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## Seasonal and spatial distribution of microplastics in sediments by FTIR imaging throughout a continuum lake - lagoon- beach from the Tunisian coast

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1        **Seasonal and spatial distribution of microplastics in sediments by FTIR**  
2        **imaging throughout a continuum lake - lagoon- beach from the Tunisian**  
3        **coast**

4  
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14        **ABSTRACT**

15        Plastics pollution in marine environment has become an issue of increasing scientific concern.  
16        This work aims to study the temporal and spatial distribution of plastics in sediments from  
17        three different Tunisian ecosystems; Rimel Beach, Bizerta lagoon and Ichkeul lake. Sediment  
18        sampling was conducted in surface (2 cm) and depth (15 cm) during spring, summer and  
19        winter. Plastics debris were separated by size fractions: macro (> 5mm), meso (1-5mm) and  
20        microplastics (<1mm) to optimize the time necessary for their characterization. Macroplastics  
21        and mesoplastics were identified using an IR Attenuated total reflectance (ATR) and  
22        microplastics with Imaging Fourier transform infrared spectroscopy (FTIR) spectroscopy after  
23        an optimized extraction protocol. Results indicate that, the lowest contamination degree with  
24        macroplastics was recorded in Ichkeul lake, 2 macro debris/m<sup>2</sup> (marine protected area,  
25        national parc of Ichkeul). Mesoparticles were only detected in lagoon of Bizerte in large  
26        quantities (4900 item/kg of sediment in surface and 680 item/kg of sediment in depth) and  
27        were identified principally as paint products. For microplastics, the repartition was quite  
28        homogeneous between the three sites with an average abundance was  $130.55 \pm 65.61$  items/kg  
29        for all seasons. The variations of microplastics abundances on the three sites could not be  
30        clearly related to the seasons. Whereas the polymer characterisation in the surface and depth  
31        sediments of the three studied areas were principally due to eight types of polymers (PVC,  
32        PET, PP, PE PS, Polyamide (PA) and polymeric methyl methacrylate (PMMA)) as reported  
33        in many other studies, surprisingly all MPs recovered in the study were smaller than 300  $\mu\text{m}$ ,

34 more than 70% being fragments. This study brings new results as regards to the state of  
35 plastic contamination in Tunisian coast and shows the importance of investigating different  
36 ecosystems in such studies.

37 Keywords: Plastic litter, macroplastics, mesoplastics, microplastics, paint products, FTIR  
38 imaging

## 39 **1. Introduction**

40 The extensive use of various plastic contaminants in the environment is a worldwide issue  
41 (Cunha et al., 2020; Kausar et al., 2020; Rashid et al., 2021; Sher et al., 2021). In recent  
42 decades, global annual plastic production has risen rapidly, with approximately 370 million  
43 tons produced in 2020 (PlasticsEurope, 2020), whereas close to 10 million tons of debris still  
44 end up in the ocean each year. Due to the physical and chemical properties of plastics, such as  
45 durability and resistance, plastic pollution becomes a serious problem and triggers adverse  
46 environmental effects (Prata et al., 2020). Plastic debris were found in different ecosystems  
47 such as oceans, lakes, rivers and beaches (Tunçer et al., 2018; Vaughan et al., 2017; Ding et  
48 al., 2019; Korez et al., 2019). Once in the environment, under the action of UV and water,  
49 they are degraded into microplastics (MPs), usually defined as particles of plastics with size  
50 under 5 mm (ref). MPs have recently increased global concern (Dikareva and Simon, 2019;  
51 Guo and Wang, 2019; F. Wang et al., 2018; Z. Wang et al., 2018; Korez et al., 2019); they  
52 studied the progressive accumulation in sediments worldwide as well as in the Mediterranean  
53 basin (Qiu et al., 2016; Vianello et al., 2013). Plastic pollution has become more prevalent in  
54 marine environments, with 40-80 percent of trash made up of debris (Barnes et al., 2009; Cole  
55 et al., 2011). Likewise, 90% of the waste collected on seabed trawls consists of plastic  
56 (Derraik, 2002; Galgani et al., 2015). The most common items, constituting over 80% of  
57 beached litter (Andrady, 2015) are cigarette butts, bags, leftover fishing gear, and food and  
58 drink containers. Most kinds of plastic (Andrady, 1994) are not biodegradable, it takes many  
59 decades, or even thousands of years to be degraded. Their degradation depends on physical  
60 factors, such as light, oxygen and temperature exposure (Swift and Wiles, 2004) or  
61 mechanical abrasion caused by wind and waves (Song et al., 2017). All these phenomena  
62 cause their fragmentation into smaller plastic items which are classed into different size  
63 categories with size limits varying among studies: macroplastics (> 25 mm); mesoplastics  
64 (from 25 mm to 5 or 1 mm); microplastics (< 5 or 1 mm) and nanoplastics (< 1µm or < 100  
65 nm) (Barnes et al., 2009; Ivleva et al., 2017). Microplastics (MP) are the most studied forms  
66 of plastic in the last years. MPs have been detected in different ecosystems around the world

67 such as sediments, beaches, lakes, rivers, and wastewater effluents (Chang, 2015). Their  
68 prevalence in the marine environment and sediments was demonstrated by Van  
69 Cauwenberghe et al., 2015. The presence of MPs in the marine environment can be related to  
70 multiple pathways. They are the result of macroplastics deterioration caused by a variety of  
71 mechanisms or directly from input of industries and domestic applications (Costa et al.,  
72 2010).

73 MPs are persistent in the environment and can present a risk for marine organisms (Wright et  
74 al., 2013). Indeed, these items can be ingested by a large range of marine biota from the  
75 trophic chain (Gall and Thompson, 2015; Gross, 2015) such as zooplankton, molluscs and  
76 fish, and lead to direct damages towards organisms, showing impacts on feeding activity,  
77 reserve depletion and inflammatory responses, with consequences for fitness, notably  
78 reproduction (Desforges et al., 2015; Dowarah et al., 2020; Paul-Pont et al., 2018; Silva-  
79 Cavalcanti et al., 2018; Zakeri et al., 2020).

80 Finally, due to their hydrophobicity, MPs can adsorb different organic pollutants such as  
81 dichlorodiphenyltrichloroethane, polybrominated diphenyl ethers and their metabolites (Frias  
82 et al., 2010; Teuten et al., 2007) and can then be passed into the organisms' tissues when  
83 ingested (Engler, 2012). This allows MPs to act as a transfer vector of toxic chemicals in the  
84 environment and its ends up consummated by humans via the food chain.

85 The Mediterranean Sea appears to be a hotspot in the world as the MP concentration in this  
86 region is approximately four times greater than the North Pacific Ocean (Sharma et al., 2021).  
87 Recently, many studies were conducted on MPs pollution in sediment of tunisian aquatic  
88 ecosystems such as Bizerte lagoon, Hergla, Chott-Mariem, the coastal area of the city of  
89 Monastir, the Gulf of Gabes and Sidi Mansour Harbor (Abidli et al., 2017, 2018; Boughattas  
90 et al., 2021; Chouchene et al., 2019a; Missawi et al., 2020; Zayen et al., 2020). MPs were  
91 found in several marine organisms (e.g.; mollusks, fish) from Tunisian coasts (Abidli et al.,  
92 2021, 2019; Boughattas et al., 2021; Zitouni et al., 2020). However, the data of contamination  
93 are difficult to compare as they were obtained using different sampling, extraction and  
94 characterization MPs protocols.

