

1 **The hidden microplastics. New insights and figures from the thorough separation and**  
2 **characterization of microplastics and of their degradation by-products in coastal sediments**

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12 **ABSTRACT**

13 The environmental pollution by plastic debris directly dispersed in or eventually reaching marine  
14 habitats is raising increasing concern not only for the vulnerability of marine species to ingestion  
15 and entanglement by macroscopic debris, but also for the potential hazards from smaller fragments  
16 down to a few micrometer size, often referred to as “microplastics”. A novel procedure for the  
17 selective quantitative and qualitative determination of organic solvent soluble microplastics and  
18 microplastics degradation products (<2mm) in shoreline sediments was adopted to evaluate their  
19 concentration and distribution over the different sectors of a Tuscany (Italy) beach. Solvent  
20 extraction followed by gravimetric determination and chemical characterization by FT-IR,  
21 Pyrolysis-GC-MS, GPC and <sup>1</sup>H-NMR analyses showed the presence of up to 30 mg microplastics  
22 in 1 kg sand, a figure corresponding to about 5.5 g of generally undetected and largely  
23 underestimated microplastics in the upper 10 cm layer of a square meter of sandy beach ! The  
24 extracted microplastic material was essentially polystyrene and polyolefin by-products from  
25 oxidative degradation and erosion of larger fragments, with accumulation mainly above the storm  
26 berm. Chain scission and oxidation processes cause significant variations in the physical and

27 chemical features of microplastics, promoting their adsorption onto sand particles and thus their  
28 persistence in the sediments.

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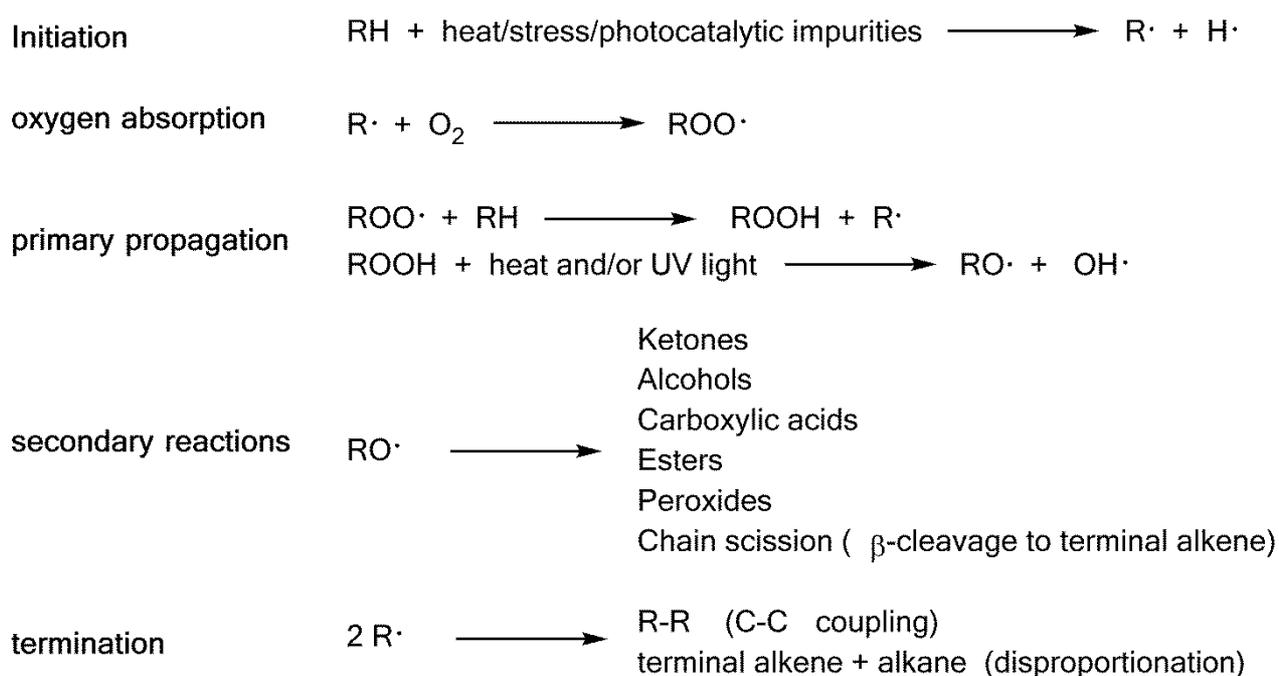
30 **Key words:** microplastics, beach sand, solvent extraction, polymer degradation, Pyrolysis-Gas  
31 Chromatography-Mass Spectroscopy, Gel Permeation Chromatography, Fourier Transform InfraRed  
32 spectroscopy

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## 34 **INTRODUCTION**

35 Since the early 1970s an increasing number of studies have been devoted to environmental issues  
36 associated with pollution from uncontrolled disposal of plastics,<sup>1</sup> with particular attention in the last  
37 years to the accumulation of plastics in marine ecosystems, the so-called marine litter. The  
38 worldwide plastics production and consumption, with further estimated 1.5-2.5 % yearly increase  
39 from the 335 Mton reached in 2016, is largely targeted to short-lived packaging applications and  
40 has been matched with increasing incautious dispersion of plastic items in the environment.<sup>2</sup> Due to  
41 the resistance of most plastics to chemical, physical and biological degradation processes, the  
42 accumulation of debris from plastics waste in marine habitats has long been recognized as a serious  
43 environmental pollution issue,<sup>3</sup> with harmful effects on planktonic organisms and marine fauna,  
44 possibly beyond those already ascertained.<sup>4-6</sup> However, in spite of the increasing scientific interest  
45 and public concern about microplastics in marine habitat, there is still a lack of knowledge  
46 concerning the intensity and quality of this kind of pollution, and particularly of the molecular  
47 features of the plastics-derived products eventually generated as a result of environmental  
48 degradation. In fact, in addition to direct hazard from entanglement or ingestion by marine fauna,<sup>7-9</sup>  
49 plastic debris may release additives<sup>10</sup> and be very efficient absorbers of polychlorinated biphenyls,<sup>11</sup>  
50 polybrominated diphenyl ethers, polycyclic hydrocarbons,<sup>12</sup> and many other persistent organic  
51 pollutants (POPs). This holds true in particular for plastic fragments smaller than 5 mm, generically  
52 referred to as microplastics, due to their larger specific surface area and the presence of surface

53 functional groups resulting from degradation and affecting POPs adsorption and release.<sup>13,14</sup> Thus  
 54 microplastics pose a more serious hazard than larger debris to living organisms, including humans,  
 55 since they can be ingested by organisms at the lowest trophic levels and result in POPs  
 56 bioaccumulation.<sup>15</sup>  
 57 Microplastics are present in the marine environment either as primary particles (manufactured as  
 58 microbeads, fibers or pellets) or as secondary fragments, the latter being the result of larger pieces  
 59 breaking down into smaller ones and of surface erosion. Fragmentation and erosion occur when  
 60 plastic debris (in particular hydrocarbon polymers, such as PE, PP and PS) is exposed to sunlight,  
 61 being the ultimate result of photo-oxidative degradation processes typically starting at the molecular  
 62 level with a photo-induced homolytic C-H bond cleavage, preferentially on tertiary carbon atoms.<sup>16</sup>  
 63 In the presence of oxygen, the newly formed carbon centered free radicals generate peroxy radicals,  
 64 rapidly converted by hydrogen abstraction into thermally and photochemically unstable  
 65 hydroperoxides. The ensuing cascade of reactions leads to polymer chain scission (molar mass  
 66 reduction) and/or crosslinking, along with the formation of various oxidized groups (scheme 1).<sup>17-19</sup>  
 67



