1 # Novel methodology to isolate microplastics from

2 # vegetal-rich samples

3 Alicia Herrera[a*], Paloma Garrido-Amador[a], Ico Martínez[a], María Dolores Samper[b], Juan López-

4 Martínez[b], May Gómez[a] and Theodore T. Packard[a]

5 [a] Marine Ecophysiology Group (EOMAR), Iu-ECOAQUA, Universidad de Las Palmas de Gran

6 Canaria, 35017 Campus Universitario de Tafira, Canary Islands, Spain.

7 [b] Instituto de Tecnología de Materiales (ITM), Universitat Politècnica de València (UPV), Plaza

8 Ferrándiz y Carbonell s/n, 03801, Alcoy (Alicante).

9

10 E-mail addresses: alicia.herrera@ulpgc.es (A. Herrera), palomagarridoamador@gmail.com (P.
11 Garrido-Amador), ico.martinez@ulpgc.es (I. Martínez), masammad@upvnet.upv.es (M. D.
12 Samper), jlopezm@mcm.upv.es (J. López-Martínez), may.gomez@ulpgc.es (M. Gómez),
13 theodore.packard@ulpgc.es (T. T. Packard).
14

15 *** Corresponding author. E-mail address: alicia.herrera@ulpgc.es** (A. Herrera)

16

17 ABSTRACT

18 Microplastics are small plastic particles, globally distributed throughout the oceans. To properly study
19 them, all the methodologies for their sampling, extraction, and measurement should be standardized.
20 For heterogeneous samples containing sediments, animal tissues and zooplankton, several procedures
21 have been described. However, definitive methodologies for samples, rich in algae and plant material,
22 have not yet been developed. The aim of this study was to find the best extraction protocol for vegetal-
23 rich samples by comparing the efficacies of five previously described digestion methods, and a novel
24 density separation method. A protocol using 96% ethanol for density separation was better than the five
25 digestion methods tested, even better than using H_2O_2 digestion. As it was the most efficient, simple,
26 safe and inexpensive method for isolating microplastics from vegetal rich samples, we recommend it as
27 a standard separation method.

28 KEYWORDS: Marine litter; microplastics; plastic extraction; density separation; organic material;
29 beach.

30 INTRODUCTION

31 Plastics are synthetic organic polymers with features, such as durability and low price, that
32 make them perfect for many applications. Unfortunately, the same characteristics that makes plastic the
33 perfect material cause it to become a serious pollution problem. Recent studies report that 4.8 to 12.7
34 million metric tons of plastic were disposed to the ocean in 2010 (Jambeck et al., 2015). At present,
35 plastic marine pollution is one of the major concerns of the scientific community and organizations
36 responsible for environmental policies at the global level (Andrady, 2011, 2010; European Parliament,
37 2008; Galgani et al., 2010, 2013; Scientific and Technical Advisory Panel, 2011).
38

39 Plastic particles smaller than 5 mm are classified as microplastics (Arthur et al., 2009).
40 Secondary microplastics are the product of degradation and fragmentation of larger plastics, while
41 primary microplastics are manufactured with size less than 5 mm, mainly for use in cosmetics, cleaning
42 products or as raw material for the production of plastic products (pre-production pellets). Due their
43 small size, microplastics can impact marine organisms including zooplankton. They can be ingested
44 directly or indirectly through the food web (Barnes et al., 2009; Setälä et al., 2014). Their consumption
45 is likely to constitute a chemical, physical, and biological hazard (Browne et al., 2008; Setälä et al.,
46 2014; Teuten et al., 2009; Von Moos et al., 2012; Wright et al., 2013; Zettler et al., 2013).
47

48 To obtain reliable and reproducible data on microplastic contamination and to investigate its
49 effects on marine biota and the environment, it would be beneficial to first harmonize and standardize

50 the sampling, extraction, and quantification methods that are being used by the scientific community
51 (MSDF Technical Subgroup on Marine Litter, 2013; Rochman et al., 2017). Sampling techniques, and
52 analytical techniques to isolate and quantify microplastic samples from different environments, have
53 been reviewed extensively (Besley et al., 2017; Hanvey et al., 2017; Hidalgo-Ruz et al., 2012; Lusher
54 et al., 2017; Miller et al., 2017; Van Cauwenberghe et al., 2015). For microplastics extraction, most
55 techniques are based on density separation via flotation (Claessens et al., 2013; Cole et al., 2015;
56 Coppock et al., 2017; Imhof et al., 2012; Thompson et al., 2004). Density separation requires highly
57 dense solutions, such as sodium chloride (NaCl, 1.2 g/cm^3), sodium iodide (NaI, 1.6 g/cm^3) and zinc
58 chloride ($ZnCl_2$, 1.6-1.7 g/cm^3) because the specific densities of the most common plastics in
59 environmental samples range from 0.01 g/cm^3 to 1.60 g/cm^3 (Table 1). Other separation strategies for
60 microplastics include evaporation, filtration, sieving, and visual sorting (Crawford and Quinn, 2017;
61 Hidalgo-Ruz et al., 2012; Masura et al., 2015; Song et al., 2014; Yamashita and Tanimura, 2007).
62 These techniques are useful for isolating microplastics from sediments, but isolating them from
63 biological material requires a different treatment. The density of the biological material (leaves, seeds,
64 wood, etc.) is, in most cases, lower than the density of the solutions used in the separation process, and
65 therefore they float together with microplastics. Another problem is that microplastics are imbedded in
66 the organic material and cannot be isolated by density only.

67 Several digestion techniques for the removal of the organic material in microplastic samples
68 have been described (Catarino et al., 2017; Claessens et al., 2013; Cole et al., 2014; Dehaut et al.,
69 2016). Many of them were specifically designed to be effective in extracting microplastics from animal
70 tissue or zooplankton. However, techniques for digesting the algal and plant component of sediment
71 samples have not been developed (Hanvey et al., 2017). This type of biological material is abundant in
72 beach samples, and can even retain microplastics on its surface (Gutow et al., 2015). Finding a way to

73 separate microplastics from this vegetal material is thus important to assess the extent of microplastic
74 pollution in the aquatic environment. A recent study suggested that dried algae and seagrasses, among
75 other residues present in the microplastic samples, could be removed by visual sorting or sieving, using
76 the naked eyed or a microscope (Crawford and Quinn, 2017; Hidalgo-Ruz et al., 2012). These
77 procedures may be acceptable for the biggest fragments, for large pieces of algae and leaves, and for a
78 small number of samples. However, for the smaller particles and for a large number of samples, these
79 procedures are time consuming and are likely to lead to underestimating the extent of microplastics
80 pollution.

