli,
98 located in the north, is facing the town's public beaches, characterized by the presence of a
99 fishing harbor, and the existence of a coastal landfill. This latter was constructed in the year
100 1999 and reached today 36 m height receiving about 400 tons of wastes/day. The second site
101 Beirut, located in the middle, is the country's capital. This site is near the most important harbor
102 in Lebanon and surrounded by three coastal landfills: One closed and two active landfills that
103 reached their maximum capacities (55 meters of height) in July 2019. The third site Sidon,
104 located in the south, is facing a river's mouth and is near a closed landfill operated between
105 1982 and 2013. With a height of 58 m, this landfill was the biggest in the country receiving 300
106 tons of wastes/day. Actually, its transformation to a public park led to the burying of all wastes
107 underground; traces of these wastes can always be observed underwater. These landfills lack an
108 appropriate leachates treatment or it is not operational, and leachates are directly dumped to
109 the sea. The sampling for all three matrices was done in May 2018 (spring).

110 **2.2. Contamination control**

111 Several precautions were followed to minimize air-borne contamination during sampling,
112 samples preparation and analysis. Cotton lab coats were worn and different procedures
113 (filtration, measurements and digestion) were done under a laminar flow hood. All surfaces and
114 equipment were rinsed before usage with filtered ethanol 70% and distilled water. Laboratory
115 supplies were made of glass (bottles, Petri dishes, Erlenmeyers, filtration system) and they were
116 covered immediately after manipulation. All solutions (distilled water, Ethanol 70%, potassium
117 hydroxide KOH 10% and zinc chloride ZnCl₂) were filtered 3 times on glass fibers filter (GF/A
118 filters, 1.6 µm porosity, Whatman, France) to eliminate unwanted particles or fibers. Controls
119 were made to follow up the contamination occurring with every step of preparation and
120 observation.

121 **2.3. Water and sediments sampling and analyses**

122 Sea surface water and sediments samples were collected during spring 2018. Sea surface water
123 was collected using a manta trawl net (52 µm mesh size) with an opening of 0.63 m and a length
124 of 0.255 m equipped with a flowmeter. The net was trawled for 10 minutes parallel to the coast
125 at about 500 m from the coast with a small artisanal vessel (3 m of length) at a speed of 2 knots.
126 On average, 100 m³ of sea water was sampled at each site (Supplementary Table 1). The net
127 was washed with filtered distilled water and all collected residues were rinsed inside clean glass
128 bottles. The collected water was filtered on a stainless-steel sieve of a mesh size of 850 µm and
129 the recovered water was vacuum filtered on GF/A filters. The suspected MPs present on the
130 sieve were sorted visually under the stereomicroscope, fixed on a microscopic slide and
131 conserved inside a clean glass Petri dish.

132 The first two centimeters of sublittoral sediments were sampled using a steel ring with two
133 stainless-steel plates, rinsed with filtered distilled water before usage. The samples were put
134 inside clean glass jars and stored at -4°C. Samples were thawed at room temperature 12 hours
135 prior to analysis. Then particles with a size range of 1 – 5 mm were visually sorted and the
136 remaining sediments underwent a density separation using filtered ZnCl₂ (d= 1.8 g/cm³) (Imhof
137 et al., 2013; Nuelle et al., 2014). Briefly, 50 mL of filtered ZnCl₂ was added to the sample; the
138 solution was then shaken for 5 minutes twice (once every 4 hours) and let rest for 16 hours. The

139 supernatant was then recovered and filtered on GF/A filters. Microplastics found were reported
140 per gram of sediments sample and per m³ in sea water samples.

141 **2.4. Biota sampling and analyses**

142 Anchovies and spiny oysters were also collected during spring 2018. Anchovies were caught
143 using purse seine nets. Ten individuals of approximately the same size (9 cm ± 0.9) were chosen
144 from each sampling site and were immediately individually conserved in aluminum foil and
145 conserved at -4°C. In the laboratory, their morphometric characteristics (total length and
146 weight, digestive tube weight) were measured and their digestive tracts were dissected,
147 conserved in aluminum foil and frozen at -4°C. For the spiny oysters, ten individuals were
148 collected by fishermen from each sampling site. In the laboratory, the whole individual was
149 removed from its shell and was conserved in aluminum foil. The net weight of the animal
150 ranged between 4.8 g to 38.2 g -YY with an average of 20.2 g ± 9.4.

151 All samples were defrosted 12 hours prior to digestion. Fish digestive tracts and oyster soft
152 tissues were digested using potassium hydroxide 10% KOH (Dehaut et al., 2016). In short, the
153 organic material of each individual was put inside separate Erlenmeyer containing solution of
154 KOH 10% and then they were incubated at 60°C for 24 hours. Then, the digestat was recovered
155 and vacuum filtered on GF/A filters and put inside clean glass Petri dishes.

156 **2.5. Stereomicroscope and micro-Raman spectroscopy**

157 All samples were first observed under a Leica M165 C Stereomicroscope with a 120x
158 magnification. Photos of items were captured using a Leica M170 HD camera connected to LAS
159 (Leica Application Suite) software. They were listed, measured, and their morphology, color and
160 shape were noted. Several criteria were taken into consideration while counting the suspected
161 items: (1) absence of cellular or organic structures; (2) a homogenous thickness across the
162 particles; and, (3) homogenous colors (Hidalgo-Ruz et al. 2012). Suspected microplastics were
163 divided into 6 size classes: <200 µm, 200-400 µm, 400-600 µm, 600-800 µm, 800-1000µm, and
164 > 1000 µm.

165 Suspected microplastics were analyzed using Micro-Raman Xplora Plus (HORIBA Scientific®
166 France). This machine is equipped with a closing chamber that isolates the filter from its
167 surrounding, hence minimizing any airborne contamination.

168 Due to the high amount of time it takes when analyzing filters under micro-Raman, five filters of
169 anchovies and five filters of spiny oysters from each site containing the highest number of
170 potential MPs were identified. Subsamples of sediments and water were analyzed in a way that
171 25% of particles were analyzed. For MPs polymers identification, two lasers were used with a
172 wavelength of 532 nm and 785 nm with a range of 200-3400 cm^{-1} . Two objectives (Olympus,
173 Rungis, France) were used: x10 and x100. Filters were either analyzed manually or using
174 ParticleFinder module for LabSpec 6. Each particle spectrum is compared to a database polymer
175 identification software (KnowItAll, BioRad®) and a personal library made with specific polymers
176 obtained from Goodfellow (France).