68  
 69 **Scheme 1.** General oxidation mechanism in hydrocarbon polymers

70

71 The overall rate of such degradation/oxidation processes is influenced by the chemical structure of  
72 the polymeric material and the environmental conditions (oxygen concentration, temperature and  
73 sunlight exposure). In particular, once the plastic debris reaches the shoreline, the rate and extent of  
74 oxidative degradation and fragmentation of the macromolecular structure are expected to be much  
75 higher than in water compartments, due to the comparatively higher temperatures and severe  
76 sunlight exposure.<sup>16,20,21</sup> At the macroscopic level, this results in surface embrittlement that  
77 promotes further mechanical erosion and thus auto-accelerated generation of micron- and  
78 submicron-sized fragments (secondary microplastics). Due to their small size and to a surface  
79 chemical composition that may differ significantly from that of the parent polymers, such fragments  
80 are quite elusive to the sampling in real environment and, consequently, to the qualitative and  
81 quantitative evaluation of this kind of pollution, which justifies the term “hidden” for a plastics  
82 fraction about which there is very little awareness even among scientists. In fact, to date most  
83 studies have been limited to sieving of the upper layer of the sea surface or of sand samples  
84 followed by mechanical or density sorting and counting of the collected plastics debris,<sup>22,23</sup> with  
85 fewer ones investigating the polymer types and/or the effects of microplastics uptake by marine  
86 organisms. In the present study, a thorough protocol based on the extraction with boiling  
87 dichloromethane (DCM) and xylene (selective solvents for polyolefins and most vinyl polymers) of  
88 the free and adsorbed polymeric fractions from sand samples was devised, with the purpose of  
89 recovering both microplastics and their degradation products. The sand samples were collected in  
90 an estuarine region nearby the Arno and Serchio rivers in the Tyrrhenian Sea (Tuscany, Italy)  
91 according to the typical zonation of a beach profile, that is, orthogonally to the shoreline and up to  
92 the depression behind the coastal dunes. The solvent extracts from the samples were characterized  
93 by Fourier transformed infrared spectroscopy (FT-IR), gel permeation chromatography (GPC),  
94 <sup>1</sup>HNMR spectroscopy, and pyrolysis-gas chromatography mass spectrometry (Py-GC/MS). The

95 obtained results could be used to draw a concentration profile of solvent-extractable microplastics  
96 and microplastics-derived degradation products in the different shore sectors.

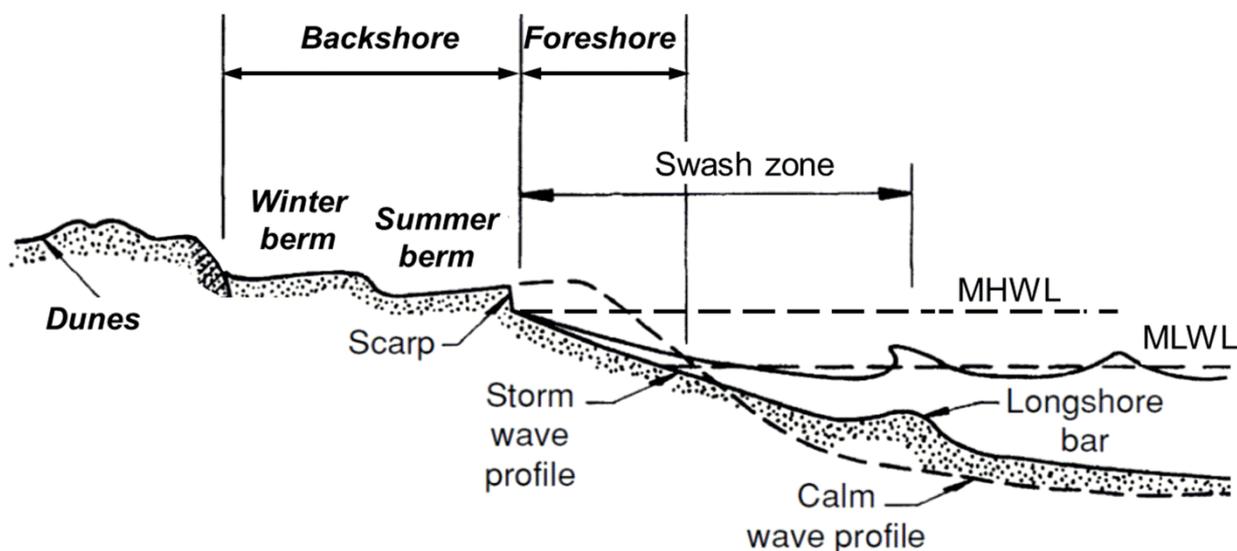
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## 98 **MATERIALS AND METHODS**

99

### 100 *Sampling site and sampling procedure*

101 Beach sand samples were collected from the shoreline of Marina di Vecchiano (Tuscany, Italy) at  
102 the end of the winter season (march 2016), that is, before the removal of natural (e.g. drift beach  
103 algae and wood debris) and artificial (plastic waste and debris) remains prior to the summer season.  
104 The selected site and sampling period thus allowed the collection of samples representative of the  
105 accumulation, during the winter season, of natural and artificial remains whose composition was  
106 clearly influenced by the nearby estuaries of the rivers Arno (about 15 km southwards) and Serchio  
107 (about 1 km southwards). The samples were collected from sixteen spots split among four transects,  
108 the latter spanning from the intertidal to the supralittoral zone (Figure 1), covering a surface area of  
109 approximately 12.000 m<sup>2</sup>. In particular, four samples were pooled in each one of the four sectors of  
110 a typical beach profile zonation:<sup>24</sup> dunes (sector A), backshore-winter berm (sector B) and  
111 backshore-summer berm (sector C), above the tidal threshold (mean high water line, MHWL);  
112 foreshore (sector D) below the tidal threshold (mean low water line, MLWL). The collected sand  
113 samples with the corresponding sampling sector and geopositioning are listed in figure S1 and table  
114 S1 of Supporting Information.



115

116 **Figure 1.** Typical beach profile and transect zonation (adapted from ref. 24)

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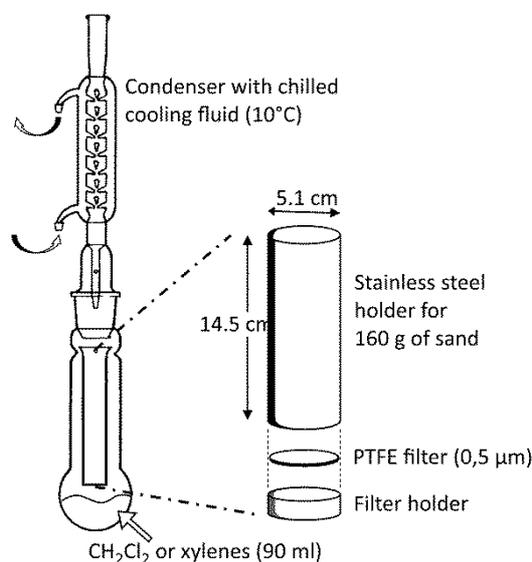
118 From each sampling spot approximately 1.5 kg sand was collected according to a core drill  
 119 procedure to a maximum 25 cm depth by using cylindrical (17 cm height, 11 cm diameter) glass  
 120 vessels, previously thoroughly rinsed with hexane. Sand samples were homogenized and then  
 121 sieved at 5 mm mesh to remove larger natural and artificial remains, dried at 60 °C in a ventilated  
 122 oven to constant weight to quantify the residual humidity, and finally sealed and stored in the same  
 123 glass vessels used for the sampling. The dried samples were then further sieved at 2 mm mesh right  
 124 before performing solvent extraction. The plastic fragments within the 2-5 mm range collected from  
 125 each sample after the sieving procedure were separately stored in similarly pre-treated glass tubes at  
 126 4 °C. Partially oxidized low density polyethylene (LDPEox) used as a reference material was  
 127 obtained by accelerated thermal aging (70 °C for 30 days) in the presence of cobalt stearate as an  
 128 oxo-degradation catalyst.