81 The objective of the present work was to find an efficient method to remove algae and plant
82 material from microplastics samples. In order to achieve this, five existing digestion protocols based on
83 HCl, NaOH, KOH and H_2O_2 treatments, and a novel density separation procedure using 96% ethanol
84 (EtOH), were tested, and their separation efficacies were calculated and compared. In addition, the
85 integrity of six types of plastic polymers (polypropylene (PP), polyethylene (PE), polyvinyl chloride
86 (PVC), polyurethane (PUR), polyethylene terephthalate (PET; polyester fibers), and polystyrene (PS))
87 subjected to the different methodologies was studied in order to confirm that these methods do not
88 damage plastic particles.

89 MATERIALS AND METHODS

90 **Sampling collection and preparation**

91 A one-liter sample was collected along the high tide line near the dunes at Famara beach, Lanzarote,
92 Spain (N 29°6.941, W 13°33.461), on January 29th, 2016 (Fig. 1a). The sample was placed in a 5 L
93 plastic container and mixed for 1 min with 3 L of sea water from the same beach. The supernatant fluid
94 was then filtered through a 1 mm aperture mesh. No measures to prevent contamination were taken

95 during sampling, because we did not have to determine the exact concentration of microplastics, but
96 only had to obtain a representative sample. After separation of the samples in the laboratory, measures
97 were taken to avoid contamination. All the procedures were done inside a fume hood. All personnel
98 wore cotton laboratory coats. In addition, all the materials used, as well as the workplace, were cleaned
99 with ultrapure water. The sample was always well protected to avoid contamination in the laboratory.
100 However, to evaluate contamination, should it occur, two clean filters were exposed during the
101 digestion procedures and density separation. They were then examined immediately after each
102 procedure under a microscope. No contamination was found on any of them.

103 **HERE SAMPLINGCOLLECTION.KMZ**

104 **SAMPLING COLLECTION**

105 The sample was composed of organic matter (mainly vegetal debris) at a concentration (w/w) of 1/6
106 and of microplastics, 5/6 (Fig. 1b). In order to avoid differences in the separation efficiencies due to the
107 different amounts of organic material present in the samples, we homogenized the sub-samples. To
108 accomplish this, the microplastics and organic matter were manually separated. Then, replicate sub-
109 samples of 6 g each, composed of 1 g of biological material and 5 g of microplastics, were taken (Fig.
110 2). Before being subjected to each of the protocols, the sub-samples were oven-dried at 60ºC and
111 weighed on a high precision balance (0.1 mg). When we were able to confirm that the treatment used
112 was safe for plastics, we were certain that any "weight loss" was due to digestion or separation of
113 organic matter.

114 **Separation efficacy**

115 Five existing protocols to digest organic matter were tested for vegetal rich samples: 3% HCl,
116 40% NaOH, 4% NaOH + SDS, 10% KOH and catalytic 30% H_2O_2 (Chemical solutions information in

117 Table 2). In addition, density separation by 96% EtOH (16.44 M) was tested (Table 2). Triplicates of
118 sub-samples composed of 1 g of biological material and 5 g of microplastics were processed with each
119 protocol.

120 **Protocol 1** corresponded to the acid digestion method tested by Cole et al. (2014). The sample was
121 previously oven-dried at 60 ºC, then 40 ml of 3% HCl (1 M) were added to sub-samples, they were
122 stirred for a minute, and finally, maintained at room temperature (20 ºC) for 24 h.

123 **Protocol 2** was based on the alkaline digestion method tested by Cole et al. (2014). As above, the
124 sample was previously oven-dried at 60 ºC, then 40 ml of 40% NaOH (10 M) were added to sub-
125 samples, they were stirred for 1 minute, and finally placed in an oven for 24 h, at 60 ºC.

126 **Protocol 3** was adapted from Dehaut et al. (2016), and consisted of alkaline sample digestion. The
127 sample was previously oven-dried at 60 ºC, then 40 ml of 10 % KOH (1.78 M) were added to the sub-
128 samples, they were stirred for 1 minute and maintained at 60 ºC for 24 h in a drying oven.

129 **Protocol 4** is based on the work of Budimir (2016), presented at MICRO 2016 International Congress.
130 In this protocol, less concentrated NaOH was added to samples together with the detergent, SDS.
131 Budimir describes an alkaline digestion procedure in which 10 ml of 4% NaOH (1M) and 5 ml of SDS
132 are added to the sub-samples, and in which only 2 hours at 50 ºC were enough to digest the biological
133 material in the samples. The original protocol was modified in order to standardize all the procedures
134 followed here. This was done by oven-drying the sample at 60 ºC, adding 40 ml of NaOH and 20 ml of
135 SDS, and mantaining it for 2 h in an oven at 50 ºC. If no visual changes were observed in the sub-
136 samples, they were maintained for 24 h at 60 ºC.

137 **Protocol 5** was based on the Wet Peroxide Oxidation (WPO) method described by Masura et al.
138 (2015). Here, only the WPO step was carried out despite Masura et al. (2015) describing several other

139 steps for the analysis of microplastics on beach sediment samples. The sample was previously oven-
140 dried at 60 ºC, 40 ml of aqueous 0.05 M Fe(II) were added to a large beaker (~800 ml) containing the
141 sample, followed by 40 ml of 30% H_2O_2 (9.79 M). After incubating five minutes at room temperature,
142 the mixture was heated to 75 ºC on a hotplate for 30 minutes. **CAUTION**: this solution can boil violently
143 if heated >75 ºC. Avoid this condition. If biological material remained in the mixture after that time,
144 another 40 ml of hydrogen peroxide should be added. In this work, on three occasions, more hydrogen
145 peroxide was added to the sub-samples.

146 **Protocol 6** was a novel method based on density separation by ethanol (see graphical abstract). The
147 sample was previously oven-dried at 60 ºC. Forty ml of 96% (v/v) EtOH (16.44 M) were added to
148 samples. They were then stirred at 600 rpm for 3 minutes and settled for 1 minute. This allowed the
149 separation of microplastics from imbedded organic material. Concentrated EtOH, at 96% has a density
150 of 0.8 g/cm^3 (at 20 °C). This is lower than the most common plastics found in samples, except for some
151 polystyrene polymers (PS), expanded foam (EPS), extruded foam (XPS) and polyurethane foam (PUR)
152 (see table 2). If the density of the biological material present in the samples is lower than 0.8 g/cm^3, the
153 biological material will float with the polystyrene and polyurethane foams while the heavier plastics
154 will sink (Figure 3). PS and PUR foams should be identified by visual detection and removed with
155 forceps. After density separation, the supernatant was removed and the remanant sample was filtered.
156 **NOTE**: use glass containers because poly(methyl methacrylate) (PMMC) can be chipped in contact
157 with 96% EtOH.