95 In this work, we provide a dataset of plastic contamination in sediments of three different  
96 types of ecosystems: the complex Ichkeul lake - Bizerta lagoon - Rimel beach in the north of  
97 Tunisia. The lake and the marshes of Ichkeul have long been recognized (with Doñana in  
98 Spain, the Camargue in France and the region of El Kala in Algeria) as one of the four main  
99 areas wetlands of the western Mediterranean basin. It is one of the few sites in the world

100 registered in three international conventions: (Biosphere Reserve in 1977, World Heritage  
101 Site in 1979 and Zone wetland of international importance in 1980 (Ramsar Convention).  
102 Ichkeul lake is connected to Bizerta lagoon via the Tinja canal about 5 km long and a few  
103 meters deep (3 meters during flooding). The Bizerta lagoon is bounded by the cities of  
104 Bizerte, Zarzouna, Menzel Jemil and Menzel Abderrahmen on the North side, Menzel  
105 Bourguiba and Tinja on the West and South side. It communicates with the mediterranean sea  
106 by a channel 7 km long and 12 m high. Sample collection was conducted during three  
107 different periods (spring, summer and winter) allowing to study also the seasonal variations in  
108 MPs concentrations. Collected plastics were characterized in terms of size distinguishing  
109 macro; meso and microplastics to optimize the times required for their identification.  
110 Chemical identification of particles was then determined though infrared ATR for macro and  
111 meso particles (> 1 mm) whereas Fourier transform micro-infrared imaging was used for MP  
112 (defined in this study as particles < 1 mm) allowing a fast and reliable identification for all  
113 particles down to approximately 80  $\mu\text{m}$ .

## 114 **2. Materiel and methods**

### 115 **2.1.Study area**

- 116 • Station 1: Rimel beach (37° 15' 20" N, 9° 55' 00" E)

117 Rimel beach is one of the most known bathing beaches in Bizerte. It's located in the urban  
118 area of Bizerte (close to 570,000 inhabitants). This beach is easily accessible as it is located  
119 very close to a highroad, and it is very frequented during summer season.

120 Station 2 : Bizerte lagoon, Menzel Abderrahmane (37° 13' 56" N, 9° 51' 39" E)

121 Bizerta lagoon is one of the largest Mediterranean lagoons covered 150  $\text{Km}^2$ , it's located on  
122 the coasts of Northern Tunisia, near of some industrial units and agricultural area. It has a  
123 huge economic and ecological importance. Moreover, it has been exploited in conchyliculture  
124 and fishing since 1964. This costal lagoon is permanently connected to the Mediterranean Sea  
125 by a straight channel of 8.5 km long and communicates with the Ichkeul lake (110  $\text{km}^2$ ) by  
126 the Tinja river (Abidli et al., 2017). It is classified number two in Tunisia in terms of  
127 production of exported seafood. However, this lagoon is submitted to many anthropogenic  
128 pressures, doting of urban, industrial (such as petrochemicals, textiles, steel, plastics  
129 processing) and agricultural activities (Abidli et al., 2017). Moreover, the direct and indirect  
130 discharges of industrial wastes make this productive ecosystem suffering, it's transformed to a

131 receptacle of several and toxic chemicals products such as pesticides, heavy metals,  
132 pharmaceutical molecules and a lot of macro plastic (Abidli et al., 2017).

133 • Station 3: lake Ichkeul (37° 10' 32" N, 9° 44' 11" E)

134 Covering 90 km<sup>2</sup>, Ichkeul Lake is a Biosphere reserve, a Ramsar site, and a UNESCO World  
135 Heritage Site (Kose, 2012; Kraïem and Ben Hamza, 2000; Stevenson and Battarbee, 1991).  
136 This lake is a permeant wetland of 9000 ha surrounded by 3000 ha of temporary marshes,  
137 situated in the northern coast of Tunisia. It is linked by a small channel (Tinja) to Bizerta  
138 lagoon (37° 18' N 09° 35' E) which in turn has an outlet to the Mediterranean Sea (Ben Salem  
139 et al., 2019). The Ichkeul Lake watershed includes five sub-basins Joumine, Ghezala, Melah,  
140 Sejnane and Douimis. This region has a typically Mediterranean subhumid climate which  
141 leads to a temporary drying out of the marshes during the summer (Casagrande et al., 2005).  
142 The average depth is 1m with a maximum of 2m registered in winter period (Casagrande et  
143 al., 2005). It has a rural environment, draining an area of 418 km<sup>2</sup>, with elevation ranging  
144 between 35 and 716 m above sea level (Aouissi et al., 2013). Sandy-clay and silty-clay soils  
145 are the two dominant soils (Aouissi et al., 2014). The salinity displays considerable seasonal  
146 changes from 3 ‰ in the innermost parts in spring to 38 ‰ at the mouth of the Tinja channel  
147 in autumn (Aouissi et al., 2013).

148 The hydrology of the complex lagoon of Bizerte and Ichkeul lake presents a seasonal  
149 variability. During winter, Ichkeul Lake is fed by freshwater from the rivers increasing the  
150 water level. At that time, large volumes of water are spilled into Bizerte Lagoon through the  
151 Tinja River. During summer, water levels in Ichkeul Lake are low due to high evaporation  
152 rates, coupled with lower river inflows. As a result, saline water from Bizerte Lagoon enters  
153 Ichkeul Lake through the Tinja River. This leads to a saline water connection between Ichkeul  
154 Lake and the Mediterranean Sea that formerly has a seasonal salinity regime, freshwater  
155 increasing with winters' rainfall and decreasing in summer. Ichkeul Lake receives principally  
156 agricultural inputs as it is surrounded by a vast agricultural area (Stevenson and Battarbee,  
157 1991).



158

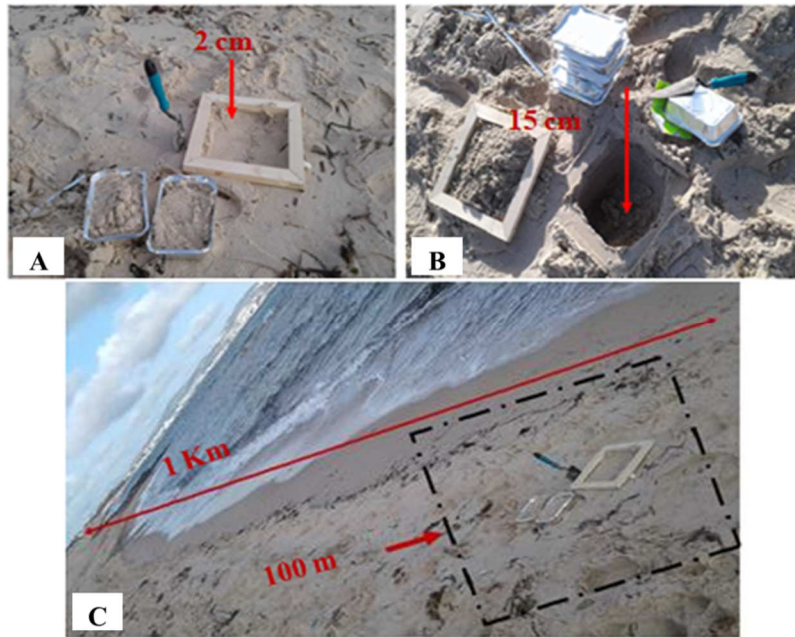
159 Figure 1: Locations of the three studied stations in the complex Ichkeul lake, lagoon of Bizerte  
160 (Northern Tunisia) and Rimel Beach (Mediterranean Sea).