177 **2.6. Data and Statistical analysis**

178 Anchovy Fulton's K condition index was calculated as an indicator of the fish general well-being:
179 $K = (\text{Body weight}/\text{total length}^3) * 100$. The feeding intensity for the fish was calculated using the
180 Gastro Somatic Index (GaSI) (Desai 1970): $\text{GaSI} = \text{stomach weight} / (\text{body weight} - \text{stomach}$
181 $\text{weight})$. The mean (\pm SD) abundances of microplastics were calculated for anchovies (MPs/ind)
182 and spiny oysters (MPs/g). After a Shapiro-Wilk test, data distribution of ingested MPs were non
183 normal and a non-parametric tests (Kruskal-Wallis and Mann-Whitney U tests) were used to
184 determine differences in microplastic numbers among species and sites. GaSI values followed a
185 normal distribution and a one way analysis of variance (ANOVA) test with a post-hoc Tukey test
186 was used. Spearman's rank correlation was used to test any correlation between the number of
187 ingested MPs and the fish condition index or GaSI. Non-metric Multidimensional Scaling (n-MDS)
188 was performed on a Bray-Curtis similarity matrix created from transformed data (square root).
189 This test was used as an ordination method alongside ANOSIM and SIMPER tests to compare the
190 contribution of each observed polymers in sea water, sediments and biota to the similarity and
191 dissimilarity between sites. Significance level was set at $p < 0.05$. All statistical tests were done

192 using SPSS software (IBM SPSS STATISTICS 20) and PRIMER v7.0 (Primer-E Ltd., Plymouth Marine
193 Laboratory, UK).

194

195

196 **3. Results and Discussion**

197 **3.1. Microplastics concentrations in sea surface waters and sediments**

198 Microplastics found along the Lebanese coastal waters consisted mainly of fragments (77.5%),
199 fibers (15.25%) and microbeads (7.25%) (Figure 2- A). For all sites, blue and red colors were the
200 most abundant representing 70% of all MPs. Concentration in the surface waters (4.3 MPs/m³)
201 were high compared to other Mediterranean regions and particularly the western part where MPs
202 concentrations vary between 0.17 and 0.62 MPs/m³ (Table 1). In the Eastern Mediterranean, the
203 MPs concentration fluctuates importantly across regions, ranging from 0.7 items/m³ in Northeast
204 Levantine coast of Turkey (Güven et al., 2017) to 7.68 ± 2.38 MP/m³ in the Israeli surface water
205 (van der Hal et al., 2017).

206 The heterogeneity of the MPs distribution and of its concentration levels could be related to
207 several factors. The mesh size of the net used could play an important role on the number of
208 microplastics caught during sampling. The high concentration of MPs found in the present study
209 maybe due to the low mesh size manta trawl net used (52 µm) compared to other studies
210 (generally between 200 and 500 µm). Also, the different methodological analyses and the lack
211 of a standard protocol could also be the cause of that difference. In fact, the water samples in
212 this study were filtered on GF/A filters and all suspected items were observed; whereas other
213 studies used organic material digestion (Gündoğdu and Çevik, 2017; Güven et al., 2017) or just
214 visual observation (Fossi et al., 2016; Lefebvre et al., 2019) which can lead to an
215 underestimation and a bias in microplastics counting. In addition, the distance of the sampling
216 location from the coast may also influence the concentration of MPs found. Our samples were
217 collected in the coastal waters (about 500 m from the shore). MPs concentration might have
218 differed if the collected samples came from the open sea (Van der Hal et al., 2017; Pedrotti et
219 al., 2016). On the other hand, the influence of hydrodynamic features such as currents, up and
220 down-welling, gyres and fronts could also be responsible for this heterogeneity in MPs

221 concentrations. The Eastern Mediterranean has been reported for its high contamination of
222 floating plastic debris with a dominance of small fragments < 2.5 cm in comparison to the
223 Western part (Cózar et al., 2015). High fluxes of plastic debris (10-30 kg/km.day) were observed
224 along the Levantine Basin coast from Egypt, Lebanon, Syria and Turkey (Constantino et al.,
225 2019). Differences between the two parts of the Mediterranean Sea can be explained by the
226 different circulation patterns: water currents in the Western part promote circulation; whereas
227 currents in the Eastern part are somewhat attractors leading to the accumulation of floating
228 debris (Mansui et al., 2015). In addition, the complexity of circulation with the existence of
229 mesoscale and sub-basin scale eddies in the Levantine basin (most specifically Shikmona anti-
230 cyclonic eddy) may also explain the high number of MPs found on the Lebanese coast (Menna et
231 al., 2012).

232 High concentrations of MPs in sediments were also found along the Lebanese coast ($2433 \pm$
233 2000 MPs/Kg d.w.). These concentrations were higher than those found in others
234 Mediterranean regions (Supplementary Table 2). This important difference can be either caused
235 by the different density separation solution used ($ZnCl_2$ used in our study is of higher density
236 than NaCl used in other studies and more microplastics can be identified) or by the mismanaged
237 waste along the Lebanese coast where high plastic items were visually observed during
238 sampling. Both micro and macroplastics (MaPs) were present in all collected sediments samples.
239 Lebanon is a potential landfall of plastics along with other Eastern Mediterranean regions
240 (Duncan et al., 2018) explaining the high abundance of macroplastics found in our samples
241 (consisting of half of the observed anthropogenic materials found). All kind of shapes were
242 found: square, rectangular, spherical and cylindrical (Figure 3). White was the dominant color in
243 Sidon and Tripoli (46.3% and 36.8%, respectively); blue color was the most abundant in Beirut
244 (23.7%). Pellets were highly abundant in two of our sampling sites (Tripoli and Sidon) which
245 explain the high abundance of white color in these two locations. Several studies investigating
246 MPs abundance in the Mediterranean sediments also reported the high presence of plastic
247 pellets among their samples (Karkanorachaki et al., 2018; Fanini and Bozzeda, 2018; Kaberi et
248 al., 2013; Turner and Holmes, 2011). The first signaled pellets on the Lebanese coast were due
249 to direct waste discharge of plastic factories on the beaches or in the rivers (Shiber, 1979). To

250 this day, and with the lack of an appropriate treatment, these industries are still releasing their
251 wastes into the aquatic system and are susceptible to unintentional losses leading to this high
252 accumulation of plastic pellets. All of which indicate their persistence whether in the Eastern or
253 Western Mediterranean and they (pellets and fragments) consist potential harm to the benthic
254 organisms inhabiting sandy beaches (Carson et al., 2011; Nelms et al., 2016).