158 After applying each treatment, samples were filtered through a Whatman® filter paper grade 4
159 (20-25 μm), oven-dried at 60 ºC and weighed on a high precision balance (0.1 mg). The efficiency of a
160 digestion protocol depends on the relative removal of organic mass during the digestion procedure. If
161 the method validation showed that plastic particles were not degraded or damaged, then any difference

162 between the samples weight, before and after being exposed to the protocols, was attributed to a loss of
163 biological material. The percentage separation efficacy (%Se) was calculated as:

164
$$\%Se = \frac{T_0 - T}{B_0} \times 100$$

165 where B_0 is the initial dry mass of biological material, T_0 is total dry mass before exposure, and T is
166 total dry mass after exposure.

167 **Statistical analysis**

168 Statistical analyses and graphics of digestion efficacies were performed with R statistical
169 software (R Core Team, 2017) and its extension, RStudio. Data normality was confirmed by the
170 Kolmogorov-Smirnov test and data homoscedasticity was assessed graphically. ANOVA and Tukey
171 post-hoc tests were applied to determine significant differences among protocols. The results were
172 represented in box plots.

173 **Method validation**

174 Each protocol was tested on plastic particles selected according to European plastics demand:
175 polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene
176 terephthalate (PET; polyester fibers), and polystyrene (PS) (PlasticsEurope, 2015). Pre-production
177 pellets were also included because they are very abundant in Canary Islands beach samples and have
178 importance in the study of marine debris (Herrera et al., 2017). A sub-sample of 7 pre-production
179 pellets collected from Famara beach were analyzed by Fourier transform infrared spectroscopy (FTIR)
180 and calorimetry to determine their composition using an infrared spectrometer (Perkin-Elmer Spectrum
181 BX from Perkin-Elmer Spain S.L., Madrid, Spain). 20 scans between 4000 and 600 cm^{-1} were
182 performed with a resolution of 32 cm^{-1} in the reflection mode. Differential scanning calorimetry (DSC)

183 was conducted in a Mettler-Toledo 821 calorimeter (Schwerzenbach, Switzerland) in air atmosphere,

184 the heating program was from 30 to 300 ºC at a heating rate of 10 ºC min^{-1} (Details in Appendix A).

185 Five pellets, and five small pieces less than 5 mm of each type of plastic polymer (PP, PE, PV,

186 PUR, PS and PET), were subjected to protocols 1 to 6 (Table 3). Each experiment was conducted in

187 triplicate. Microplastics were visually inspected under a stereomicroscope, counted, measured and

188 photographed before and after experimentation (t1 and t2, respectively). Recovery rates were calculated

189 for pellets and fragments of polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC),

190 polyurethane (PUR) and polystyrene (PS). Polyester (PET) fibers were not counted, only visually

191 inspected for changes.

192 Microplastics´ images were compared among t1 and t2 in order to detect changes in colour,

193 size, number, and shape, to determine "destructive" effects of digestion procedures (Cole et al., 2014;

194 Dehaut et al., 2016; Nuelle et al., 2014). Using the software ImageJ 1.50b, microplastics length and

195 area were digitally measured, and colour histograms were plotted (Appendix A, Fig. 4).

196 RESULTS

197 Sampling collection and preparation

198 The biological material was mainly composed of vegetal debris composed of leaves, seeds,

199 wood, seaweed, and seagrasses (Fig. 2b). Seagrass *Cymodocea nodosa* and algae *Sargassum vulgare*

200 were identified in the sample. Other material like leaves, seeds and wood pieces could not be

201 identified.

202 Separation efficacy

203 Visual examination of all the samples after the extraction procedures (Fig. 4) revealed
204 qualitative differences, especially among samples subjected to protocol 6. Biological material remained
205 almost the same after applying protocols 1, 2 and 3, while the material exposed to protocols 4 and 5
206 was partially digested. However, the density separation by the 96% EtOH (protocol 6) showed that
207 microplastics were separated almost completely from biological material (Fig. 4f). The ANOVA test
208 revealed significant differences among the separation efficacies of protocols ($F=140.6$; 5 df; p-value $<$
209 0.001). A Tukey post-hoc test showed no significant differences between efficacies of protocols 1, 2
210 and 3 (p-value $>$ 0.01) (Fig. 5). The separation efficacy (*%Se)* showed significant differences, ranging
211 from 9 to 97% (means and standard deviation are presented in Table 4). Protocols 1, 2, 3 and 4, were
212 not efficient at digesting algae and plant debris, with the mean *%Se* ranging from 9 to 40.9%.
213 Protocol 5 was the one that obtained a greater digestion efficiency, with an average *%Se* of 64.6
214 $\pm$ 7.1%, but the highest *%Se* and the most efficient separating microplastics from algae and plant
215 material was protocol 6. This simple procedure incorporated density separation using 96% EtOH. After
216 the ethanol addition, an average of 97% of biological material floated and separated from the
217 microplastics that had sunk to the bottom. Polystyrene extruded foam and expanded foam floated along
218 with the biological debris, but were easily detected and removed from the sample with forceps.

219 **Method validation**

220 From the analyzed pellet sub-sample, 6 pellets were identified as polyethylene (PE) and 1 pellet
221 was identified as polypropylene (PP) (Detailed results can be found in Appendix A).

222 The recovery rates were 100% in all treatments for pellets and fragments of polypropylene (PP),
223 polyethylene (PE), polyvinyl chloride (PVC), polyurethane (PUR) and polystyrene (PS). Polyester
224 fibers could not be recovered after subjected to 40% NaOH treatment (protocol 2). Microplastics were

225 successfully recovered after the experiments, and their colours, shapes and sizes remained intact,
226 except for fibers subjected to protocol 2. Changes in shape, size and colour were observed in polyester
227 fibers subjected to this protocol (40% NaOH) by visual examination and by comparing colour
228 histograms before and after treatments (Fig. 6).