161

### 162 1.1.Sampling

163 Samples collection was conducted in March 2019, July 2019, and January 2020. Ospar  
164 sampling strategy (OSPAR 2011) has been adapted and applied. In each site, 2 kg of  
165 sediments were randomly sampled to a depth of 2 cm and 15 cm using a stainless-steel shovel  
166 and a 25×25 cm wooden frame with a 2 cm height (Fig. 2), and then these sediments were  
167 transferred into aluminium tray. Different macroplastics were collected from the three studied  
168 areas.





169

170 Figure 2: Photos of sediment sampling in Surface (A) and depth (B) using Oscar protocol (C).

171 **1.2.Sample treatment**

172 Collected macroplastics were stocked at -4 °C for analysis. For analytical reason, as they can  
 173 be manipulated easily with tweezers for identification using IR-ATR, mesoplastics were  
 174 defined in this study as the particles with sizes > 1 mm. Their extraction was realized by  
 175 sieving the sediment on a 1 mm metallic sieve. Then MPs (< 1 mm) were isolated from  
 176 sediment samples according to Nuelle et al., (2014). Firstly, all sediment samples were dried  
 177 at 70 °C for 24 h to a constant weight. For microplastics extraction, a saturated salt solution  
 178 was added to 100 g of dried sediment samples from each site. All samples were stirred with a  
 179 clean glass rod for 2 min and settled for 24 h. Then, the density separation, 200 ml of H<sub>2</sub>O<sub>2</sub>  
 180 (30%) was added, to mix with final supernatants in the conical flask. The process of chemical  
 181 degradation continued for 24 h at 65 °C with an agitation at 100 rpm, in order to fully digest  
 182 organic matters in the extracts. Finally, for filtration, the solution was poured through a metal  
 183 grid with a porosity of 100 µm connected to a vacuum pump and then rinsed with Milli Q  
 184 water. To avoid artificial and suspended plastic contamination in the laboratory, all  
 185 instruments and containers were thoroughly rinsed with ultrapure water and 95% ethanol. Lab  
 186 gowns and gloves were worn throughout the sample collection and laboratory analysis  
 187 process. Meanwhile, blank experiments were conducted. Experiments showed that no piece of  
 188 microplastics was found in the blank controls.

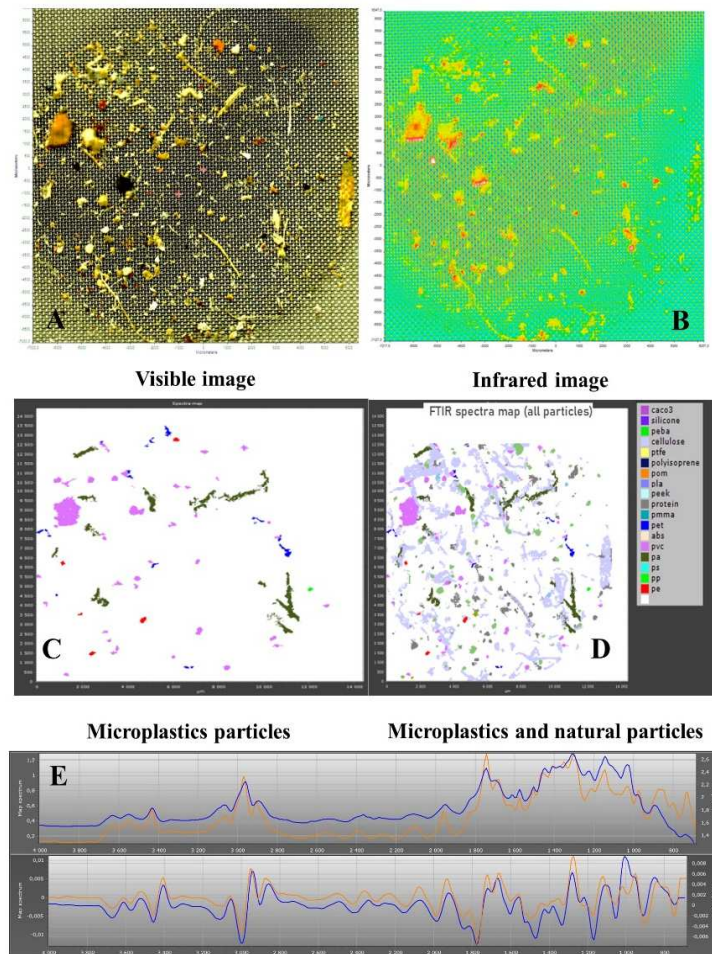
189 **1.3.Macro, meso and microparticles characterisation and quantification**



190 To define the subsample, debris of each sample were first examined under a stereomicroscope  
191 (Leica microsystems) and pooled if they were strongly similar in shape, colors, curves and  
192 borders. Macroplastics were identified using an Attenuated Total Reflection Fourier  
193 Transform Infrared spectrometer (ATR-FTIR Vertex70v, Bruker) equipped with a diamond  
194 crystal ATR accessory and a denudated triglycine sulfate (DTGS) detector. The system was  
195 operated by OPUS 7.5 (Bruker Optik GmbH) during measurements and analyses. Background  
196 and sample spectra were recorded in absorbance mode in the  $4000\text{-}600\text{ cm}^{-1}$  region with  $4\text{ cm}^{-1}$   
197 resolution and 20 scans. For identification, spectra were compared to Perkin Emer library.  
198 After each analysis by ATR-FTIR, the sample holder was cleaned with ethanol or acetone.  
199 As a lot of mesoparticles were found in the samples, a first step was to verify their polymeric  
200 nature. Thus, they were placed in different aqueous solutions (zinc chloride  $\text{ZnCl}_2$ , sodium  
201 chloride  $\text{NaCl}$  and  $\text{H}_2\text{O}_2$  30%) to check their density. The spectra of these mesoparticles were  
202 then acquired by (ATR-FTIR Vertex70v, Bruker) with the same operating conditions than for  
203 the macroplastics. They were also characterized by Scanning electron microscopy (SEM) with  
204 energy dispersive X-ray spectroscopy (EDX) to deeply investigate their chemical  
205 composition. The MEB-EDX is a non-destructive and rapid technique used to identify the  
206 chemical elements present in the selected areas. In this work, MEB-SEM analysis was  
207 performed using Jeol Model, USA.

208 MPs isolated on the metallic grids were then characterized by Fourier transform micro-  
209 infrared spectroscopy (micro-FT-IR, PerkinElmer Spotlight 400, USA). The system was  
210 operating by SPECTRUM IMAGE. Each filter was scanned 1 time for one hour in the wave  
211 number range of  $4000\text{-}750\text{ cm}^{-1}$ . The infrared image data were analysed with  
212 SIMPLEV1.0.0 (Fig. 3), and the target infrared spectrum was compared with the standard  
213 spectral library (PerkinElmer Library).

214



215

216 Figure 3: Microplastics identification. (A) : example of analyzed sample, (B) : infrared image , (C) :  
 217 only MPs detected in the filter, (D): all detected particles include MPs and (E): example of spectral  
 218 identification of a PET particle (orange) VS library spect.