129

130 ***Solvent extraction of sand samples***

131 Sand samples were submitted to a solvent extraction procedure in order to determine the content of  
 132 plastics and their degradation products. Extraction of 160 g of dried sand from each sample was  
 133 carried out with 90 mL refluxing dichloromethane (DCM, 99.9%, stabilized with amilene,

134 Environment Analysis grade, Romil-SpS, Cambridge, UK) for 3 hours, using a modified  
135 Kumagawa-type apparatus consisting of a stainless steel cylinder (14.5 cm height, 5.1 cm diameter)  
136 fitted at the bottom with a stainless steel holder bearing a 0.5  $\mu\text{m}$  pore size, 47 mm diameter PTFE  
137 filter (Fluoropore, purchased from Sigma Aldrich), and suspended inside a 1000 mL flask, as  
138 depicted in Figure 2. Before each extraction the apparatus was conditioned by refluxing 90 mL  
139 DCM for 3 hours to remove any contaminant. The setup of the extraction apparatus allows  
140 percolation of the hot extracting solvent throughout the sample maintained at a temperature close to  
141 that of the boiling solvent (here 37  $^{\circ}\text{C}$ ), thus preventing incipient re-precipitation of extractable  
142 fractions that may be less soluble at room temperature.



143

144 **Figure 2.** Kumagawa-type extraction apparatus

145

146 Selected DCM-extracted sand samples were then submitted to a second extraction at 135-140 $^{\circ}\text{C}$   
147 with refluxing xylenes (98.5%, Sigma-Aldrich) to collect the DCM-insoluble, less degraded  
148 polyolefin fraction. For each sand sample the solvent extraction was carried out in triplicate. The  
149 volume of the resulting solutions was reduced to 5 mL in a rotatory evaporator, transferred to 10  
150 mL weighed glass vials and finally evaporated to dryness under a nitrogen stream.

151 In order to exclude exogenous contamination of the extracts from the apparatus and extracting  
152 solvent, the overall procedure was initially assessed for quality assurance by performing the

153 extraction in the absence of sand sample; the same test was then repeated periodically for quality  
154 control. In particular, preliminary blank extraction tests were performed by fitting the stainless steel  
155 cylinder with one of three different filters, respectively: PTFE syringe filter (0.45  $\mu\text{m}$  pore size, 25  
156 mm diameter; Agilent Technologies), Chromafil® Xtra H-PTFE syringe filter (0.2  $\mu\text{m}$ , Macherey-  
157 Nagel GmbH, Germany) and Fluoropore membrane filter (PTFE, 0.5  $\mu\text{m}$ , Sigma Aldrich). The  
158 latter type was chosen for the subsequent extractions as it was the only one that in the blank tests  
159 did not result in the release of any detectable organic residue both by gravimetry ( $< 0.01$  mg) and by  
160 FT-IR analysis. The effectiveness and exhaustiveness of the extraction procedure was validated by  
161 performing in triplicate additional extraction tests on beach sand samples further contaminated with  
162 known amounts of expanded poly(styrene) (EPS) and artificially oxidized low density  
163 poly(ethylene) (LDPEox), respectively, as internal reference materials (see Supporting Information  
164 for a detailed description of these reference materials and of the validation procedure). In a first  
165 stage, DCM extraction tests were performed the average recovery rate of the additional amounts of  
166 polymeric powders dispersed in the sand samples was found to be nearly quantitative for both EPS  
167 ( $94.9 \pm 6.3$  %) and for the DCM-soluble LDPEox fraction ( $96.5 \pm 2.2$  % of the 10.7 % recovery  
168 determined by separate solubility tests of pure LDPEox). In a second stage, the DCM extracted  
169 samples were further extracted with boiling xylene to achieve a further over 90 % recovery of the  
170 residual polyolefin (table S2 and S3 of Supporting Information).

171

### 172 *Analytical techniques*

173 Gravimetric analysis of solvent extracts was performed on dried samples conditioned 7 hour at  
174 60 °C in a ventilated oven, followed by cooling at room temperature in a desiccator before weighing  
175 with a Kern Model ALS 220-4 analytical balance (0.01 mg precision). This procedure was repeated  
176 up to constant weight and the precision of the measurements was validated by considering the  
177 pooled standard deviation with 95 % confidence.

178 Fourier transformed infrared spectroscopy (FT-IR) spectra were recorded at  $4\text{ cm}^{-1}$  resolution with a  
179 Nicolet Avatar 360 spectrophotometer. Soluble fractions were analyzed as films cast onto KBr disks,  
180 while the material scraped off the surface of the sieved plastics debris ( $>5\text{ mm}$ , cleaned with  
181 d.i. water in an ultrasonic bath and oven-dried at  $60^\circ\text{C}$ ) was grinded with KBr in a mortar and  
182 converted in disks at 2000 psi.

183 For the Pyrolysis-Gas Chromatographic-Mass Spectroscopy (Py-GC/MS) analyses a system  
184 comprising a multi-shot EGA/PY-3030D pyrolyzer (Frontier Lab) connected with a HP Agilent  
185 6890 gas-chromatograph coupled with a Mass Selective 5973 single quadrupole mass spectrometer  
186 was used. Single-shot pyrolysis was performed by a heat jump to  $650^\circ\text{C}$  and interface temperature  
187  $300^\circ\text{C}$ . After split (1:10) injection the chromatographic separation was performed with a 30 m (i.d.  
188 0.25 mm) 5% diphenyl 95% polysiloxane HP-5ms capillary column (Agilent) fitted with a 2 m (i.d.  
189 0.32 mm) deactivated pre-column, using high purity (99.9995 %) helium as the gas carrier at 1.2  
190 mL/min flow rate; temperature program: 5 min at  $40^\circ\text{C}$ , then heating at  $10^\circ\text{C}/\text{min}$  up to  $300^\circ\text{C}$ ,  
191 and a final 10 min at  $300^\circ\text{C}$ . The operating conditions were set up according to those optimized for  
192 a PS standard (24 kg/mol) and for the DCM soluble fraction of the reference partially oxidized  
193 LDPE. Mass spectra from the literature<sup>25</sup> and mass-spectral libraries (NIST5, Wiley 2.4.0) were  
194 used for the identification of pyrolysis products and the interpretation of pyrolysis profiles.

195 Analytical quality assurance for Py-GC/MS methodology was achieved as follows: i) for each Py-  
196 GC/MS analysis of the sand extracts a blank Py-GC/MS was previously run using the same  
197 procedure and instrumental conditions, the same cup and the same glass fiber to be used for the real  
198 sample. This routine step achieves the regular cleaning of the sample cup and of the glass fiber from  
199 potential contaminations; besides, it allows monitoring and identifying memory effects from  
200 previous analysis, which were never found in any of the analyzed samples under the adopted  
201 conditions; ii) procedure blanks were run daily by analyzing the concentrated extract of the solvent  
202 resulting from a blank extraction performed with the same extraction apparatus, the same solvent  
203 and solvent volume, the same conditions adopted for the extraction, and the same concentrations

204 steps adopted for the sand samples. The contaminants identified from the analyses of the procedure  
205 blanks were only palmitic (hexadecanoic) acid and stearic (octadecanoic) acid. No pyrolysis  
206 products related to the presence of any synthetic polymers or other natural materials were detected  
207 in the pyrolytic profiles of procedure blanks.