229 DISCUSSION

230 Claessens et al. (2013) developed a nitric acid digestion-based method for animal tissue using
231 22.5 M HNO_3 to digest mussels (The HNO_3 concentration reported by Claessens et al. (2013) was 22.5
232 M (~ 95%). This is probably an error in the manuscript). It employed overnight organic matter
233 oxidation at room temperature, followed by 1 h heating at 60 ºC and by 1 h boiling at 100 ºC, and
234 finally a warm filtration (aprox. 80 ºC). This acidic digestion technique resulted in high digestion
235 efficacies of tissues, but when tested for polystyrene spheres and nylon fibers, nylon rope fibers could
236 not be recovered. Other authors have also reported damage to plastic particles using HNO_3 digestion
237 (Avio et al., 2015; Catarino et al., 2017; Dehaut et al., 2016). For this reason, although several nitric
238 acid methods have been recently used (De Witte et al., 2014; Van Cauwenberghe and Janssen, 2014;
239 Vandermeersch et al., 2015), here, because they cause damage, nitric acid methods were not included.

240 An alternative to strong-acid digestion is the use of non-oxidizing acids or alkaline hydrolysis.
241 Cole et al. (2014) compared the use of HCl at concentrations of 1 M and 2 M, NaOH at different
242 concentrations (1 M, 2 M, 5 M, 10 M) and the enzyme, Proteinase-K, in digesting marine plankton in
243 samples containing polyethylene, polyester, nylon, polystyrene and unplasticised polyvinyl chloride
244 (uPVC) based microplastics. Methodologies using 1 M (3%) HCl and 2 M (6%) HCl to digest
245 zooplankton had the lowest efficacies of 82.6% and 72.1% respectively (Cole et al., 2014). In the study

246 of Nuelle et al. (2014), none of the biogenic organic particles like chitin carapaces or leaves had
247 dissolved or were discoloured, even at a higher concentration (20% HCl) than the one used here.

248 Methodologies using NaOH, however, have shown a wide range of efficacies (Claessens et al.,
249 2013; Cole et al., 2014; Nuelle et al., 2014) depending on the concentration and the procedure
250 followed. The optimized alcaline digestion protocol proposed by Cole et al. (2014) (NaOH 40% during
251 24 hs at 60ºC) showed a digestion efficacy of 91.3%. However, after the treatment, several polyester
252 fibers were lost, nylon fibers were partially destroyed, and physical changes were observed in
253 polyethylene fragments and uPVC granules. In addition, Dehaut et al. (2016) reported degradation in
254 cellulose acetate (CA), polycarbonate (PC) and PET. The digestion efficacy of the same protocol used
255 by Cole et al. (2014) (protocol 2), in our samples was lower and varied from 3.4 to 14%. Furthermore,
256 damage in the polyethylene fibers was also detected. The higher efficacies, previously found by Cole et
257 al. (2014) were not found here when digesting vegetal material, probably due to the different
258 composition of the organic material. Biogenic matter of plant origin is composed mainly of cellulose,
259 hemicellulose and lignin, compounds that are very difficult to digest.

260 In protocol 4, we used less concentrated NaOH solution (4%) to avoid damage in fibers. As
261 previously shown by Budimir et al. (2016), the SDS detergent improved the 4% NaOH digestion
262 efficacy. We obtained a digestion efficacy of 40%, however, it was significantly lower than in
263 protocols 5 and 6. Finally, because 40% NaOH caused drastic changes in the color and shape of
264 polyethylene fibers (Fig. 6) and because of its low digestion efficacy, even with SDS, NaOH is not
265 recommended for digesting vegetal material.

266 The strong base, KOH, was also investigated. Foekema et al. (2013) used 10% KOH to dissolve
267 the contents of fish stomachs, intestines and esophagus. To completely dissolve the organic material, 2

268 or 3 weeks of incubation time was necessary (Foekema et al., 2013; Lusher et al., 2016; Tanaka and
269 Takada, 2016). This protocol was then modified and adapted by Dehaut et al. (2016), who shortened
270 the incubation time to 24 h and applied higher temperatures (60 ºC). They obtained high digestion
271 efficacies, ranging from 99.6% to 99.8%, when applying this protocol to mussels (*Mytilus edulis*),
272 velvet crabs (*Necora puber*) and black seabreams (*Spondyliosoma cantharus*) tissues. They also proved
273 that KOH had no detrimental effects on plastic polymers except cellulose acetate, which was altered in
274 shape and size after every digestion protocol tested (Dehaut et al., 2016). Similar results were found by
275 Kühn et al. (2017). They confirmed the resistance of most plastic polymers to KOH, with the exception
276 of cellulose acetate from cigarette filters, some biodegradable plastics, and a polyethylene sheet. The
277 10% KOH method described by Dehaut et al. (2016) has been used to digest organic matter in fish
278 gastrointestinal tracts and in shellfish tissues (Rochman et al., 2015; Štindlová et al., 2017). Here we
279 use the protocol modified by Dehaut due to the shortened digestion time and high digestion efficiencies
280 obtained. Long exposure of 2 or 3 weeks to 10% KOH could improve the digestion of vegetal organic
281 matter, but it is not suitable for large-scale beach monitoring programs due to the time needed to
282 process the samples. Protocol 3 (10% KOH) appeared promising before testing (Lusher et al., 2017),
283 but when applied to algae and plant material, its digestion efficacy was found to be lower (24 ± 2.8%)
284 than reported previously for animal tissues and for the gastrointestinal tract of fish. As has been
285 mentioned previously, this could be due to the different composition of plant and algae material, since
286 they contain cellulose, hemicellulose, and lignin that are more resistant to 10% KOH.