219 **2.2.Statistical analysis**

220 All data were expressed as mean  $\pm$  standard error of the mean (SEM). One-way analysis of  
 221 variance (ANOVA) was used to assess significant differences among different groups.  
 222 Probability levels  $<0.05$  (Kruskal Wallis tests) were considered as statistically significant.  
 223 Data were analysed by Origin 2018 software.

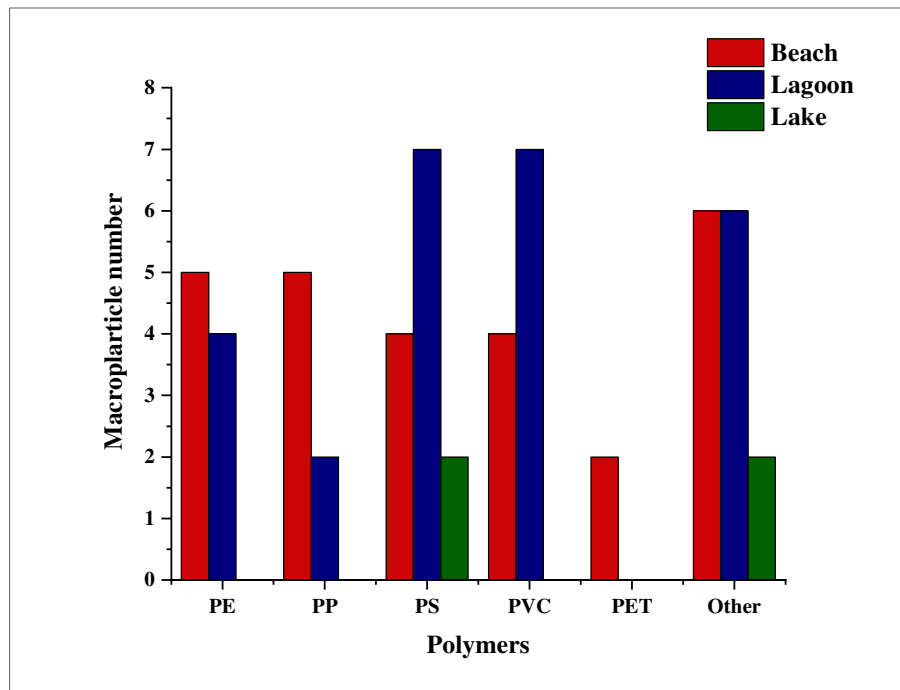
224 **3. Results**

225 **3.1. Characterisation of macroplastics**

226 In total, 56 (26; 26 and 4 sampled respectively at Rimel Beach, Bizerte Lagoon and Ichkeul  
 227 Lake) debris  $> 5$  mm were sampled from the three studied areas in March 2019 campaign.  
 228 Macroparticles collected from each site were identified by ATR (Fig. 4). Five main types of  
 229 polymers were detected: PS (14.28%), PVC (19.64%), PET (16.07%), PP (14.28%), PE

230 (3.57%) (Fig. 4) and 32.14% were identified as other kind of polymers and paint products.  
 231 Sampled plastics were also classified by colours. The predominant colours are as follow: blue  
 232 (22.9%) > white (20.8%) > transparent (20.8%) > pink (10.4%) > yellow (8.3%) > orange  
 233 (8.3%) > black (6.2%) > red (2%).

234  
 235



236  
 237

Figure 4: Abundance and characterisation of macroplastics.

### 238 3.2.Characterisation of (meso)plastics

239 Mesoparticles were defined here as particles ranged from 1 mm to 5 mm. Our study showed  
 240 that mesoparticles were only present in Bizerta lagoon station. In 50g of sediment, 245 items  
 241 were detected in surface (4900 item/kg of sediment) and 34 items in depth (680 item/kg of  
 242 sediment). To identify these mesoparticles, different flotation tests were realized (with sodium  
 243 chloride NaCl and zinc chloride ZnCl<sub>2</sub>). Surprisingly, contrarily to most commercial  
 244 polymers, these mesoparticles did not float and remained at the bottom of the tube after  
 245 flotation tests with sodium chloride and zinc chloride. The identification with ATR-FTIR  
 246 confirmed that 80.27% of the totality were paint products and only 1.97% were identified as  
 247 polymer particles (17,71% were not clearly identified). The identification results for surface  
 248 and depth sediments are detailed in [Tables 1 and 2](#). To get a better characterisation of these  
 249 painting products, electronic scan microscopy coupled with energy dispersive X-ray  
 250 Spectroscopy (SEM-EDX) was used. [Fig.5](#) shows a SEM picture of blue particle and a

251 superposed cartography, the brown and green colours indicating the presence of the oxygen  
252 and titanium. The spectrum sum is indicated in (Fig. 5), the principal compounds of paintings  
253 (oxygen and titanium) were detected in the atomic composition (33% and 6.43%  
254 respectively).

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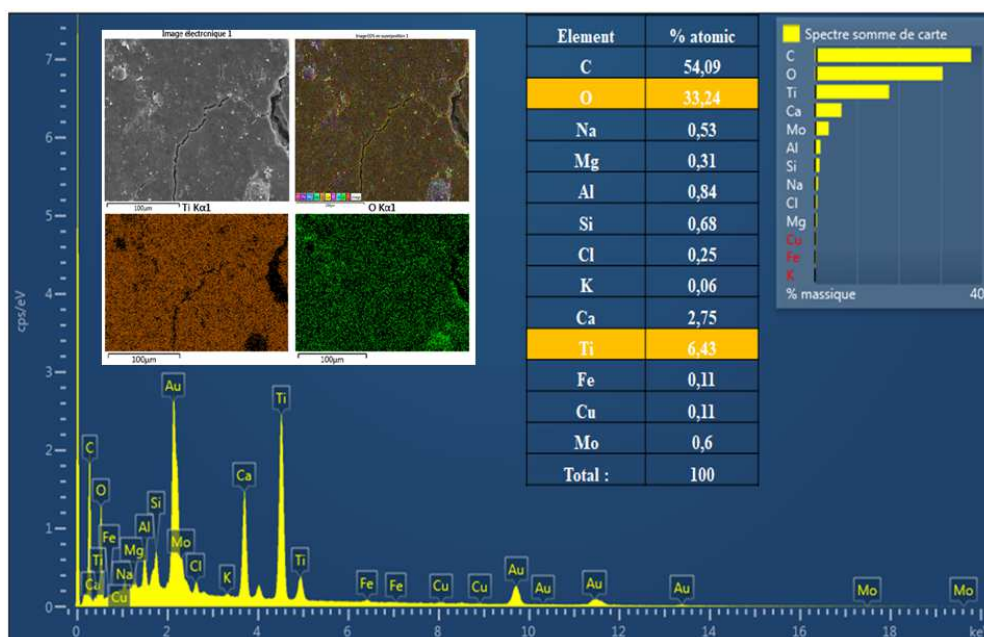
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Figure 5: MEB-EDX characterization of mesoparticles.

266

Table 1: ATR diamond identification (nature, colour) expressed as percentage of meso-particles collected in 50 g of surface sediments from Bizerta lagoon.