255 Along the Lebanese coast, inter-site differences in MPs concentrations were observed. Surface
256 water samples showed that Sidon was the most contaminated site with 6.7 MPs/m³ (Figure 2-
257 A). This site is characterized by a high presence of microbeads with 1.16 microbeads/m³. Beirut
258 had the lowest concentration of MPs with 2.35 MPs/m³ consisting mainly (90%) of fragments.
259 The high number of MPs and pellets observed in the south of Lebanon can be explained by site's
260 localization near the biggest landfill. Although this landfill is closed since 2013, results of our
261 study provide preliminary evidence that landfill isn't the final sink of plastics, but a potential
262 source of microplastics (He et al., 2019). Fragmentation and release of microplastics is a long-
263 term process. Microplastics inside leachates from active and closed landfills have been
264 identified (He et al., 2019). After the stop of post-closure care, microplastics in leachate would
265 be discharged directly into coastal waters. This can pose serious environmental problems since
266 microplastic is not listed as pollutant in the any country's regulations of landfill. This area is also
267 characterized by the presence of the mouth of a major river, Al-Awali River. During sampling, we
268 observed fragments and other wastes being brought into the coastal waters via the river runoff.
269 This river extends for 48 km of length and collects all the urban water from nearby villages due
270 to the non-existence of sewage treatment system. These microplastics are prone to be washed
271 from surface waters onto beaches and, therefore, integrate within the sediments (Barnes et al.,
272 2009; Poeta et al., 2014). The general circulation along the coast of Lebanon is dominant
273 northwards and the water movements are strongly related to surface currents and seasonal
274 metrological factors (Goedicke, 1972). This northwards movement might explain why MPs and
275 MaPs were the most abundant in sediments samples of Tripoli (4.68 MPs/g and 4.08 MaPs/g,
276 respectively) (Figure 2- B). The high levels of MPs found in the surface water in Sidon and Beirut
277 might have been carried by surface circulation ending up washed on Tripoli beaches.

278

279 3.2. Microplastics in anchovies and spiny oysters

280 Microplastics can be ingested by a large variety of marine species and more specifically by filter-
281 feeders (Van Cauwenberghe and Janssen, 2014; Catarino et al., 2018; Collard et al., 2015) raising
282 the alarm for commercial species that are highly consumed by humans. In our samples, 83.3% of
283 European anchovies analyzed had ingested MPs. Such high level of ingestion is in agreement
284 with what was described for anchovy from the Adriatic Sea (90%) (Renzi et al., 2019a). However,
285 in the Western Mediterranean Sea, lower occurrences of MPs were described in anchovy
286 digestive tracts (11 to 40 %) (Collard et al., 2015; Compa et al., 2018; Lefebvre et al., 2019)
287 (Table 2). In the present study, anchovies ingested an average of 2.5 ± 0.3 MPs/individual which
288 was higher than that found in anchovies collected on the Northwestern Mediterranean coasts
289 such as Spanish coasts (0.18 ± 0.20 items/individual) (Compa et al., 2018) and Gulf of Lions (0.11
290 to 0.85 MPs/individual) (Collard et al., 2015; Lefebvre et al., 2019) but lower than those
291 collected in the Adriatic Sea (6.62 ± 3.67 items/individual) (Renzi et al., 2019a). Anchovies
292 caught in the Western Mediterranean Sea had lower ingested MPs than those caught in the
293 Eastern and Central Mediterranean confirming the higher pollution of these regions (Table 2).
294 For instance, Japanese anchovies, *Engraulis japonicus* from the heavy polluted area of Tokyo
295 bay displayed the same MPs ingestion (2.30 ± 2.50 MP/ind Tanaka and Takada, 2016) as in our
296 study.

297 Three shapes of plastics were found: fragments, films and fibers (Figure 2 – C; Figure 3). There
298 was a slight dominance of fragments (1.3 ± 0.36 fragments/fish) over of fibers (1.1 ± 0.17
299 fragment/fish), whereas films had the lowest occurrence (0.1 ± 0.1 films/fish). The items had
300 different colors: black, blue, red, white, transparent, and green. For the three sites, blue
301 fragments were the most abundant representing more than 50%; whereas for the fibers, black
302 was the dominant color. This predominance of blue plastic items has been previously reported
303 in fish and bivalves (Romeo et al., 2015; Güven et al., 2017; Compa et al., 2018; Digka et al.,
304 2018).

305 Along the Lebanese coast, anchovies from Beirut had ingested the highest number of MPs items
306 (2.9 ± 1.9 MPs/individual) and those from Tripoli the lowest number (2.3 ± 1.6 items/individual)
307 (Supplementary Table 3). However, there were no significant differences between the numbers

308 of ingested items at the three analyzed sites ($P > 0.05$). No relationship was highlighted between
309 the concentration of MPs in anchovy digestive tracts and in the surface water as it has been
310 described in the western Mediterranean (Compa et al., 2018; Lefebvre et al., 2019). As a pelagic
311 species, anchovy are highly moving fish so they potentially did not ingest MPs at the site they
312 were collected, making correlations harder to underline. Gastro-Somatic Index (GaSI) and Fulton
313 condition index were higher in Beirut yet not significantly different (Supplementary Table
314 3). There was no correlation between GaSI and the number of ingested items ($r = 0.067$; $P >$
315 0.05). Fulton condition index and the number of ingested items showed a weak but not
316 significant correlation ($r = 0.316$; $P = 0.089 > 0.05$). In the western Mediterranean Sea, recent
317 studies also revealed that anchovies' MPs ingestion was not related to the body condition
318 (Compa et al. 2018; Lefebvre et al., 2019).

319
320 Spiny oyster is a Lessepsian species of high commercial value in Lebanon. The collected
321 individuals had an average of 7.2 ± 1.4 items/individual and 0.45 ± 0.3 items/g ww. To date, this
322 is the first study done on MPs ingestion in *S. spinosus* in the Mediterranean and worldwide.
323 Therefore, our results can only be compared to other oysters or bivalves species. Oysters from
324 the present study had the same average of items found in *Crassostrea gigas* collected from the
325 Atlantic Ocean with an average of 0.47 ± 0.16 particles/g ww (Van Cauwenberghe and Janssen,
326 2014), yet lower than that found in wild mussels (*Mytilus galloprovincialis*) collected from the
327 central Tyrrhenian Sea (Mediterranean) with a an average of 7.2 items/g ww (Renzi et al.,
328 2018a) and from the Adriatic Sea with an average of 1.06–1.3 items/g ww (Gomiero et al.,
329 2019). Our samples were less contaminated that those collected from the Mediterranean which
330 is probably due to the difference in the analyzed species and not the geographical MPs
331 concentration in the environment. Oysters tend to have more flesh than mussels explaining the
332 difference in MPs ingestion per gram of ww.