287 In summary, protocols 1 to 4 were inefficient in digesting vegetal material and were not
288 considered further.

289 Oxidizing treatments using hydrogen peroxide (H_2O_2) have been widely used in microplastics
290 studies (Avio et al., 2015; Dubaish and Liebezeit, 2013; Güven et al., 2017; Liebezeit and Dubaish,

291 2012; Majewsky et al., 2016; Mathalon and Hill, 2014; Mintenig et al., 2017; Nuelle et al., 2014; Tagg
292 et al., 2015; Zhao et al., 2017). Some of these studies (Liebezeit and Dubaish, 2012; Mathalon and Hill,
293 2014; Tagg et al., 2015) obtained high efficacies using H_2O_2 to digest biogenic and organic matter
294 without altering the microplastic polymer chemistry. Nuelle et al. (2014) compared different solvents
295 (H_2O_2, HCl and NaOH) to digest biogenic matter of animal and plant origin. In their studies, samples
296 of organic matter and microplastics, were subjected to 4 ml of different solvents (30% H_2O_2, 35%
297 H_2O_2, 20% HCl and NaOH (20, 30, 40 and 50%)) for 7 days. Results showed that with NaOH and HCl
298 solutions none of organic particles had dissolved completely or became transparent. However, both
299 30% H_2O_2 and 35% H_2O_2 solutions engendered visible changes in organic particles, mostly of animal
300 origin. According to Nuelle et al. (2014), after 7 days 35% H_2O_2 treatment, 92% of the biogenic
301 material had been dissolved completely or had lost its colour. As a result, this digestion procedure was
302 considered safe for plastic polymers. This method may be promising for digesting organic matter of
303 plant origin. However, we aim to improve it by finding a method that, in addition, reduces sample
304 processing time.

305 Avio et al. (2015) tested two methodologies using H_2O_2 (Avio´s protocols 4 and 6) for
306 extracting microplastics from the gastrointestinal tract of the fish mullet (*Mugil cephalus*). Avio´s
307 protocol 4 was based on 7 days digestion of dried samples in 30% H_2O_2, and Avio´s protocol 6 was a
308 new method based in a density separation with NaCl 1.2 g/cm^2 followed by digestion of organic matter
309 with 15% H_2O_2. They obtained extraction efficiences of 70% for the 30% H_2O_2 and 95% for the new
310 method. To validate the new protocol polyethylene and polystyrene particles were analyzed by FT-IR
311 before and after the extraction procedure. Their results have confirmed that microplastics were
312 efficiently extracted without any damage to the polymers (Avio et al., 2015). These findings cannot be
313 compared with our observations, because they did not report the digestion efficiency data. Furthermore,

314 we used the (WPO) method that, in addition to the 30% H_2O_2-based digestion, employs 0.05 M Fe (II)
315 as a catalyst. Masura et al. (2015) recommend this method as suitable for determination of
316 polyethylene, polypropylene, polyvinyl chloride, and polystyrene in organic-matter rich samples from
317 water, beach sediments and bed sediments. Recovery rates or digestion efficiencies were not reported.
318 Here, we tested this protocol because it reduced processing time and had been used successfully,
319 previously, to digest vegetal organic matter from beach samples (Masura et al., 2015), organic debris
320 from water samples (Free et al., 2014; Masura et al., 2015; McCormick et al., 2014) and wastewater
321 (Sutton et al., 2016). Masura et al. (2015) described a complete procedure to follow, from which we
322 only selected and tested the part corresponding to the WPO, since our objective was to test the
323 digestion of the organic material. The complete protocol might be more efficient than found here.

324 Dyachenko et al. (2017) test the effectiveness of the catalytic WPO procedure on non-plastic
325 contaminants, such as human hair, cotton clothing fibers, cigarette filters, and toilet paper fragments,
326 that are commonly found in wastewater. None of the contaminants analyzed, because they were
327 composed by cellulose fibers, were digested by the catalytic WPO method. Here, among the five
328 digestion protocols tested, protocol 5 (WPO), yielded the highest digestion efficacy, with values of 64.6
329 ± 7.1%. However, a high remnant of biogenic material were observed (Fig. 4b), probably due to the
330 fact that cellulose and other compounds were not digested, as has been demonstrated by Dyachenko et
331 al. (2017). These investigators proposed an optimized WPO method by performing a sequence of
332 catalytic WPO. After each digestion cycle the solution was filtered through a 0.125 mm sieve and then
333 rinsed with hexane (HPLC grade) three times followed by a rinse with deionized H_2O. The optimized
334 WPO method could probably improve digestion of plant organic matter, but it would also require more
335 processing time, which is a potential difficulty for the analysis of a large number of samples.

336 Proteinase-K treatment was 97% effective in digesting the plankton and did not damage the
337 microplastics (Cole et al., 2014). A recent study found digestion efficacies of 88% in animal tissues
338 using trypsin (Courtene-Jones et al., 2017). Enzymatic methods were not included in the present work
339 because of their high price. However, they apparently do not harm microplastics and yield high
340 digestion efficacies (Cole et al., 2014; Courtene-Jones et al., 2017; Lusher et al., 2017), but processing
341 many samples using an enzyme approach would not be cost-effective. Nevertheless, cellulase might be
342 a suitable alternative for the digestion of algae and vegetal material in case none of the other methods
343 proved sufficiently effective.

344 Finally, the novel methodology based on density separation, tested as protocol 6, succeeded in isolating
345 the microplastics except for the polystyrene and polyurethane foams (EPS, XPS and PUR). They were
346 recovered by visual detection and physical removal. This protocol did not damage any type of plastic
347 and, in addition, was inexpensive and required less time than the other protocols. In addition, according
348 to chemical resistance chart (Thermo Scientific Nalgene, 2018), 96% EtOH at 20°C does not cause
349 damage to most plastic polymers after 30 days of exposure. Damage was only reported in polyethylene
350 terephthalate copolymer (PETG) and the Flexible PVC after 7 days of exposure. Therefore, these
351 polymers should not be affected by a brief exposure of < 10 min. Immediate damage occurs only in
352 polymethyl methacrylate (acrylic) (PMMA) and styrene acrylonitrile (SAN), but these polymers are
353 rarely found in marine debris. Other chemical resistance charts (Bürkle GmbH, 2018; Curbell Plastics,
354 2013) showed a partial resistance of PE and PC to 96% EtOH exposure at 20°C, but not one of them
355 report the exposure time.

356 Furthermore, the 96% EtOH protocol was safe and did not require any specific equipment,
357 protocol 6 was therefore, considered the best option for the extraction of microplastics from vegetal-
358 rich samples. The density separation methods currently used are based on solutions with higher density

than most plastics polymers (NaCl (1.2 g/cm^3), NaI (1.6 g/cm^3), $ZnCl_2$ (1.6 1.7 g/cm^3)), these solutions are not suitable for the separation of organic matter, because they float together with plastics. This is why this method is proposed. It consists of using a 96% EtOH solution that is less dense than the density of most plastics, but denser than biogenic material of plant origin. This difference allows plastics to sink and organic matter to float, making their separation easy. This method is not effective in separating the sediment from the plastic, because both have a higher density than EtOH. This means that the 96% EtOH method should be used after separating the sediments by density using NaCl, NaI or $ZnCl_2$. The review of analytical techniques for quantifying microplastics in sediments, published by Hanvey et al. (2017), shows the importance of performing a matrix removal step. The authors indicate that organic matter is ubiquitous in sediment samples, however matrix removal was carried out in only 5 of 43 microplastic studies that they listed. According to the results obtained here, we recommend including a density separation step using 96% ethanol to remove vegetal matter. This step is suitable to be included in the protocols for extracting microplastics from beach samples, in order to harmonize the methodologies to meet the monitoring requirements of the European Marine Strategy Framework Directive (MSFD, 2008/56/EC).