267

Particles Nb	Nature	Colour	Percentage (%)
Paint product			
172	Undercoat paint	Blue	70.20
10	Eggshell paint	Red	4.08
8	Uniplus 1-77	Pink	3.26
8	Eggshell paint	Green	3.26
3	Eggshell paint	Grey	1.22
1	Undercoat paint	Green	0.40
Polymers			
2	Rubber white	White	0.81
2	Bisphenol A polycarbonate 20% glass	Yellow	0.81
Not identified			
23	Cork	Orange	9.38
4	Dibutyl sobocole	Yellow	1.63
4	Not identified	green	1.63
3	Uniplus 1-77	Beige	1.22
3	Uniplus 1-77	white	1.22
2	Not identified	Black	0.81
<b>Total = 245</b>			

268

269 Table 2: ATR diamond identification (nature, colour) of meso-particles collected in 50 g from the  
 270 depth sediment of Bizerta lagoon.

Particles Nb	Natures	Colours	Percentage (%)
Paint product			
34	Undercoat paint	Blue	79.06
1	Artist water colour	Transparent	2.32
Polymers			
1	Rubber white	White	2.32
Not identified			
4	Not identified	Black	9.3
2	Uniplus 1-77	Pink	4.65
1	Cork	Orange	2.32
<b>Total = 43</b>			

271

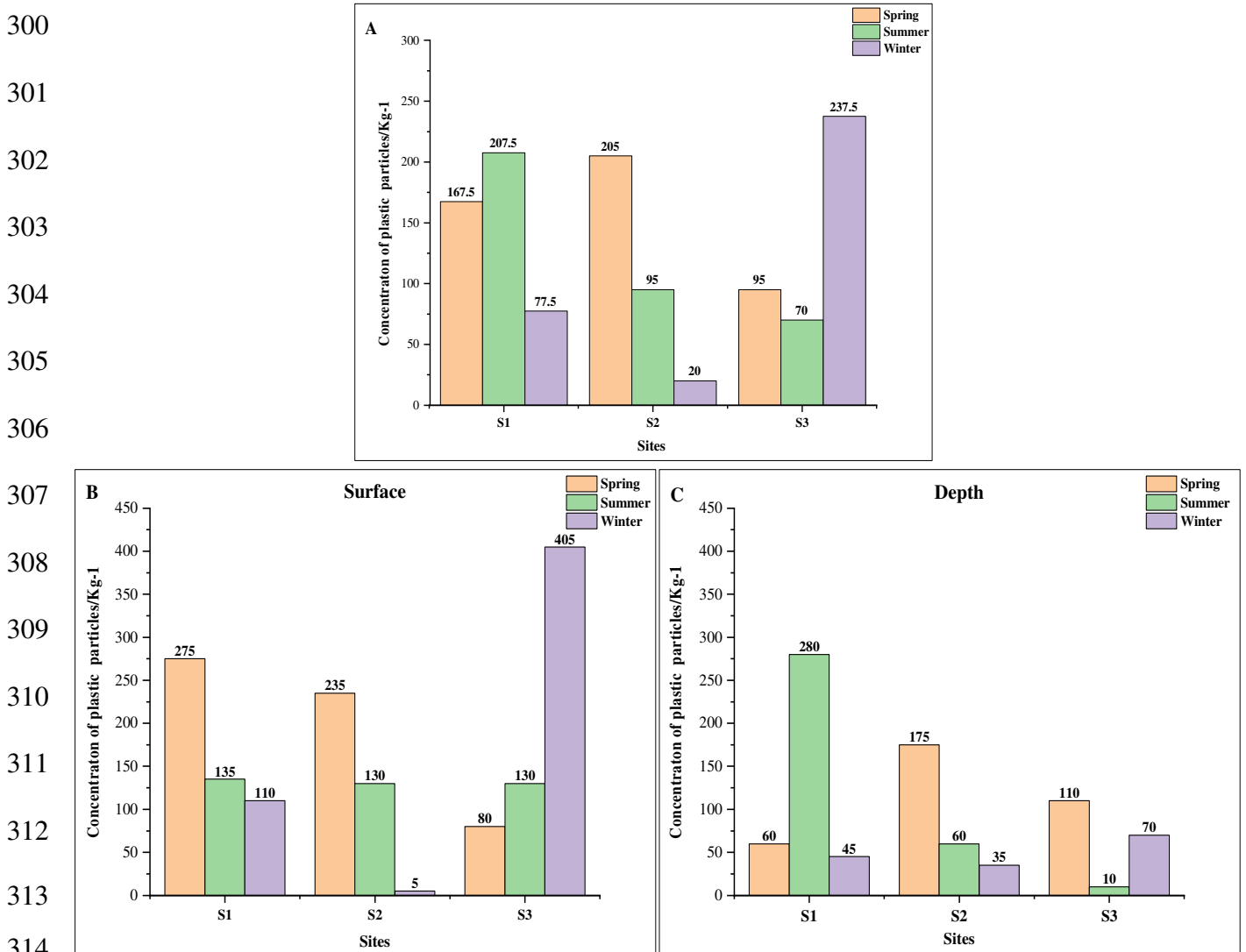
### 272 3.3. Characterisation of microparticles

#### 273 3.3.1. Effect of spatial and temporal variations in abundance of microplastics

274 Out of the 3229 suspected particles in all of the samples, a total of 467 particles were  
 275 identified as MPs in the sediments of all studied areas and sampling periods; the average  
 276 abundance between surface and depth samples was ( $130.55 \pm 65.61$  items/kg). Among the  
 277 particles that were not identified as plastics, most of them were identified as calcium  
 278 carbonate, cellulose, protein, silica fragments etc. The quantities of MPs found in sediment  
 279 samples from the three sampling sites in Tunisia, both in the dry and the rainy seasons, are  
 280 detailed in (Fig.6A). The concentrations for the three sampling areas Rimel beach S<sub>1</sub> ( $130.55$   
 281  $\pm 65.61$  items/kg), Bizerta lagoon S<sub>2</sub> ( $106.66 \pm 65.55$  items/kg) and Ichkeul lake S<sub>3</sub> ( $134.16 \pm$   
 282  $68.88$  items/kg) were not significantly different. The highest MP abundance was found at S<sub>3</sub>,  
 283 reaching 237.5 items/kg (Fig.6A) in winter and the minimum was 20 items/kg observed at S<sub>2</sub>  
 284 also in winter.

285 Spatially, overall, the average abundance of MPs in surface ( $167.22 \pm 85$  items/kg, Fig. 6B)  
 286 was 1,8 higher than in depth ( $93.88 \pm 62.96$  items/kg, Fig. 6C). MPs presence in surface  
 287 sediments of S<sub>1</sub>, showed a significative decrease ( $p < 0.05$ ) from spring to winter ( $275 \pm 35$  items/kg  
 288 in spring vs  $110 \pm 28$  items/kg in winter). The same trend was registered for S<sub>2</sub>. In contrast, at  
 289 S<sub>3</sub> location, MPs concentration increased from spring ( $130 \pm 14$  items/kg) and summer ( $80 \pm$   
 290  $0$  items/kg) to reach a maximum in winter ( $405 \pm 63$  items/kg).

291 Seasonally, our result showed that the average abundance of MPs on the three sampling sites  
 292 in spring ( $150.83 \pm 84.44$  items/kg) and winter ( $134.16 \pm 90.27$  items/kg) was higher than in  
 293 summer ( $106.66 \pm 73.33$  items/kg). A higher abundance of MPs in surface sediment during  
 294 spring was registered in both S1 and S2 respectively ( $275 \pm 35$  items/kg) and ( $235 \pm 21$   
 295 items/kg) compared with S3 ( $80 \pm 0$  items/kg) ( $p < 0.05$ ). During summer there was no  
 296 significant difference between the beach, lagoon, and lake ( $p > 0.05$ ). For the winter samples,  
 297 the statistical analysis showed that there was a significant difference in the abundance of MPs  
 298 between the different ecosystems, the highest level was found in the lake (S3) with ( $405 \pm$   
 299  $65$  items/kg) compared with the other studied sites (Fig.6A).