333 Oysters from all sites had the presence of fragments, films and fibers with 86.3% of analyzed
334 individuals having suspected MPs in their soft tissue (Figure 2- C). Due to a difference in
335 individual total body soft tissue weight, the number of items was also reported to the number
336 of items per gram. Oysters from Beirut had the highest items concentration with 8.3 ± 4.4

337 items/individual (or 0.78 ± 0.62 items/g) out of which fibers constituted more than 50%. Sidon
338 had the highest number of fragments/individual reaching 4.3 ± 4.4 fragments/individual. Tripoli
339 had the lowest number of items/g with 0.22 ± 0.13 items/g. Beirut was significantly different
340 when comparing to the other sites ($P < 0.05$). The observed items had six different colors
341 similar to those found in the anchovies; with blue color being dominant in the oysters' samples.

342 **3.3. Size class distribution and polymers characterization**

343 In the water samples, the size class $< 200 \mu\text{m}$ dominated in Tripoli (48.78%) and Sidon (51.8%)
344 whereas for Beirut, the size class $> 1000 \mu\text{m}$ dominated (31.7%) (Figure 3 – A). Suspected items
345 $< 1000 \mu\text{m}$ represented a percentage of 88%, 69.3% and 87% at Tripoli, Beirut and Sidon,
346 respectively. All sediment samples had a high abundance of the size class $> 1000 \mu\text{m}$
347 representing more than 70% of the samples (Supplementary Figure 1). For the biota, the
348 smallest size class $< 200 \mu\text{m}$ was the most abundant in all sites (Figure3 - B). For anchovies, the
349 ingested size class 200-400 μm was the second most dominant in Tripoli and Beirut with a
350 percentage of 34%. The size class 600-800 μm was only present in Sidon's anchovies, whereas
351 the size class $> 1000 \mu\text{m}$ was absent in Tripoli. These results agree with the feeding ecology of
352 the European anchovy which is a filter-feeder that feeds on planktonic organisms generally < 2
353 mm (Bacha and Amara, 2009). No relationship between the size of the anchovies and the size of
354 the ingested items was observed. Although Beirut's anchovies were significantly ($P < 0.05$) bigger
355 than those from the other two sites, they ingested items of a similar size than those ingested in
356 Tripoli and Sidon. This indicates that the size of ingested items may be related to the available
357 particles during feeding rather than the morphometric characteristics of organisms (i.e. mouth
358 opening size). Plastics of all size classes were present inside the oyster's body soft tissue. The
359 smallest size class (0-200 μm) was the most abundant for all sites. The two size classes: 200-400
360 μm and $> 1000 \mu\text{m}$ were the second most abundant between the ingested MPs. Even though
361 there was a significant difference in spiny oyster's net weight ($P < 0.05$) between sites, all MPs
362 items size classes were present in all collected individuals. This confirms that the size of ingested
363 items is not related to the individual weight, but to the available particles existing in the
364 surrounding water.

365

366 Among the suspected MPs analyzed, 94% were identified as microplastics. Nine types of
367 polymers were identified in our samples (Figure 4). Six types were common to all three matrices
368 (water, sediments and biota): polypropylene (PP), polyethylene (PE), polystyrene (PS),
369 polyamide (PA), polyethylene terephthalate (PET) and polyurethane (PUR). In addition to these
370 polymers, polyvinyl chloride (PVC) was present in water and sediments; polylactic acid (PLA) was
371 only present in the water whereas acrylonitrile butadiene styrene (ABS) was only present in
372 biota. Each of the three matrices had a specific polymer that dominated over the others: PE was
373 the most abundant in water samples (70.83%, 50% and 47.37% at Tripoli, Beirut and Sidon,
374 respectively). Polyethylene is the most common plastic on a global scale and consequently the
375 most dominant plastic debris in the Mediterranean Sea and worldwide, deriving mainly from
376 plastic bags and bottles (Suaria et al., 2016). It is well documented that plastics as PE float in the
377 water surface, as its density is lower than this of the water. Polymers with a density higher than
378 that of water (PVC, for example) were observed in the surface water. The existence of polymers
379 denser than seawater have been already reported (Suaria et al., 2016; Enders et al., 2015) with
380 no clear mechanism leading to their existence. During sampling, there was strong waves and the
381 water was agitated which might explain their presence due to the high velocity and turbulent
382 mixing which increases the chances of denser polymers to persist on the sea surface (Moritomi
383 et al., 1982; Suaria et al., 2016). Polypropylene was the most abundant in sediments (48.11%,
384 55.88% and 43.33% at Tripoli, Beirut and Sidon, respectively), and polystyrene dominated in the
385 biota specimens (having a percentage equal to or above 25% for anchovies and spiny oysters).
386 Polypropylene was observed in all anchovies and spiny oysters of all sites except in the
387 anchovies of Beirut where PE was observed instead. Polymers distribution didn't have a pattern
388 with shapes or colors. For example, pellets found were mostly white (several were colored) yet
389 their polymer type was of PE, PA, and PP. The same for fragments, different colors were related
390 to different polymers with no similar pattern.

391 Comparison of the polymers composition identified in water, sediments and oysters indicated a
392 dissimilarity between groups (ANOSIM, $R=0.796$, significance level = 0.2%). Cluster dendrogram
393 led to the existence of two distinct groups (a and b) regrouping on the one hand the water and
394 sediment samples and on the other hand the biota (anchovies and oysters) (Supplementary

395 Figure 2). Non-metric multidimensional scaling (n-MDS) showed the dimensional distance
396 separating these groups (a and b) based on their polymer composition. Water and sediments
397 formed 'group a' and were characterized by the high presence of PP and PE. Whereas 'group b'
398 composed by biota samples, and were characterized by the high abundance of PS. Similarity of
399 Percentages (SIMPER) indicated that PE, PP, PA and PUR contributed to 72.12% of dissimilarity
400 between these two groups.