FUNDING SOURCES

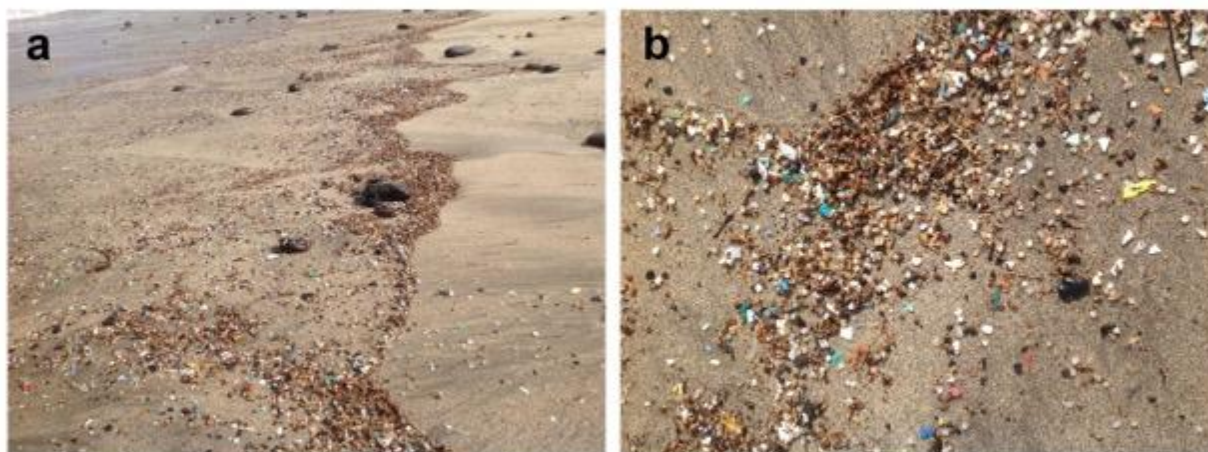
This work was funded by projects PLASMAR (MAC/1.1a/030), with the support of the European Union (EU) and co- financed by the European Regional Development Fund (ERDF) and the INTERREG V-A Spain-Portugal MAC 2014-2020 (Madeira-Azores-Canarias), MICROTROFIC (ULPGC2015-04) awarded to A.H. by ULPGC and BIOMAR (CEI-39-20162105-01) awarded to M.G. by CEI Canarias: Campus Atlántico Tricontinental. A.H. was supported by a postdoctoral fellowship granted by Universidad de Las Palmas de Gran Canaria (ULPGC-2014). T.T.P. was supported by

381 TIAA-CREF (USA), Social Security (USA), and Canary Islands CEI: Tricontinental Atlantic Campus
382 program.

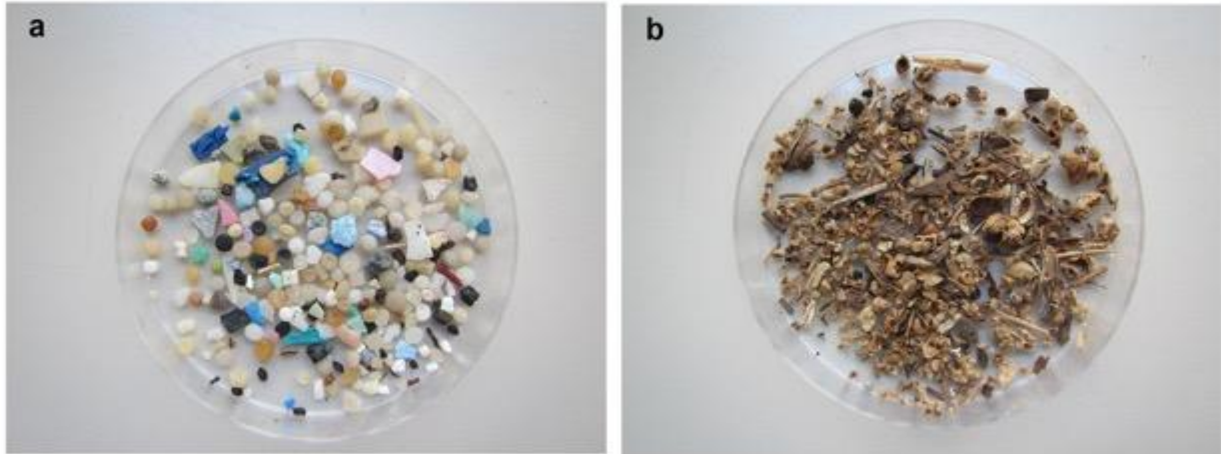
383 ABBREVIATIONS

384 B, Biological material; C_2H_6O, Ethanol; EtOH, Ethanol; EPS, Expanded foam; XPS, Extruded foam;
385 HCl, Hydrochloric acid; H_2O_2, Hydrogen peroxide; Fe(II), Iron (II); HNO_3, Nitric acid; CA, cellulose
386 acetate; PE, Polyethylene; PET, Polyethylene terephthalate; PETG, polyethylene terephthalate
387 copolymer; PMMA, polymethyl methacrylate (acrylic); PP, Polypropylene; PS, Polystyrene; PUR,
388 Polyurethane; PVC, Polyvinyl chloride; SAN, styrene acrylonitrile; KOH, Potassium hydroxide; SE,
389 Separation efficacy; NaCl, Sodium chloride; NaI, Sodium iodide; NaOH, Sodium hydroxide; SDS,
390 Sodium dodecyl sulfate; T, Total dry weight; uPVC, Unplasticized polyvinyl chloride; WPO, Wet
391 Peroxide Oxidation; $ZnCl_2$, Zinc chloride.