315 Figure 6: Number of microplastics collected from the sediment surface during sampling by total,  
 316 season and sites (A). Abundance of microplastic in surface and depth of sediment from the three  
 317 sampling sites (S1: Rimel beach, S2: Bizerte lagoon S3: Ichkeul lake).

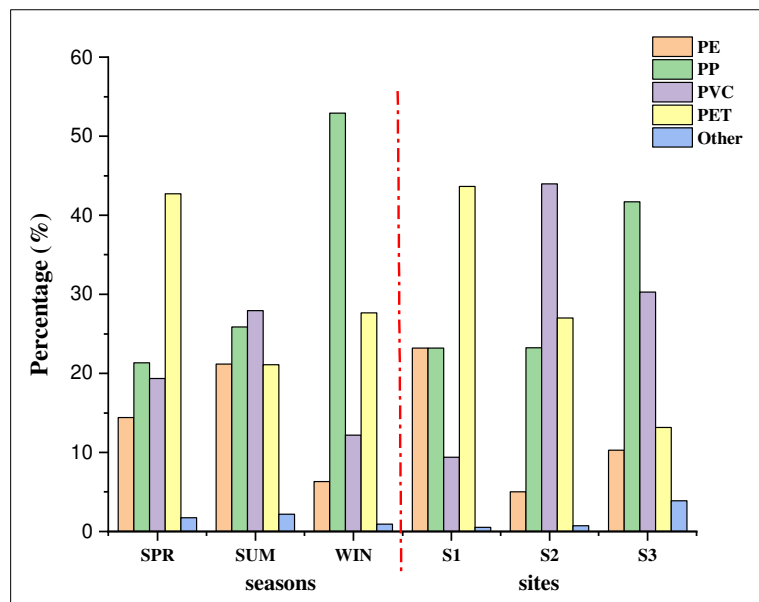
318



319 3.3.2. Characterisation of microplastics

320 3.3.2.1.Polymer type

321 More than 97.86% of the polymer types of MPs in sediments of the three studied areas were  
322 PP (31.38%), PET (29.22%), PVC (23.85%) and PE (13.40%). Only four other polymers  
323 were detected (PS, PA, ABS and PMMA) and represented only 2.14% of the total (Fig. 7).  
324



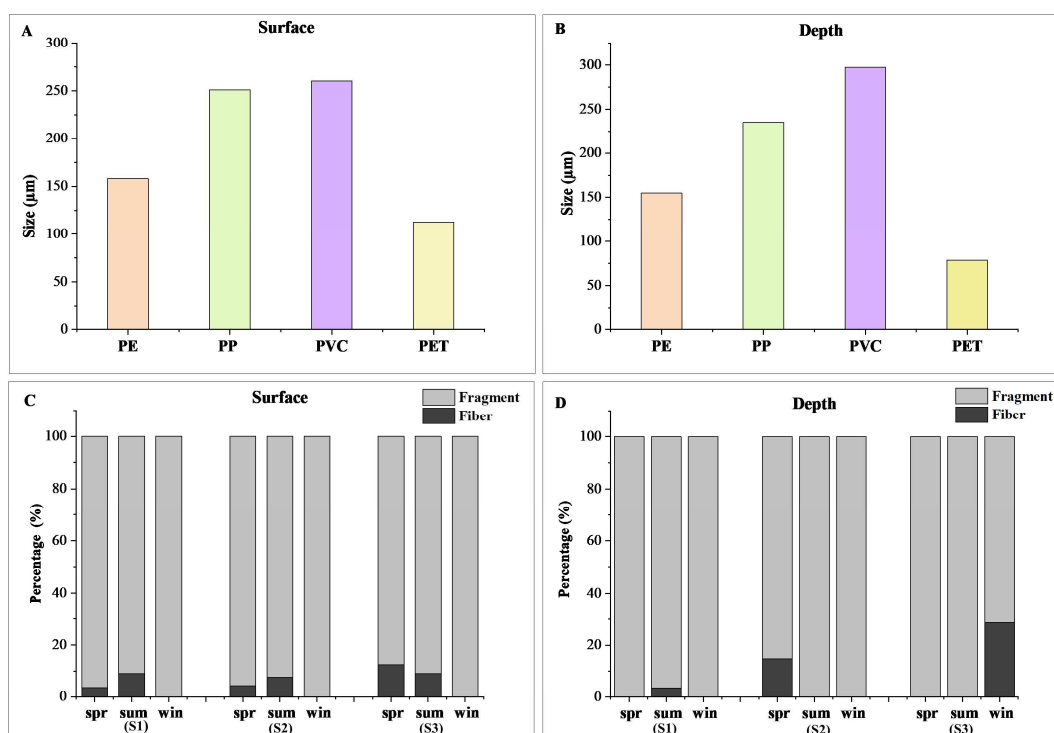
325  
326 Figure 7: Polymers distribution according to seasons and sites. Seasons; SPR: spring, SUM: summer  
327 and WIN: winter. Sites; S1: Rimel beach, S2: Bizerte lagoon and S3: Ichkeul lake.

328  
329 3.3.2.2.Size

330 The MPs sizes detected in the samples ranged from 78.35 to 297.25  $\mu\text{m}$  (Fig. 8A, B). For MPs  
331 in surface the sizes were between 112.79 to 260.22  $\mu\text{m}$ . In the depth, the registered sizes were  
332 from 78.33 to 297.25  $\mu\text{m}$ .

333 3.3.2.3.Shape and colors

334 In this work, the totality of detected MPs in the surface and depth sediments of the three  
335 studied surfaces were fragments (for example, 92.3% in the surface of Bizerta lagoon in  
336 summer and 71.42% (Fig. 8E and F) in the depth of Ichkeul lake in winter). Colors cannot be  
337 determined using the micro-FT-IR, PerkinElmer Spotlight 400.



338

339 Figure 8: Classification of MPs by size (A, B), shape (C, D). Seasons; SPR: spring, SUM: summer  
 340 and WIN: winter. Sites; S1: Rimel beach, S2: Bizerte lagoon and S3: Ichkeul lake.

341

## 342 4. Discussion

### 343 4.1. Macroplastics

344 Plastic contamination is considered as a very challenging issue for the environment. This  
 345 study aimed at assessing the distribution, types and abundance of macroplastic debris in  
 346 Rimel beach, lagoon of Bizerte and Ichkeul lake in north Africa (Tunisia). The macroplastic  
 347 abundances are the same in the beach and lagoon with 26 items 100 m<sup>-2</sup> (0.6 macro items m<sup>-2</sup>  
 348 ), and 13 times lower level of macroplastic was detected in the lake area with only 2 items 100  
 349 m<sup>-2</sup> (0.02 macro items m<sup>-2</sup>). Our results of macroplastic contamination in Bizerte lagoon near  
 350 of Menzel Abderahman harbour, are consistent with (Abidli et al., 2017; Zitouni et al., 2020).  
 351 These results they are close to those reported by (Jeyasanta et al., 2020) in India or in the  
 352 same range than those reported in previous investigations as 51 macro items per 100 m<sup>2</sup> in  
 353 Bermuda (Sciacca and van Arkel, 2015), 33 in the Azores, and 26 on the beaches of Easter  
 354 Island, all these beaches being on the Atlantic Ocean.