401
402 **3.4. Microplastics potential entry into the Mediterranean Basin via coastal landfills, and their**
403 **fate and distribution in the coastal environment**

404
405 This study showed a high microplastics pollution in areas located in the vicinity of coastal
406 landfills. Even though some of them are no longer active, but their waste continues to find its
407 way into the sea due to mismanagement and lack of proper treatments. Some of the polymers
408 found in our samples such as polyethylene, polypropylene and polystyrene were observed in
409 transparent, white and different colors. They are widely used in packaging, consumer products,
410 pipes and fitting, and are the common polymers found in landfills and their leachates (Kazour et
411 al., 2019; He et al., 2019; Praagh et al., 2018). The high abundance of these types of polymers in
412 sea surface water and sediment samples can be traced back to mismanaged landfills along with
413 accidental garbage disposal. Even though Lebanon is considered as a potential sink of plastics in
414 the Eastern Mediterranean (Duncan et al., 2018), it should also be considered as an important
415 microplastics source in the Mediterranean basin. In most studies, whether in the surface water,
416 sediments or biota, fibers were generally the dominant microplastic shape found (Lefebvre et
417 al., 2019; Renzi et al., 2019b; Compa et al., 2018). In the present study, fragments constituted
418 more than 77%, 90% and 50% of items found in surface water, sediments and biota samples,
419 respectively. The same pattern was found in a study done near an abandoned coastal landfill,
420 where fragments constituted the majority of found items (Kazour et al., 2019). Such dominance
421 of fragments in the analyzed samples highlights on the potential role of Lebanese coastal
422 landfills in fragments input into the Mediterranean Basin. Other factors can also lead to this
423 severe microplastics contamination and, most precisely, on fragments abundance on the

424 Lebanese beaches. Munari et al. (2017) indicated a high number of fragments (approximately
425 90% of the samples) was observed near beaches affected by riverine run-off. All of the sampled
426 Lebanese beaches are affected by riverine runoff as well as a significant anthropogenic pressure
427 leading to a major microplastics pollution along the beaches (100 times higher than other
428 reported studies, see Supplementary Table 3). These microplastics are prone to re-enter the
429 surface water with waves hitting the shoreline (directed by wind speed and direction) increasing
430 their chance to end up in nearby regions via the northward general water circulation pattern.
431 The above stated factors increase microplastics availability to marine organisms leading to
432 potential human health risks. In Lebanon, an average of 19.7g of fish and seafood per person is
433 consumed (Nasreddine et al., 2006) making it around 7 kg per caput per annum. Converting the
434 average of ingested MPs/g in oysters to the number of seafood ingested by a Lebanese person,
435 we would have approximately 31, 500 MPs ingested per person (4500 MPs/kg of oysters). This
436 is similar to that obtained by Catarino et al. (2018) (13,731 and 68,415 particles/y/person) yet
437 lower than that obtained by Van Cauwenberghe & Janssen (2014) (11, 000 MPs/y/person).
438 European anchovies are eaten wholly by the Lebanese population as *Bizree* (juvenile pelagic fish
439 caught by purse seiners) as well as spiny oysters that are served in various Lebanese restaurants
440 raising the concern on the human consumption of these contaminated seafood.

441

442 **4. Conclusion**

443 These results should be considered as a preliminary analysis on MPs levels recorded off the
444 Lebanese coast in three different matrices. This is the first contribution to characterize the area
445 and to understand the degree of its MPs pollution. More studies are necessary to evaluate the
446 ecological impacts of these MPs on the marine biodiversity in the Levantine Basin. The levels of
447 MPs found were alarmingly high in comparison to other Mediterranean regions. The high
448 number of ingested items by both anchovies and oysters could suggest a potential health
449 concern since these two species are wholly consumed (without being degutted). This study
450 highlighted the role of landfills in MPs entry into the coastal marine environment. Coastal
451 landfills and mismanaged wastes are important issues existing in 80% of the Mediterranean

452 Basin explaining the elevated number of MPs found in this semi-enclosed basin. The whole
453 Mediterranean should be regarded as an important case study for microplastics pollution where
454 more initiative should be taken to insure an appropriate waste management and, hence,
455 decreasing plastics pollution.

456

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463

464

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466

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Figure 1: Localization of the sampling sites (Tripoli, Beirut and Sidon) along the Lebanese coast.