392 ARTWORK

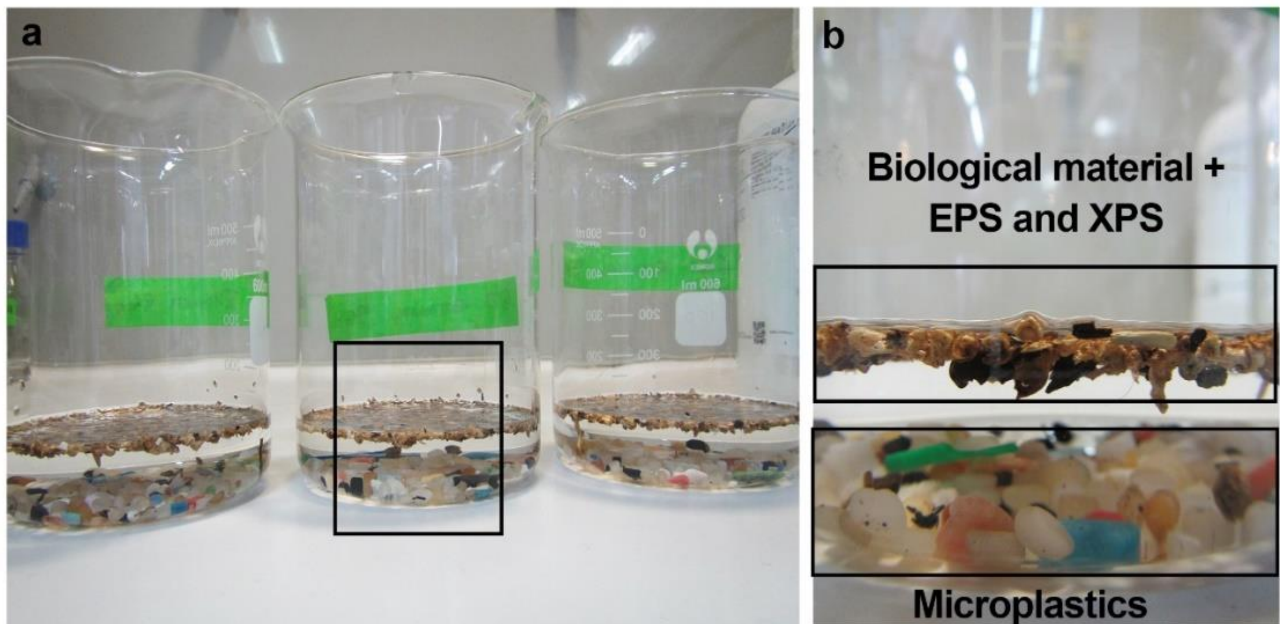


393 Figure 1. (a) Microplastic pollution from the high tide line in Famara, Lanzarote. (b) Detail of
394 microplastics and organic debris.

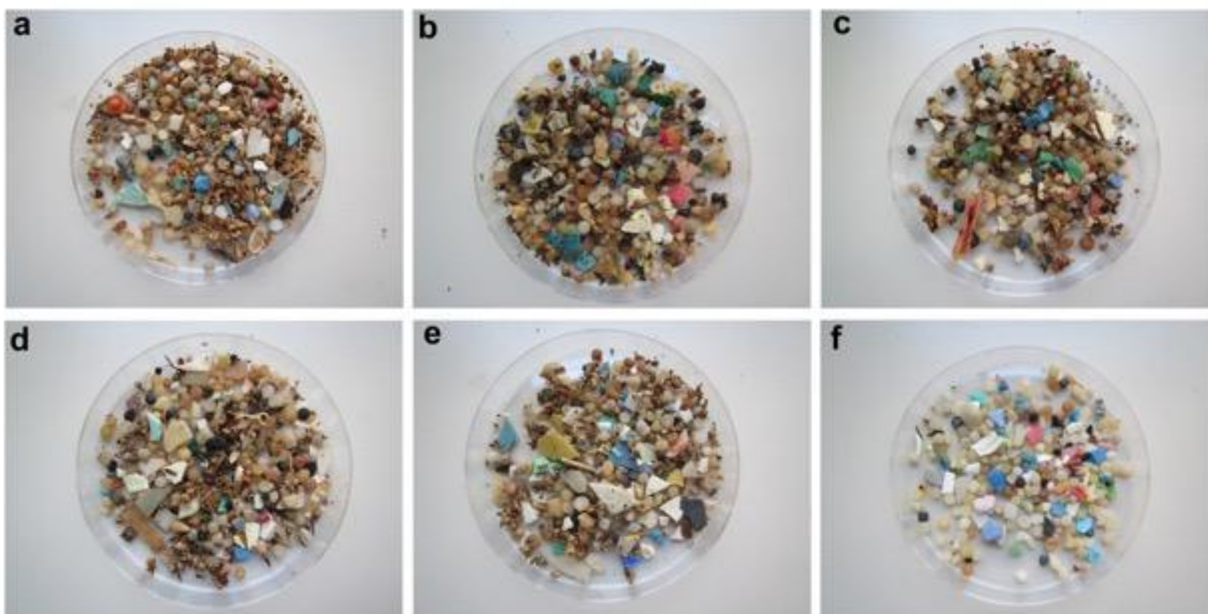


395

396 Figure 2. Samples were composed of (a) 5 g of microplastics and; (b) 1 g of biological material.



397 Figure 3. Density separation by 96% EtOH. (a) samples subject to protocol 6 (96% EtOH (b) shows the
398 organic matter floating together with the EPS and XPS foams, the rest of the microplastics are
399 deposited in the bottom.