208 Molecular weight and molecular weight distribution of the polymeric fractions from the solvent  
209 extracts of the sand samples were determined by Gel permeation chromatography (GPC) using a  
210 Jasco apparatus with a PU-2089 Plus pump and injector, column oven, and RI-2031 Plus refractive  
211 index detector and UV-2077 Plus multiple wavelength UV/Vis detector operating at 254, 260 and  
212 340 nm. Two distinct chromatographic runs were performed on each sample, using either two in  
213 series PLgel Mixed-E or two PLgel Mixed-D columns (Polymer Laboratories, Varian, Inc),  
214 respectively, thermostated at 30 °C. Analyses were performed with chloroform eluent at 1.0  
215 mL/min flow rate, after filtration of the DCM solutions through 0.2 µm PTFE filter membranes  
216 without detectable retention of insoluble fractions. Calibration was performed with two sets of  
217 polystyrene standards (Polymer Laboratories: 0.8, 1.68, 2.4, 4.0, and 10.3 kDa for the setup with  
218 Mixed-E columns; 1.79, 4.85, 23.8, 52.1, 254.0, and 293.3 kDa for the setup with Mixed-D  
219 columns).

220 <sup>1</sup>H-NMR analyses were performed on 40 mg samples in 800 µL CDCl<sub>3</sub> using a Bruker Avance400  
221 spectrometer operating at 400 MHz.

222

## 223 **RESULTS AND DISCUSSION**

224 Due to the harsh exposure to sunlight and abrasive environment, shoreline sand contaminated by  
225 plastics debris contains a variety of minute particles showing a wide range of sizes and chemical  
226 characteristics. These may range from relatively intact micrometer- to millimeter-scale particles,  
227 physically blended with sand particles, to waxy and/or variously oxidized material adsorbed or  
228 adhering to sand granule surface. For the above reasons, separation methods commonly adopted for  
229 the quantitative and qualitative determination of microplastics in marine sediments,<sup>26,27</sup> while

230 effective in the case of pristine plastics pellets or plastics debris at least a few mm in size, may be  
231 less accurate and reliable in case of extensively degraded and oxidized plastics particles with size  
232 that may be typically the few micrometer or even sub-micrometer range.

233 In order to investigate in detail the spatial distribution and chemical features of microplastics in  
234 shoreline sediments, sand specimens were collected from a beach in the northern Tuscany coast of  
235 Italy (figure S1 in Supplementary information). Four sectors of the typical zonation of a beach  
236 profile (dunes, backshore-winter berm, backshore-summer berm and foreshore) were pooled and the  
237 sand samples were sieved down to 2 mm mesh to remove larger natural and artificial remains before  
238 further processing. A new protocol was then devised to effectively extract the polymeric and  
239 polymer-derived material from the inorganic matrix and to analyze quantitatively and qualitatively  
240 the various fractions that could be separated by selective solvent extraction and chromatographic  
241 methods. Dichloromethane (DCM) was used for the primary extraction since it is a good solvent for  
242 poly(styrene) (PS), low molecular weight aromatic and aliphatic hydrocarbons up to paraffin wax,  
243 and low-to-medium molecular weight oxidized polyolefins;<sup>28</sup> in such solvent most polysaccharides,  
244 lignin and other biomolecules, as well as high molecular weight semi-crystalline polyolefins, are  
245 insoluble. Further extraction was performed with boiling xylene to recover the less degraded  
246 polyolefin fraction. Quantitative and qualitative multi-technique analyses on the various extracts,  
247 along with blank extractions of sand samples added with known amounts of reference PS and  
248 partially oxidized LDPE (LDPEox) were performed for QA/QC and validation of the  
249 exhaustiveness and selectivity of the extraction procedure towards synthetic hydrocarbon polymers  
250 and their degradation products (see Supporting Information for the validation tests).

251 The average amounts of polymer-containing solids extracted with DCM, expressed as mg of extract  
252 from 1 kg of dry sand sample, are reported in table 1.

253 The results clearly show a preferential accumulation of DCM extractables in the winter berm and  
254 dunes zones, that is, the shore sectors at or beyond the outer limit of the swash zone, in which the  
255 stranded plastics waste is not effectively removed by tidal fluctuations and sea storms.<sup>29</sup> The long

256 term accumulation results then in the progressive degradation of polymeric material exposed to  
257 relatively harsh photo-oxidative and thermo-mechanical conditions.

258

259 **Table 1.** Average amount of DCM extracts within each sampling sector.

Sampling sector	Polymer-containing solids in DCM extract <sup>(a)</sup> (mg/kg)
A (Dunes)	30.2 ± 2.2
B (Backshore-winter berm)	11.9 ± 5.2
C (Backshore-summer berm)	7.1 ± 1.0
D (Foreshore)	5.9 ± 0.4

260 <sup>(a)</sup> Each figure is the average of 12 measurements resulting from three replicates on each one of the  
261 four samples of the same sector but different transect; the amount of polymer-containing solids is  
262 expressed as mg per kg of dry sampled sand (sand dry density about 1.8 g/cm<sup>3</sup>).

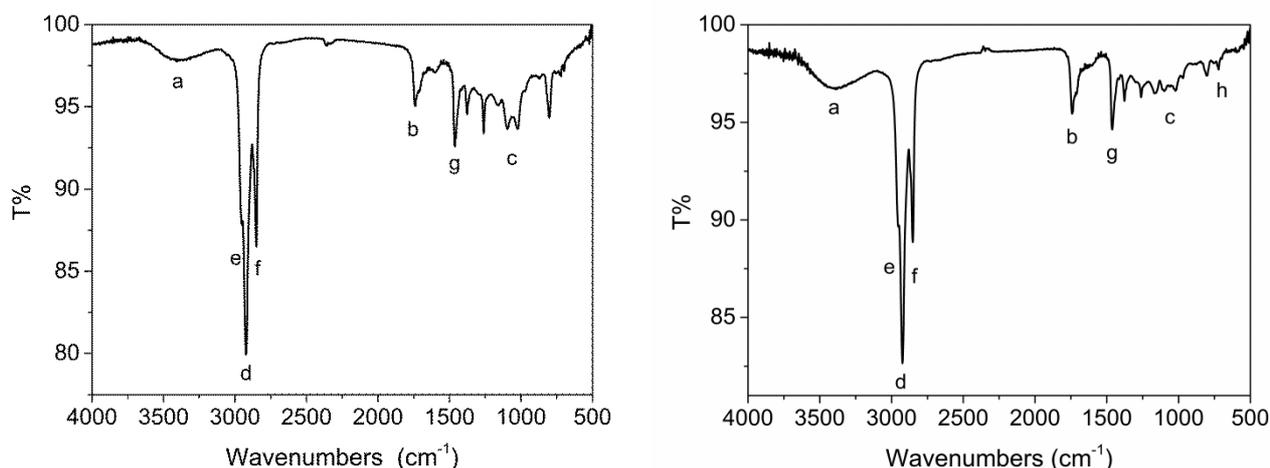
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264 Selected samples already extracted with DCM were also submitted to a further extraction with  
265 boiling xylenes (130-140°C) in order to ascertain the presence of high molecular weight polyolefins,  
266 which are insoluble in boiling DCM. It is worth mentioning that xylene extraction tests performed  
267 on reference sand samples contaminated with a known amount of LDPEox gave over 90%  
268 polyolefin recovery (see table S2 and S3 in Supporting Information). Gravimetric analysis of the  
269 xylene extracts of sand samples suggests that the presence of intact (that is, neither significantly  
270 fragmented nor oxidized) polyolefin is nearly negligible, an indication that the smaller polyolefin  
271 fragments are already highly oxidized and thus extractable in DCM. While unexpected, this  
272 apparently surprising result can be explained if we consider that the degradation mechanism leading  
273 to the formation of micrometric and sub-micrometric plastic particles involves a complex pattern of  
274 reactions (scheme 1), turning the most common main chain hydrocarbon commodity polymers, such  
275 as poly(ethylene) (PE), poly(propylene) (PP) and poly(styrene) (PS) (that is, those most used in