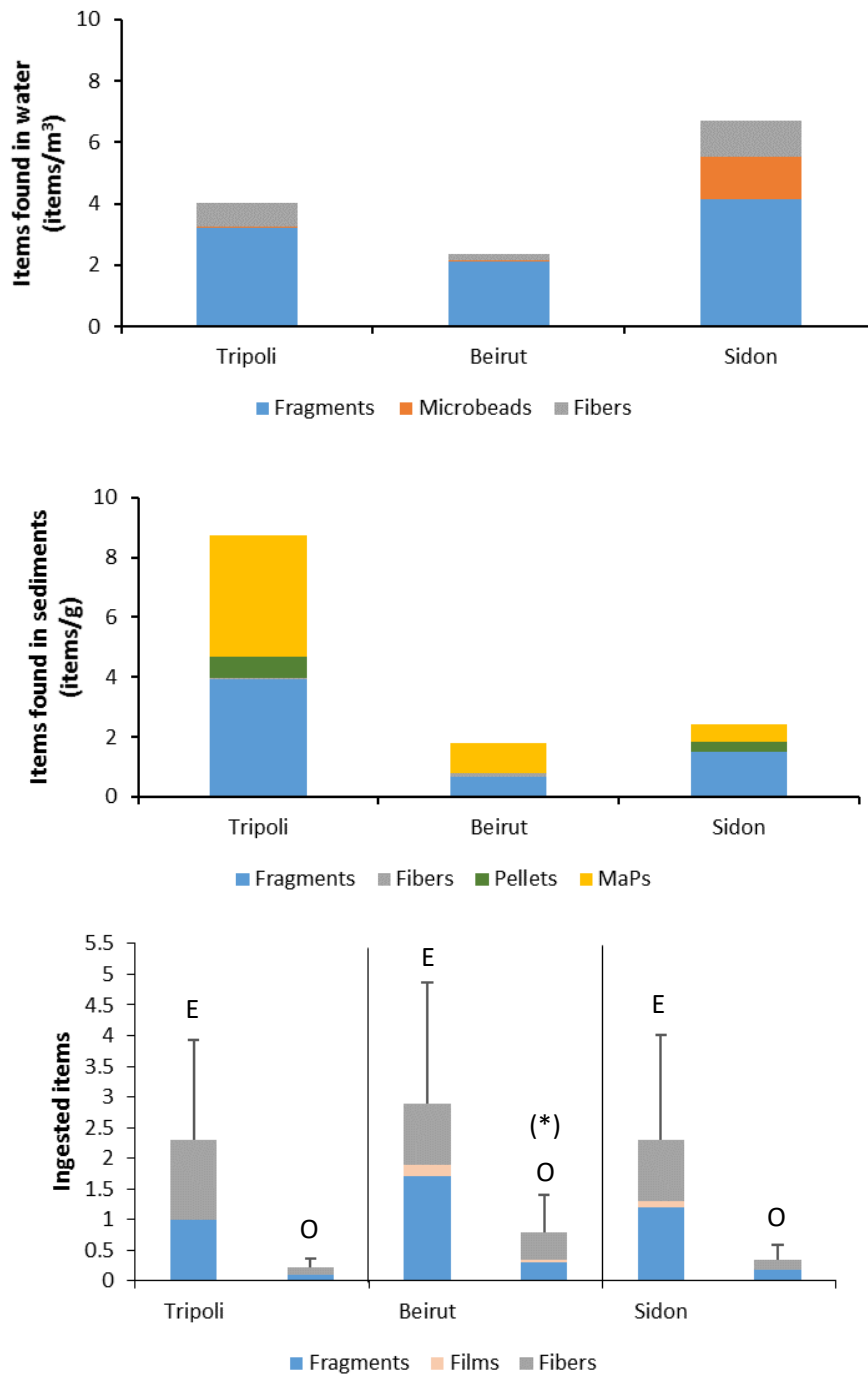


Figure 2: A) Concentration of suspected microplastics (MPs/m³) according to their shapes found in the surface water; B) Suspected microplastics (MPs) and macroplastics (MaPs) concentration (items/g) found in one gram of sublittoral sediments; C) Average number (\pm SD) of ingested suspected microplastics by shape categories in the digestive tract of (E) European anchovies (MP/ind) and in the soft tissues of (O) spiny oysters (MP/gram) at the three sampling sites (* indicate statistically significant difference).

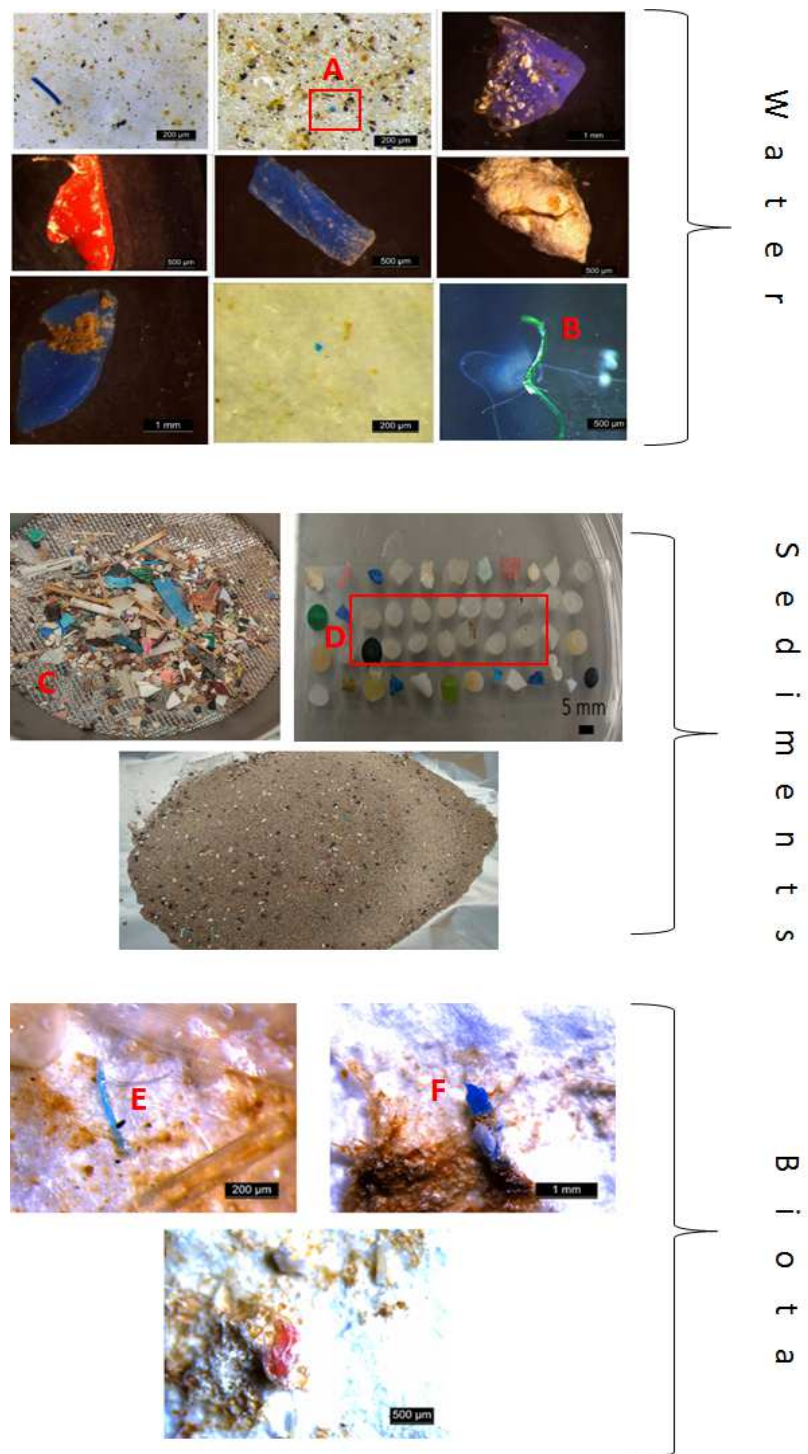
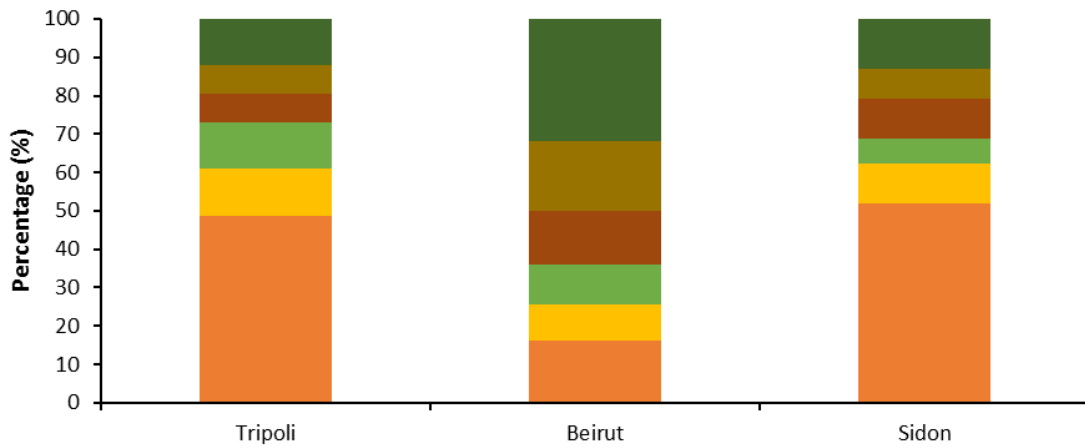
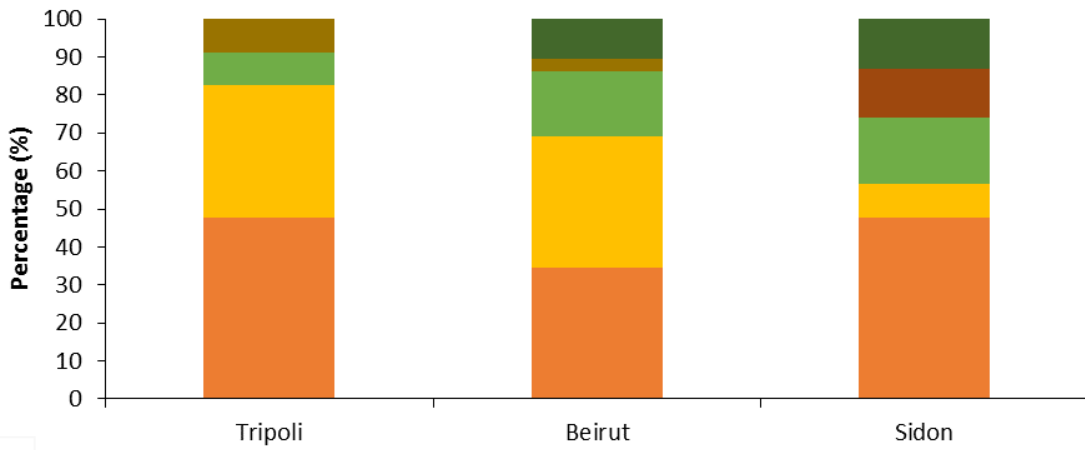