355 The concentration of plastic litter in Rimel beach and lagoon of Bizerte are related with  
356 human activities, such as tourism activities (Blettler et al., 2017; Browne et al., 2011; Imhof et  
357 al., 2017; Jeyasanta et al., 2020; McDermid and McMullen, 2004), urbanization (Ariza et al.,  
358 2008; Do Sul et al., 2011) and recreational usage of beaches (Gao et al., 2021). Rimel beach is  
359 one of the most famous and attractive beach for local and foreign visitors because of the  
360 proximity of beach to the road, especially during the summer season which may be the reason  
361 of the highest distribution recorded. Whilst it is possible that meteorological factors (i.e. wind  
362 and rain) further influence macroplastic accumulation in urban shoreline environments (see  
363 GESAMP, 2019), human activity may be a central direct driver of macroplastic accumulation  
364 in these systems, owing to high proximal household densities. Beach cleaning activities in  
365 Tunisia are mainly carried out by local authorities as in other countries. However, voluntary  
366 participation by citizens at these events is relatively low. Moreover, it has been demonstrated  
367 that beaches lacking cleaning and trash bins exhibited significantly more macroplastics,  
368 supporting earlier findings of (Rayon-Viña et al., 2018). Indeed, Blettler et al. (2017) found  
369 that plastic pollution was more associated with domestic solid wastes in their freshwater study  
370 system. In the other hand, Bizerte lagoon is one of the most famous and suitable areas for  
371 shellfish farming and recreational fishing used by many fishermen. So, these items found on  
372 the beach were probably discarded by the users of this area. This macroplastic contamination  
373 in Bizerte lagoon near of Menzel Abderahman harbour close to those reported in (Abidli et  
374 al., 2017; Zitouni et al., 2020), may confirm that maritime harbours represent a source of  
375 plastic litter in the studied region. The majority of macroplastics fragments showed signs of  
376 deterioration and were strongly weathered implying that they had probably been disposed in  
377 the environment for a long time (GESAMP, 2015).

#### 378 **4.2.Mesoplastics**

379 Another original aspect of this study was the identification, for the first time in this area, of  
380 mesoparticles (ranged from 1 to 5mm) (Napper and Thompson, 2018) from paint products.  
381 Mesoparticles were only detected in Bizerta lagoon. Using observation through stereo  
382 microscope, they could be considered as plastic fragments and might lead to a considerable  
383 over-estimation of the plastic contamination in such areas. This study emphasizes again the  
384 necessity to use notification techniques such as IR. Indeed, using IR, ,and confirmed by Fluo-  
385 X and MEB-EDX, we showed that 79.97% and 70.06% of these mesoparticles were identified  
386 as paint products in surface and depth sediments respectively. For the same reasons already  
387 cited previously, lagoon of Bizerte is the seat of several shellfish farms, known by the fishing

388 harbour of Menzel Abderrahmen. It can be hypothesized that the high contamination of paint  
389 product might be the result of harbour activities. Harbours and especially vessels have been  
390 reported as main sources of some litter types originating from fishing activity in general such  
391 as antifouling paints, cleaning and maintenance of ships' hulls, in addition to abandoned  
392 fishing gear (Edyvane et al., 2004). In many studies paint product are considered as plastic  
393 (Abdel Ghani et al., 2022; Pradit et al., 2022; da Silva et al., 2022) or resulting from primary  
394 plastics (Li et al., 2016).

395

### 396 **4.3. Microplastics**

#### 397 4.3.1. Spatial abundance of MPs

398 Until now, no studies in North Africa, and more precisely in Tunisia have focused on  
399 comparing the contamination by plastics in different ecological ecosystems such as Rimel  
400 beach, Bizerta lagoon and Ichkeul lake. Our survey showed that MPs were present in the three  
401 sampling sites and during the three sampling periods (winter, summer, spring), with variations  
402 in terms of abundance. The registered spatial distribution of MPs between sites could be  
403 influenced by urbanization, the presence of industries, and the nature of the ecosystems.

404 The average abundance of MPs in sediments of Rimel bathing beaches ( $173.33 \pm 34.12$   
405 items/kg) was in the same range of these reported from the Barra beach Aveiro (Portugal),  
406 along the mediterranean Sea and on the Phuket Beach in Thailand (Table 3). This result was 16 times  
407 higher than the one reported in Tarragona coast (western Mediterranean) (Table 3). Nevertheless,  
408 the concentrations were in the low range of what has been observed for the Carolina beach in  
409 Virginia ( $1410 \pm 810$  items/kg). These differences could be explained by differences between  
410 locations of demographic and industrial densities, or from geomorphology and dynamics of  
411 the sampled beach.

412 The concentration of MPs in Bizerta lagoon was in the range of  $118.33 \pm 34.38$  items/kg.  
413 These results were 4 times lower than those found by (Abidli et al., 2018),  $461.25 \pm 29.74$   
414 items/kg in MB (Bizerta lagoon). However, MPs abundance in Bizerta lagoon was 2 times  
415 higher than those founded in the Mar Menor lagoon in Spain (Table 3). This differences may  
416 be explained by the geological position of Bizerta lagoon, the density of human population in  
417 MA (more than 16,000 inhabitants), the significant maritime traffic, and industrial activities  
418 such as a petroleum refinery (Tunisian Refining Industries Company), a lubricants processing

419 unit (Sotulub), a cement plant (Cements of Bizerte), and the park of economic activities of the  
 420 free zone of Bizerte, which hosts several industrial and service companies (Abidli et al., 2017).  
 421 Quantified concentrations of MPs in this study were lower than those reported by (Abidli et  
 422 al., 2017), who founded in the same site  $7960 \pm 6840$  items/kg which is 67 times higher than  
 423 our results. These differences could be due to the sampling period but also extraction and  
 424 identification methods used. Bizerta lagoon is well-known for its fishing activities (harbour  
 425 with a fishing fleet of 181 coastal boats), which can confirm the high degree of pollution, with  
 426 even paint particles being classified as microplastics.

427 Ichkeul lake presented a high level of MPs ( $205 \pm 65$  items/kg). Despite, lake Ichkeul is a part of  
 428 a national parc of Ichkeul and it's one of the protected wetlands in the world (Ramsar  
 429 convention 2011), this ecosystem, is closely related by the Bizerta lagoon via Tinja river  
 430 (Abidli et al., 2017) favouring this high level of contamination. MPs detected in the present study  
 431 for Ichkeul lake were 7 times higher than those reported by (Hengstmann et al., 2021) who  
 432 collected  $1410 \pm 822$  items/kg in Lake Tollense sediments (Germany) (Table 3). In contrast,  
 433 present results are in the range with those registered by (Mao et al., 2021) in Wuliangshuai  
 434 Lake, (northern China) reporting values ranging from 165 and 724 items/kg (Table 3).