276 packaging and also the foremost source of marine pollution<sup>2</sup>), into increasingly polar, partially  
277 oxidized polymers with lower molecular weight, and promoting their solubility in DCM. In  
278 particular, it is expected that PP and PS, bearing one reactive tertiary C-H bond every other main  
279 chain carbon, be even more susceptible to oxidative degradation and fragmentation than PE, the  
280 latter having either no side chains, as in HDPE (High Density PE), or only a few branches, as in  
281 LDPE and LLDPE (Low Density PE and Linear Low Density PE, respectively). Indeed, not only  
282 the rate but also the outcome of the degradation processes are associated with the chemical structure  
283 of the polymer. For instance PS, when exposed to UV irradiation in the presence of oxygen,  
284 undergoes a relatively fast degradation resulting in yellowing, the latter caused by the buildup of  
285 extended conjugation including oxidized intermediates (e.g. carbonyl groups). Such process, which  
286 takes initially place within a thin surface layer where oxygen is more easily absorbed, results in  
287 chain breaking and formation of intermediates bearing oxidized groups vicinal to aromatic rings  
288 that may act as free radical scavengers (similarly to hindered phenol stabilizers<sup>30</sup>), thus protecting  
289 the underlying bulk polymer from further oxidation, at least until the embrittled, photo-oxidized  
290 surface layer is mechanically removed by physical or mechanical ablation. On the contrary,  
291 degradation in polyolefins typically proceeds throughout the polymer bulk because of their higher  
292 oxygen permeability and the absence of degradation intermediates with any photo-stabilizing  
293 activity. As a result, both polyethylene and, particularly, polypropylene undergo extensive  
294 fragmentation with molecular weight reduction of several orders of magnitude,<sup>31</sup> making them more  
295 easily soluble in a low boiling, moderately polar solvent such as DCM. A rough estimation based  
296 on the figures from Table 1 suggest the presence of up to 8,5 g of generally undetected and largely  
297 underestimated microplastics (including the highly oxidized/degraded fraction) in the upper 10 cm  
298 layer of a square meter of sandy beach.

299 For the identification of the polymer types and the evaluation of the structural modifications caused  
300 by weathering, chemical characterization of the DCM extracts was carried out by FT-IR and <sup>1</sup>-  
301 HNMR spectroscopy as well as Pyrolysis-GC/MS and Gel Permeation Chromatography.

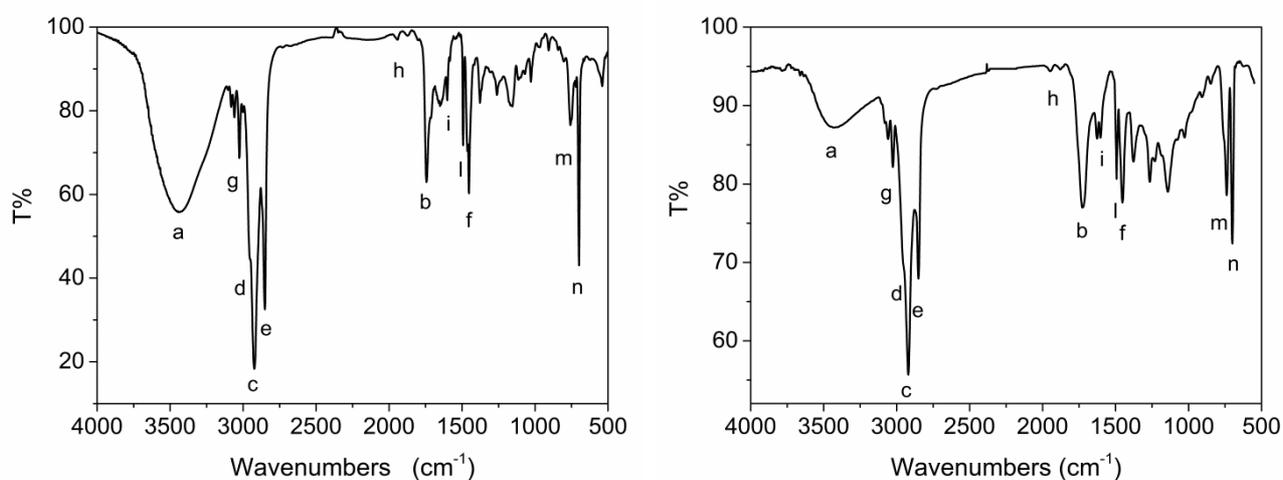
302 Representative FT-IR spectra of DCM extracts from the samples collected in the foreshore (sector  
 303 D) and summer berm (sector C) are reported in Figure 3. Both spectra present the characteristic  
 304 strong absorptions from linear aliphatic moieties (some branching is detected from the presence of a  
 305 weak methyl stretching absorption at  $2950\text{ cm}^{-1}$ ), with additional ones typical of oxidized moieties  
 306 such as aliphatic carbonyl (C=O stretching at  $1720\text{-}1740\text{ cm}^{-1}$  with shoulder down to  $1660\text{ cm}^{-1}$   
 307 from conjugated ketone C=O stretching) and hydroxyl groups (broad O-H stretching peaking at  
 308  $3430\text{-}3400\text{ cm}^{-1}$  and C-O stretching  $1020\text{ cm}^{-1}$ ). No significant absorption bands of aromatic  
 309 moieties (e.g. from PS) were detected in the samples from these two sectors within the swash zone.  
 310



311 **Figure 3.** Representative FT-IR spectra of the DCM extracts from sand samples of foreshore (left)  
 312 and summer berm (right). Main peaks: oxidized groups at  $3400$  (a, broad  $\nu_{\text{OH}}$ ) and  $1740\text{-}1710$  (b,  
 313  $\nu_{\text{C=O}}$ ),  $1100$  and  $1020$  (c,  $\nu_{\text{C-O}}$ )  $\text{cm}^{-1}$ ; aliphatic hydrocarbon at  $2923$  (d,  $\nu_{\text{asym,CH}_2}$ ) with shoulder at  
 314  $2950$  (e,  $\nu_{\text{asym,CH}_3}$ ),  $2850$  (f,  $\nu_{\text{sym,CH}_2}$ ),  $1461$  (g,  $\delta_{\text{CH}_2}$ ) and  $720$  (h,  $\delta_{\text{CH}_2,\text{rock}}$ )  $\text{cm}^{-1}$ .

315  
 316 On the other hand, the spectra of DCM extracts from both the winter berm (sector B) and the dune  
 317 (sector A) samples, as exemplified in the representative spectra reported in figure 4, showed a  
 318 higher complexity. In particular, the spectra of two out of the four winter berm extracts and those of

319 all the dune ones show, in addition to the absorptions from aliphatic hydrocarbon (linear and  
 320 branched) and from oxidized (alcohol, carbonyl and carboxyl derivatives) structures previously  
 321 described, several absorptions from aromatic hydrocarbon structures at 3080-3000, 1945-1800,  
 322 1600, 1490 and 740-700  $\text{cm}^{-1}$  that can be ascribed to styrenic polymers. The relative intensity of the  
 323 aromatic absorption bands with respect to those typical of linear alkane varied significantly among  
 324 the various extracts from sectors A and B. In general, the highest relative content of aromatic  
 325 compounds was detected in the DCM extracts from the dune samples.