Figure 3: Microplastics of different shapes and colors collected along the Lebanese coast in three different matrices: e.g. A) blue microbead and B) green fiber found in the collected surface water, C) Macro and microplastics collected on the 2 mm sieve and D) white and black pellets collected in Tripoli sediments sample, E) blue fiber and F) blue fragment observed on biota filter.

A)



B)



C)

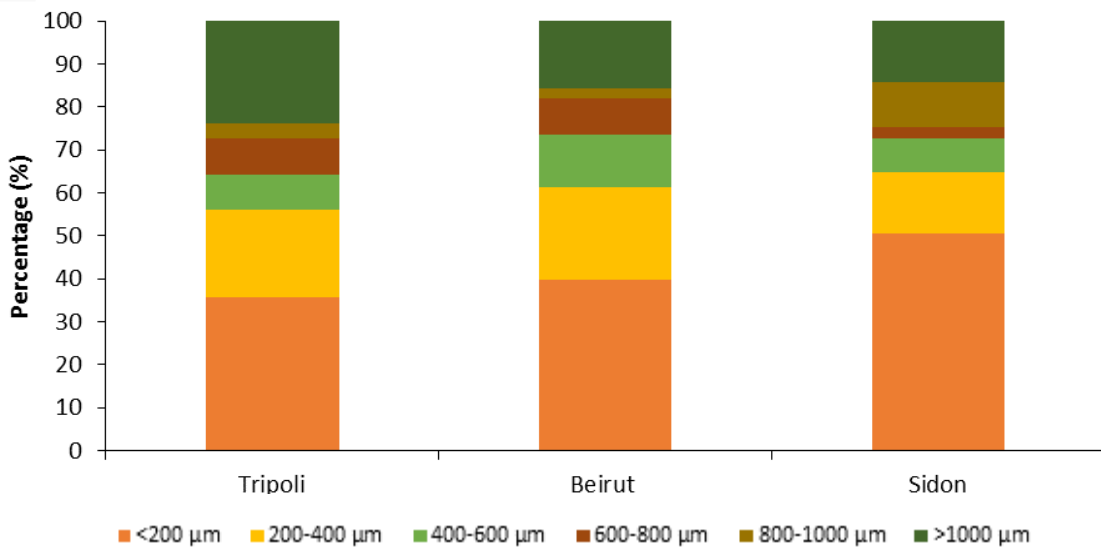


Figure 4: Proportion of size class composition of suspected microplastics in the A) Water surface, B) anchovies and C) spiny oysters from the different sampling sites