435

436 Table 3: Microplastic abundance in different beaches, lagoons and lakes in the world.

Location	Abundance items/Kg	Authors
<b>Rimel beach (Mediterranean), Tunisia</b>	$173.33 \pm 34.12$ items/kg	This work
Sidi Mansour Harbor in Southeast Tunisia	37.72 to 798.19 items/kg	(Chouchene et al., 2019b)
Mediterranean Sea	$166 \pm 205$ items/kg	(Constant et al., 2019)
Barra beach Aveiro, Portugal	100 items/kg	(Chouchene et al., 2021)
Odisha beaches, southeastern coast of India	$378.3 \pm 39.7$ items/kg	(Patchaiyappan et al., 2021)
Beach along Phuket coastline, Thailand	$188.3 \pm 34.5$ items/kg	(Akkajit et al., 2021)
Natural beach, Cox's Bazar, Bangladesh	$368.68 \pm 10.65$ items/kg	(Hossain et al., 2021)
Tarragona coast (western Mediterranean)	10.7 items/kg	(Expósito et al., 2021)
Virginia and North Carolina beaches	$1410 \pm 810$ items/kg	(Dodson et al., 2020)
<b>Bizerta lagoon (MA)</b>	$118.33 \pm 34.38$ items/kg	This work
The littoral zone of the north Tunisian coast	$316.03 \pm 123.74$ items/kg	(Abidli et al., 2018)
Bizerte lagoon, Tunisia	$7960 \pm 6840$ items/kg	(Abidli et al., 2017)
The Mar Menor lagoon (SE Spain)	$53.1 \pm 7.6$ items/kg	(Bayo et al., 2019)
<b>Ichkeul lake, Tunisia</b>	$205 \pm 65$ items/kg	This work
Lakeshore sediments	$1410 \pm 822$ items/kg	(Hengstmann et al., 2021)
Wuliangshuai Lake, northern China	Between 165 and 724 items/kg	(Mao et al., 2021)

437

#### 438 4.3.2. Seasonal abundance of microplastics

439 The abundance of MPs in surface sediment of Rimel beach and lagoon Bizerte present a  
440 decreasing profile among the three sampling periods, showing a higher level in spring and  
441 summer compared to winter. This result was confirmed by the study of (Gao et al., 2021).  
442 High temperatures and intense UV light registered during summer also made the MPs or  
443 plastics in the bathing beaches more likely to be broken into smaller MPs (Acosta-Coley and  
444 Olivero-Verbel, 2015; Andrady, 2015). However, regarding Ichkeul lake, MPs levels increase  
445 from spring, summer and winter, ( $80 \pm 0$  items/kg), ( $130 \pm 14.14$  items/kg) and ( $405 \pm 63.63$   
446 items/kg) respectively. Thus, seasonal variations in MPs concentrations in Ichkeul lake could  
447 be explained by the fact that this ecosystem receives many rivers discharges such as Douimis,  
448 Sajenan, El melah, Ghezela and Joumine, which can provide the increase of MPs levels.  
449 (Prata et al., 2020), showed that more particles were collected during wet seasons (464 and  
450 232 particles) than dry periods (19 and 18 particles). Furthermore, other researchers found  
451 that the abundance of MPs was higher during the wet season in summer than during the dry  
452 season in winter (Browne et al., 2010; Eo et al., 2019).

453 In depth, Rimel and Bizerta lagoon presented a lower level of contamination among the  
454 different seasons as compared to the surface sediment. However, in summer, Rimel beach  
455 presented a high number of MPs, that could be explained by the increase of population during  
456 this period. For Ichkeul lake, the abundance of MPs was lower comparing to previous sites  
457 than the one detected in surface sediment in summer and even winter.

#### 458 4.3.3. Characterization of MPs: types, shapes, sizes

459 For all sampling periods, PP, PET and PVC were the most detected polymers in surface and  
460 depth sediment of the three studied areas. In dried seasons, there was a predominance of PET,  
461 PVC and PE respectively which was already observed in other studies (Gao et al., 2021).  
462 However, in winter, our results showed a predominance of PP and PET, which was consistent  
463 with those of (Prata et al., 2020).

464 In the present study, the size of MPs varied from 78.33 to 297.25  $\mu\text{m}$ . These findings are in  
465 line with those of many other studies (Blašković et al., 2017; Chouchene et al., 2019b; Lots et  
466 al., 2017; Missawi et al., 2020). Furthermore, the results of the present study indicate that the  
467 Northern Mediterranean zone has a significant concentration of MPs, which might be related  
468 to high population density and waste input in the coastal sites (Lots et al., 2017). This  
469 phenomenon of small size dominating has been confirmed in some previous studies (Imhof et  
470 al., 2016; Xiong et al., 2018). Small-size MPs are mostly decomposed from large-scale

471 plastics by mechanical, chemical, biological processes after oxidation under UV light. The  
472 abrasion after contact with sand may explain the reason of these small sizes MPs. Another  
473 possibility is that MPs are broken during the analysis process leading to particles. Concerning  
474 the lowest sizes reported in the study, the grids used to extract the MPs before identification  
475 were chosen with 100  $\mu\text{m}$  mesh size as a good compromise between the time of IR imaging,  
476 the instrument resolution (more than to 10  $\mu\text{m}$ ) and the quantity of mineral and organic  
477 products retained after filtration. Hence, it was not surprising that the smallest MPs identified  
478 in the samples were around 70  $\mu\text{m}$ , MP smaller than this size being not extracted.

479 The totality of observed MPs in this study were fragments and fibers. The percentage reached  
480 in same sites (Rimel beach, Bizerta lagoon and Ichkeul lake) was 100% of fragments. These  
481 findings suggest that secondary MPs deriving from the mechanical fragmentation of bigger  
482 plastic debris, as well as photolysis, thermo-oxidation, thermo-degradation, and  
483 biodegradation processes (Laglbauer et al., 2014; Zhao et al., 2018) were the most common  
484 sources of plastic debris in the studied locations. (Abidli et al., 2018, 2017) also showed that  
485 fibres and fragments were the most abundant MP types in the lagoon of Bizerte. Therefore,  
486 MP contamination in the populated areas of Rimel beach and Bizerta lagoon (MA) could be  
487 attributed to sewage input and fragmentation of large plastic particles into smaller ones.  
488 Furthermore, more than 78 % of the particles found in Menzel Jemil (Bizerte lagoon) were  
489 fragments, which are likely connected to fragmentation procedures of larger plastics and  
490 electrical plates that are discarded by numerous companies that make plastic and electronic  
491 plates (Abidli et al., 2018) in this sector.

## 492 **5. Conclusion**

493 Marine environment burden is enhancing day to day due to illegal and unfair disposal  
494 of huge number of plastics all around the world. Even remote and preserved ecosystems are  
495 now damaged by plastic contamination. Results of this work provide first insights of the state  
496 of plastic contamination along the Tunisian coast, for all sizes of plastics from macro to  
497 microplastics. For this, an analytical procedure based on the separation of macro, meso and  
498 microplastics with a cutoff of 1 mm between meso and micro was set up to allow accurate  
499 identification of plastic particles with lower times of assessment. The obtain results showed  
500 that in the studied area, the mesoparticles (between 1 and 5 mm) were mostly paint products  
501 and were present in high quantities in Bizerta Lagoon, an area famous for its fishing activities.  
502 Additional survey is necessary to fully understand the transfer of those different plastics  
503 throughout this area and influence of parameters such as hydrology or economic activities.



504 Further studies are also necessary to take into consideration risk assessment associated with  
505 plastic collected in this area.

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**Sample collection : OSPAR protocol**



**Methodology**

**Macroparticles (>5 mm)**

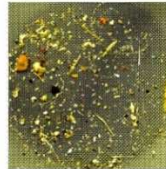


**Mesoparticles (1 – 5 mm)**



**Microparticles (<1 mm)**

**FT-IR Imaging**



**Identification & Characterization**