326 **Figure 4.** Representative FT-IR spectra of the DCM extracts from sand samples of winter berm  
 327 (left) and dune (right). Main peaks: oxidized groups at 3450-3400 (a, broad  $\nu_{\text{OH}}$ ) and 1745-1710 (b  
 328  $\nu_{\text{C=O}}$ )  $\text{cm}^{-1}$ ; aliphatic hydrocarbon at 2923 (c,  $\nu_{\text{asym,CH}_2}$ ) with shoulder at 2950 (d,  $\nu_{\text{asym,CH}_3}$ ), 2850 (e,  
 329  $\nu_{\text{sym,CH}_2}$ ), 1460 (f,  $\delta_{\text{CH}_2}$ )  $\text{cm}^{-1}$ ; aromatic (PS) at 3080-3025 (g,  $\nu_{\text{CH,arom}}$ ), 1945-1800 (h, arom.  
 330 overtones), 1600 (i,  $\nu_{\text{C-C,arom}}$ ), 1492 (l,  $\nu_{\text{C-C,arom}}$ ) 740 (m,  $\delta_{\text{CH,arom}}$ ) and 700 (n,  $\delta_{\text{C-C,arom}}$ )  $\text{cm}^{-1}$ .

331

332 In addition to the DCM extractable fractions, the larger plastic debris within the 2-5 mm size range  
 333 separated during the sieving procedure of the sand samples were also analyzed by FT-IR  
 334 spectroscopy. These larger fragments were collected from the winter berm and dunes sectors in  
 335 which they were present in a sizable amount, that is, outside the range of the swash zone, in which  
 336 they are deposited and carried back to the sea continuously by surface waves and currents.<sup>32</sup> All the

337 collected larger fragments from the 4 sampling sites (4 transects) of each sector were found to  
338 belong to only three polymer classes: PE, PS and PP, respectively, the most abundant one being PE  
339 (10 fragments of PE in total, against 3 fragments for PP and 3 for PS, details of the weight and  
340 FTIR identification of the recovered plastic fragments, most of them being from disposable flatware  
341 and packaging, are listed in Table S4 and Figure S3 of Supporting Information). In all cases the FT-  
342 IR analysis revealed extensive oxo-degradation, indicating the progress of plastics degradation in  
343 the backshore and dune accumulation sectors. The spectra were generally comparable with those  
344 from artificially aged PE,<sup>33</sup> PP,<sup>34</sup> and PS reference samples. The similarity of the spectral features of  
345 the larger (2-5 mm) plastics fragments and of the DCM extracts from the same sampling sites  
346 indicates that accumulation of highly degraded plastic material is the ultimate product of the  
347 fragmentation and surface erosion of plastics debris embrittled by photo-oxidative, thermal and  
348 mechanical weathering.

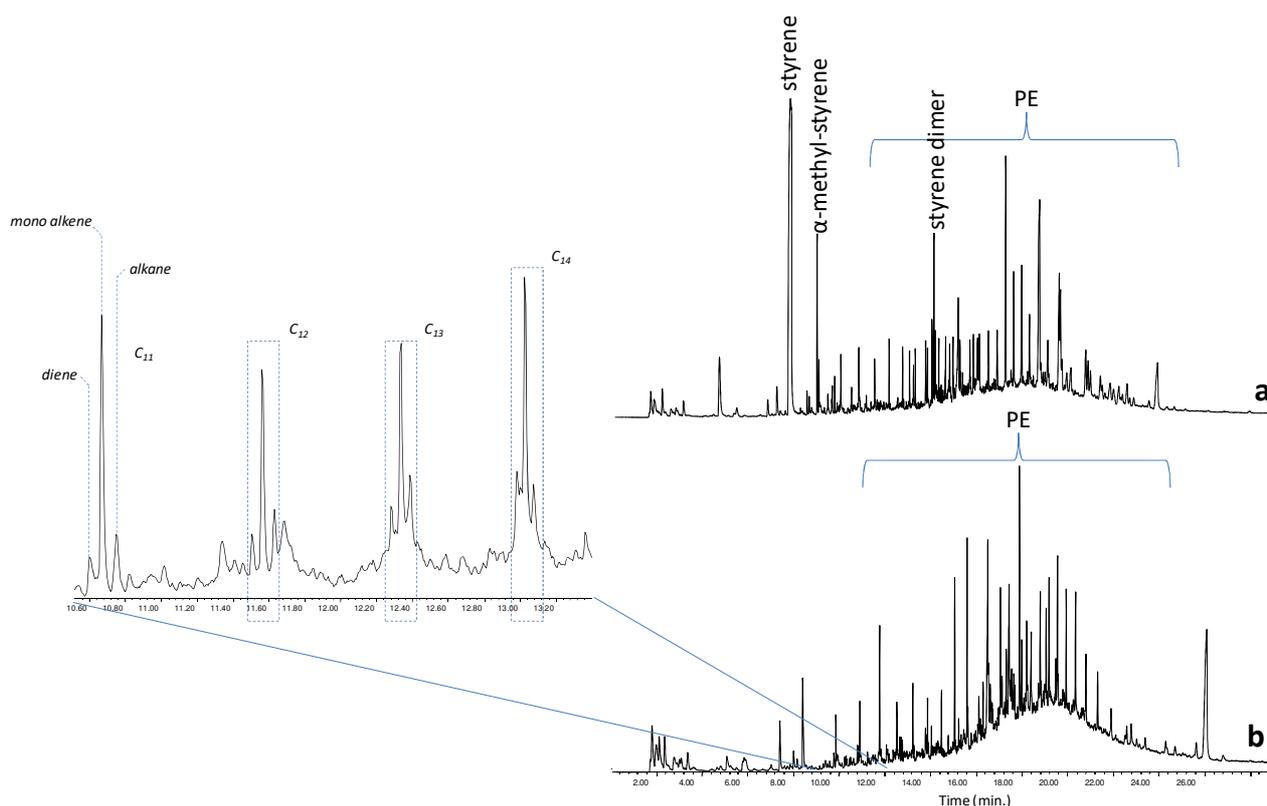
349

### 350 **Analysis by Pyrolysis-GC/MS**

351 Analytical pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC/MS) was used to  
352 obtain information on the chemical composition of the DCM extracts at a molecular level. The  
353 pyrolytic profiles of the analyzed samples featured specific markers clearly related to the presence  
354 of polystyrene (PS) and polyethylene (PE), as identified based on the literature<sup>35</sup> and on the analysis  
355 of standards (Supporting Information, Figure S5). In particular, all the samples were found to  
356 contain PE markers, while those of PS were only found in the backshore sectors A and B, in  
357 agreement with the evidences from the FTIR analyses as discussed before. Figure 5 shows the  
358 pyrolytic chromatographic profiles obtained for samples G3040001 (dunes) and G3040008  
359 (foreshore), representative of the two types of recorded profiles. Identification of pyrolysis products  
360 from their MS features is reported in Table S5 of Supporting Information .

361 All the markers of PS (styrene,  $\alpha$ -methyl styrene, 1-ethyl-2-propenyl-benzene, diphenylethylene,  
362 1,2-diphenylpropane, and low oligomeric species) feature the tolyl ion fragment with m/z 91, while

363 PE can be identified by the typical pyrolytic profile characterized by a series of clusters  
364 corresponding to linear hydrocarbon fragments (from 6 to 36 carbon atoms), each one comprising a  
365 mono-unsaturated hydrocarbon main peak with the common ion fragment  $m/z$  55 as the marker, and  
366 two ancillary peaks deriving from the saturated and di-unsaturated analogues (common ion  
367 fragments  $m/z$  57 and  $m/z$  55, respectively). Due to the specific fragmentation pattern of the PE  
368 polymer, we can exclude the presence of a paraffin wax. In fact, under the adopted Py-GC/MS  
369 conditions, the distribution of peaks in the pyrolysis profile of paraffin wax differs from that of PE  
370 and PP for the shape of the hydrocarbon peaks clusters: in the case of paraffin wax the pyrolysis  
371 products are only saturated hydrocarbons with molecular weights higher than those from PE. This  
372 was confirmed by the analysis of reference materials.