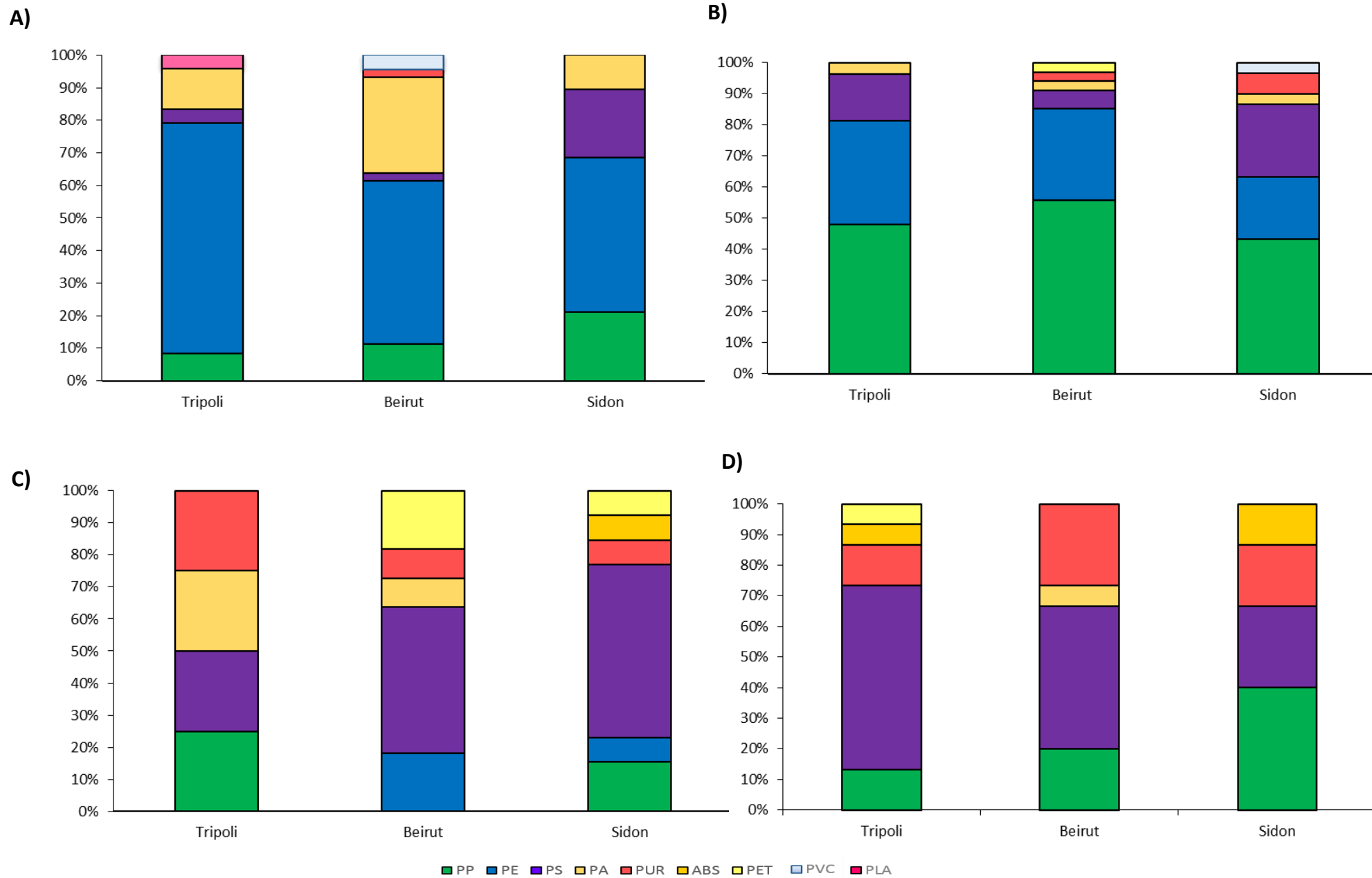


Figure 5: Proportion of different polymers identified by micro-Raman spectroscopy in the three analyzed compartments: A) water; B) Sediments, C) anchovies and (D) spiny oysters.

Table 1: Literature data on microplastics abundance in Mediterranean surface waters and instrumental analysis methods used for detection of MPs. To compare with our results, only studies expressing concentrations in m³ are presented.

Location	Sampling nets	Mesh size (μm)	Identification method	MPs abundances	Reference
NW Mediterranean (the Rhône River and the Têt River)	Manta trawl net	333	Stereomicroscope/FTIR	0.18- 0.19 items/m ³	Constant et al., (2018)
NW Mediterranean Sea (Ligurian Sea and Sardinian Sea)	WP2 standard net	200	Stereomicroscope	0.62 \pm 2 items/m ³	Fossi et al., (2012)
NW Mediterranean Sea (Gulf of Lion)	WP2 plankton net	200	Stereomicroscope/FTIR	0.23 \pm 0.20 items/m ³	Lefebvre et al., (2019)
NW Mediterranean Sea (Gulf of Asinara and the Sardinian Sea)	Neuston net	200	Stereomicroscope	0.31 \pm 1.17 items/m ³	Fossi et al., (2016)
NW Mediterranean Sea (North)	WP2 standard ring net	200	Stereomicroscope	0.17 \pm 0.32	Panti et al., (2015)

Western of Sardinia; Gulf of Asinara)				items/m ³	
NW Mediterranean Sea (Tuscany coastal water)	Manta trawl net	330	Stereomicroscope/FTIR	0.26/m ³	Baini et al., (2018)
Central-Western Mediterranean Sea (Sardinian Sea)	Manta trawl net	500	Binocular stereomicroscope	0.15 items/m ³	De Lucia et al., (2014)
Eastern Mediterranean (Turkish Mediterranean Coast)	Manta trawl net	333	Microscopic observation/FTIR on 24 items	0.7 items/m ³	Güven et al., (2017)
Eastern Mediterranean (Iskenderun Bay)	Manta trawl net	333	Microscopic observation	7.26 items/m ³	Gündoğdu, (2017)
Eastern Mediterranean Sea (Iskenderun Bay and Mersin Bay)	Manta Trawl net	333	Microscopic observation	2.73 items /m ³	Gündoğdu and Çevik, (2017)

South Eastern Mediterranean Sea (Israeli surface waters)	Manta Trawl net	333	Stereomicroscope	7.68 ± 2.38 items/m ³	van der Hal et al., (2017)
Lebanese coast	Manta Trawl net	52	Stereomicroscope/Raman	4.3 ± 2.2 items/m ³	Present study

Table 2: Average (\pm SD) of ingested items found in the digestive track of European anchovies (*Engraulis encrasicolus*) collected in different areas of the Mediterranean Sea.

Sites	Digestion method	Concentration (items/ind)	Occurrence	Polymers Identification method	Main polymer	Reference
Spanish Western Mediterranean coasts	None. Visual observation	0.07 (\pm 0.35) to 0.33 (\pm 0.26)	15%	FTIR	PET	Compa et al., (2018)
Western Mediterranean Sea (Iberian Peninsula coast and the Balearic Islands)	None Visual observation	0.07 \pm 0.26	2.56%	None	-	Rios-Fuster et al., (2019)
North Western of the Mediterranean Sea (Gulf of Lions)	None. Visual observation	0.11 \pm 0.31	11%	ATR-FTIR	PET	Lefebvre et al., (2019)
Northwestern Mediterranean Sea	NaClO	0.85	40%	Raman spectroscopy	PE, PP, and PET	Collard et al., (2015)
Northern Mediterranean (Adriatic Sea)	H ₂ O ₂	1.25	91%	μ -FT-IR	PVC	Renzi et al., (2019a)
Eastern Mediterranean Sea (Lebanese coast)	KOH 10%	2.5 \pm 0.3	83.4%	Raman Spectroscopy	PS	Present study