373  
374 **Figure 5.** Chromatograms from the Py-GC/MS analysis of: a) sample G3040001 from sector A  
375 (dunes); b) sample G3040008 from sector D (foreshore). PE=polyethylene pyrolysis products  
376 (detailed peak identification is reported in Table S5 of Supporting Information).

377

378 It is worth pointing out that no markers of natural polymers, such as levoglucosane (indicating the  
379 presence of polysaccharides), phenols (from lignin), or toluene (from humic acids)<sup>36-38</sup> were  
380 detected.

381 The nearly exclusive presence of highly degraded polystyrene and polyolefins as the polymeric  
382 material retrieved by sieving at <2 mm size and exhaustive solvent extraction (and thus would go  
383 nearly undetected by the usual mechanical or density sorting procedures<sup>39</sup>) suggests the occurrence  
384 of preferential transport, and eventually of deposition ashore, of the lower density fully hydrocarbon  
385 polymers, as opposed to other polymeric materials (polyesters, acrylic fibers, polyamide, etc.). The  
386 latter, once dispersed in a water compartment, may end up more easily and quickly in deep  
387 sediments rather than being transported ashore.<sup>40</sup>

388

### 389 **Molecular weight and molecular weight distribution**

390 Further insights on the level of polymer degradation was obtained from gel permeation  
391 chromatography (GPC) analyses. Two different GPC column sets were used in twofold analyses  
392 performed on each sample, each set being suited for the separation and molecular weight (MW)  
393 evaluation of in the higher and lower MW range, respectively, as summarized in Table 2.

394 In Figure 6, the elution profiles of representative foreshore and summer berm samples present  
395 almost exclusively a broad and structured peak at high retention times (RT), associated with highly  
396 degraded, low MW material with either a highly aromatic or a highly oxidized hydrocarbon  
397 structure, or both, as highlighted by the intense signal from both the RI (concentration dependent)  
398 and UV<sub>254</sub> (structure and concentration dependent) detectors. The much weaker RI signal at lower  
399 RT, with an even weaker or absent UV<sub>254</sub> signal, suggests that the samples most exposed to the surf  
400 washout retained mainly the low molecular weight, highly oxidized fraction, possibly because of  
401 effective adsorption onto the sand particle surface, while comparatively larger and less oxidized  
402 high MW material is removed by the wave activity.

403

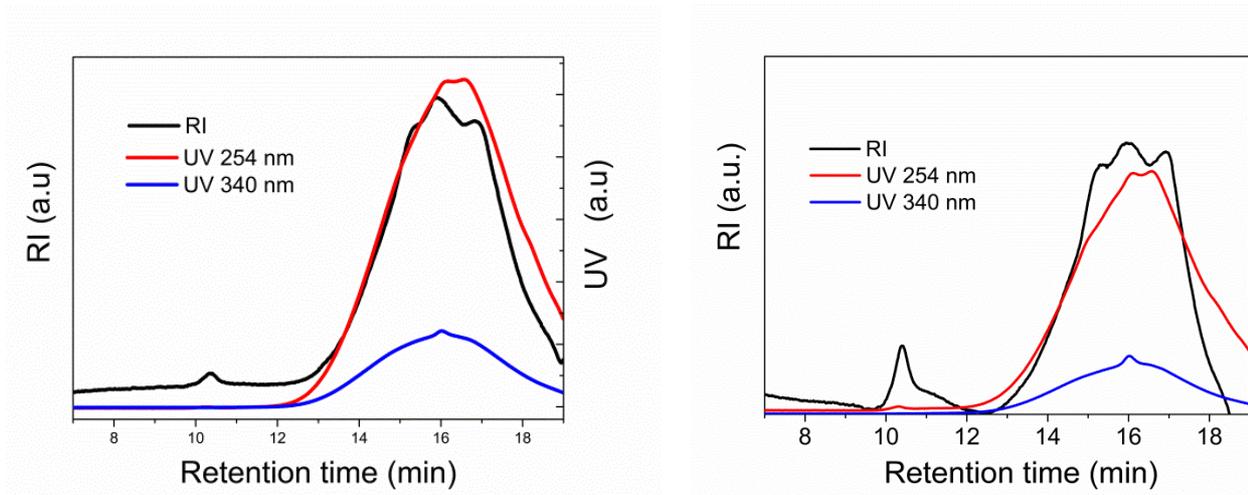
404 **Table 2.** Results from the GPC analyses on the DCM extracts.

Sampling sector	Sample code	High RT <sup>(a)</sup>		Low RT <sup>(b)</sup>	
		$\bar{M}_w$ (kDa)	PDI <sup>(c)</sup>	$\bar{M}_w$ (kDa)	PDI <sup>(c)</sup>
A (Dunes)	G3040001	1.1	2.46	160.1	1.86
A (Dunes)	G3040002	0.2	1.97	154.2	2.12
A (Dunes)	G3040012	1.3	1.80	73.4	2.70
A (Dunes)	G3040016	0.9	1.98	128.2	2.02
B (Backshore-winter berm)	G3040003	1.4	3.00	111.3	1.74
B (Backshore-winter berm)	G3040004	1.1	2.73	n.d.	-
B (Backshore-winter berm)	G3040011	0.9	2.27	85.5	1.98
B (Backshore-winter berm)	G3040015	0.7	2.18	173.4	1.76
C (Backshore-summer berm)	G3040005	1.0	2.45	n.a.	-
C (Backshore-summer berm)	G3040007	1.0	2.29	n.a.	-
C (Backshore-summer berm)	G3040010	1.2	2.78	n.a.	-
C (Backshore-summer berm)	G3040014	0.9	2.51	n.a.	-
D (Foreshore)	G3040008	1.1	2.68	n.a.	-
D (Foreshore)	G3040009	1.0	2.58	n.d.	-
D (Foreshore)	G3040013	0.9	2.53	n.d.	-
D (Foreshore)	G3040006	1.0	2.57	n.d.	-

405 a) high retention time, low molecular weight fraction analyzed with Mixed-D GPC columns.

406 b) low retention time, high molecular weight fraction analyzed with Mixed-D GPC columns.

407 c) polydispersity index



408 **Figure 6.** GPC profiles (Mixed-E columns, low molecular weight range) of DCM extracts from the  
 409 foreshore (left) and the summer berm (right) sand samples (the Mixed-D column set was not used  
 410 for these samples since only a minor fraction of high MW polymeric material was detected).

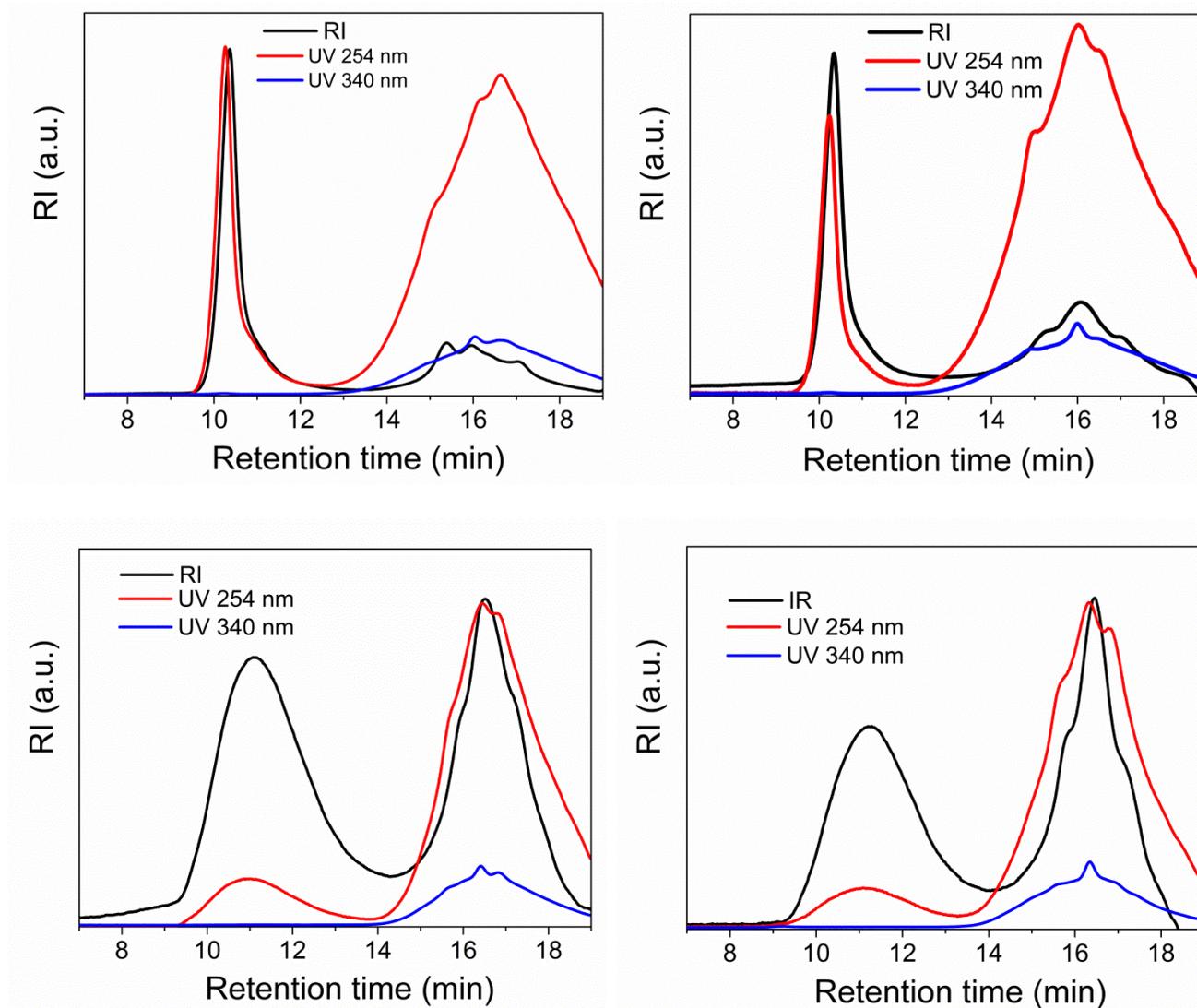
411

412 The GPC profiles of representative winter berm and dune samples (Figure 7) present a well  
 413 resolved additional broad peak at low RT with comparably intense signals from RI and UV<sub>254</sub>  
 414 detectors, suggesting the presence of high MW partially oxidized PS. The large polydispersity index  
 415 (PDI) from both the high MW (mostly polystyrene and its oxidized derivatives) and the low MW  
 416 (polyolefin and polystyrene oxidized derivatives ) fractions may be considered as the result of the  
 417 heterogeneity of the source of pristine polymers. In fact, random chain cleavage occurring upon  
 418 oxidative degradation of polyolefins is typically accompanied by a PDI decrease, consistent with  
 419 the PDI of the low MW fraction still lower than those typically found for virgin polyolefins.

420 The actual composition of the two fractions of the dune sample G3040012 was investigated by  
 421 collecting separately the low RT and the high RT eluates ( $\bar{M}_w=73$  kDa and  $\bar{M}_w=1.3$  kDa with  
 422 highest threshold of about 10 kDa, respectively, table 2). The pyrolytic profile and MS  
 423 fragmentation pattern obtained in the pyrolysis-GC/MS analysis of the low RT, high MW fraction  
 424 showed the presence of PS only, in agreement with the strong signal of the UV<sub>254</sub> GPC detector. On  
 425 the other hand, the Py-GC/MS analysis of the high RT, low MW fraction indicated the presence of

426 oligomeric products of partial degradation from both PS and PE. These results were confirmed by  
427 the FT-IR and <sup>1</sup>H-NMR analyses of the two GPC fractions (figure S6 and S7 in Supporting  
428 Information and comments therein).

429



430 **Figure 7.** GPC profiles (above: Mixed-E columns; below: Mixed-D columns) of DCM extracts  
431 from the winter berm (left) and the dune (right) sand samples. The narrower peak at low r.t. in the  
432 GPC traces recorded using the Mixed E column set is an artifact due to exclusion volume effects.

433

434 It is apparent that further, more in-depth and geographically distributed investigations are needed to  
435 evaluate the actual extent of this elusive form of pollution, and to assess if a clear correlation exists  
436 with the type and amount of larger plastic fragments collected at the same site.

437 However, the results presented here clearly show that the pollution of water basins by polymeric  
438 materials may still be largely underestimated. In fact, to date the scientific community and  
439 environmental agencies have mainly focused their research either on the concentration and  
440 distribution of macroscopic (>2 mm) objects and debris or on the potentially dangerous effects on  
441 the biota of micron- and submicron-sized particles. On the other hand, it is apparent that over 50  
442 years of uncontrolled dispersion of plastics in the environment must have resulted in accumulation  
443 of degraded polymers in both benthonic and shoreline sediments, a “hidden” reservoir of exogenous  
444 and potentially hazardous materials that are not easy to identify and quantify as they are  
445 intermingled within a complex and highly variable matrix.

446 It is worth stressing out that the proposed procedure cannot be intended as a general protocol for the  
447 exhaustive extraction and quantification of all types of plastics and plastics degradation products.  
448 Nonetheless, it was shown to be sufficiently selective, reliable and accurate for the purpose of  
449 qualitative and semi-quantitative determination of smaller polymer fragments accumulating  
450 preferentially at the seashore because of their low density (thus mainly from larger polyolefin and  
451 polystyrene items). The analytical data indicate that slow fragmentation and chemical degradation  
452 of this polymeric fraction may results in up to 30 mg/kg of microplastics (including a large portion  
453 of weathered polymeric or oligomeric species) where long term accumulation occurs, that is beyond  
454 the swash zone, corresponding to about 5.5 g (or half a 500 mL plastic bottle) within just the upper  
455 10 cm layer of a square meter of sandy beach !

456 Higher density polymers, such as polyester, polyamide and PVC, are more likely to end up in the  
457 benthic sediment rather than on the shoreline ones and will be targeted in a forthcoming paper.  
458 Finally, further concern should arise from the fact that microplastics, because of their extremely  
459 large and increasingly polar surface area, may also effectively stick or adsorb onto sand particles,

460 and act as effective POPs (pesticides, chlorinated and polycyclic aromatic compounds)  
461 accumulators. Further investigations now in progress will deal with these latter issues.

462

#### 463 **ACKNOWLEDGEMENTS**

464 The University of Pisa supported this research through the PRA 2017\_17 program.

465

#### 466 **SUPPORTING INFORMATION.**

467 Geopositioning of sampling site, raw data on extraction procedures, FTIR spectra and Py-GC/MS  
468 chromatograms of reference polymers, list of collected larger (> 2 mm) plastic fragments, FTIR and  
469 <sup>1</sup>H-NMR spectra of low and high MW polymer fractions from chromatographic separation.

470

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