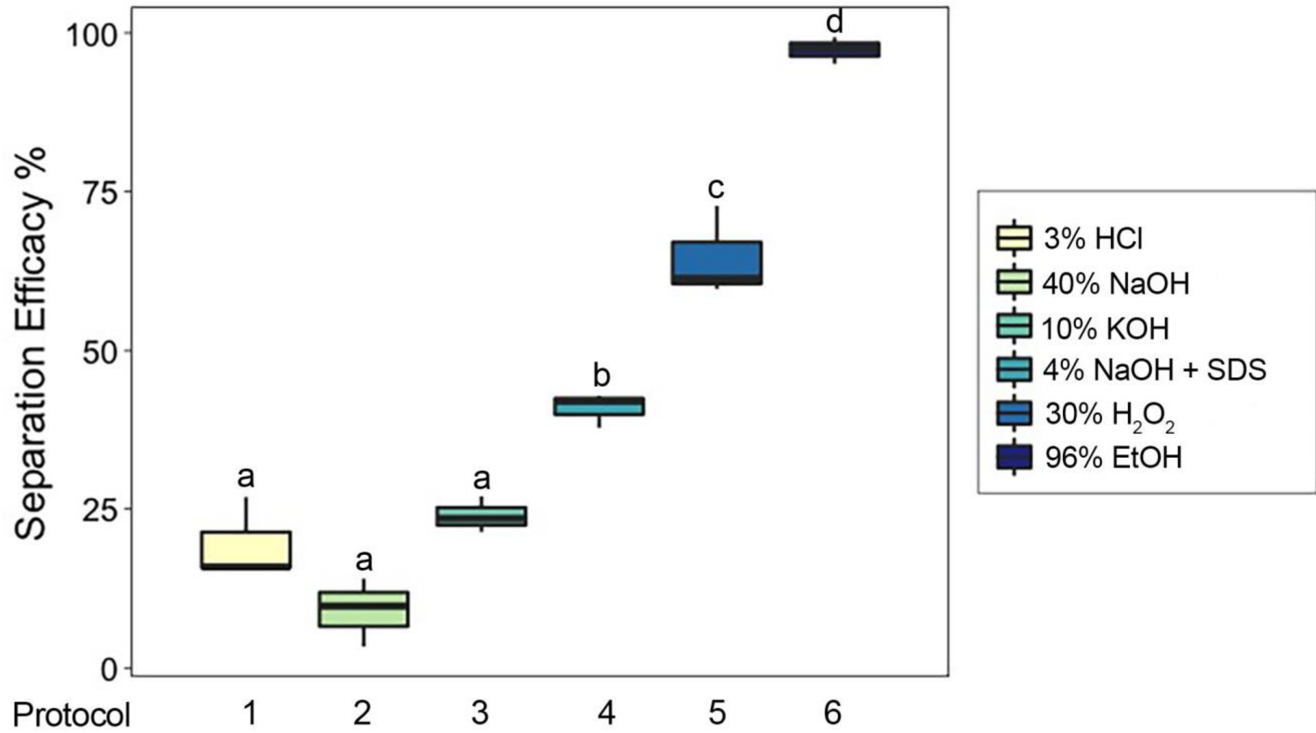


400 Figure 4. Microplastic samples that contained algae and plant debris after being subjected to: (a) HCl
401 protocol 1, (b) NaOH protocol 2, (c) KOH protocol 3, (d) NaOH+SDS protocol 4, (e) H_2O_2 protocol 5,
402 and (f) EtOH protocol 6.

403

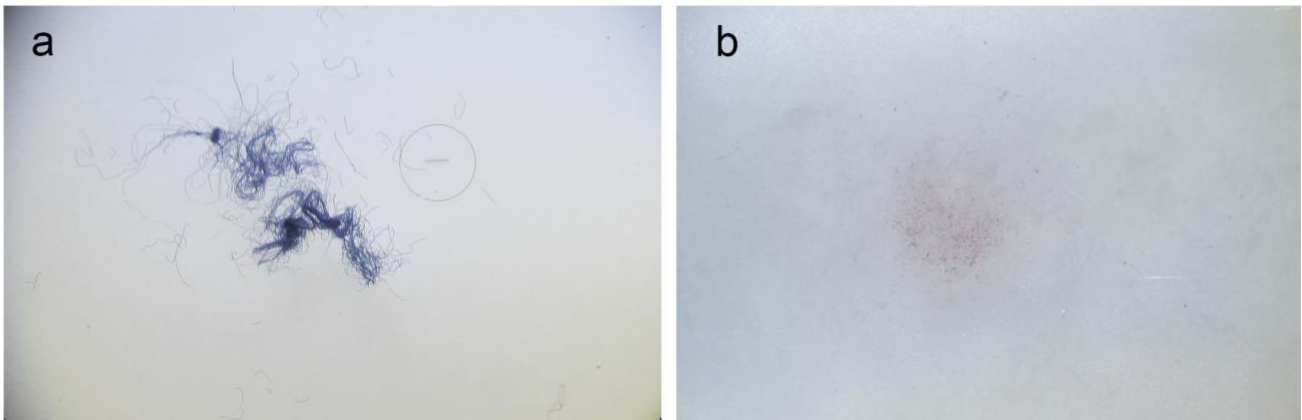
404

405



406 Figure 5. Separation efficacy (%) of the protocols. The central thick line of each box designates the
407 median, the box height shows the interquartile range, and the whiskers indicate the lowest and the
408 highest values. Different letters indicate significant differences (Tukey post-hoc test p-value < 0.01).

409



410 Figure 6. Photographs of polyester fibers, (a) before and (b) after being subjected to 40% NaOH
411 (protocol 2).

412 TABLES

413 Table 1. Density ranges of common plastic polymers (modified from Crawford and Quinn, (2017)) and

414 96% ethanol.

Plastic polymers	Abbreviation	Density in g/cm^3
Polystyrene (expanded foam)	EPS	0.01-0.05
Polystyrene (extruded foam)	XPS	0.03-0.05
Polypropylene	PP	0.88-0.91
Low-density polyethylene	LDPE	0.92-0.94
High-density polyethylene	HDPE	0.94-0.97
Nylon 6.6	PA	1.05-1.10
Polyvinyl chloride	PVC	1.45-1.70
Polyethylene terephthalate	PET	1.40-1.60
Polystyrene	PS	1.04-1.05
Polystyrene (30% glass fibers)	PS	1.40-1.50
Polyurethane	PUR	1.20-1.40
Polyurethane (foam)	PUR	0.03-0.80
Ethanol 96%	EtOH	0.805-0.812

415

416

417 Table 2. Protocols applied. Original units (in bold) for the digestion solutions have been converted to

418 their % (v/v or w/v) concentration equivalent.

Protocol	Methodology	Solutions	Concentration	Temp.	Exposure time	Adapted from
Protocol 1	Digestion	40 ml HCl (PANREAC 471020)	3% (v/v) HCl (**1 M**)	20 ºC	24 h	Cole et al. (2014)
Protocol 2	Digestion	40 ml NaOH (Scharlau SO0420)	40% (w/v) NaOH (**10 M**)	60 ºC	24 h	Cole et al. (2014)
Protocol 3	Digestion	40 ml KOH (Scharlau PO02660500)	10% (w/v) KOH (1.78 M)	60 ºC	24 h	Dehaut et al. (2016)
Protocol 4	Digestion	40 ml NaOH (Scharlau SO0420) 20 ml SDS (Acros	4% (w/v) NaOH (**1 M**) SDS 0.5% (w/v)	60 ºC	24 h	Budimir et al. (2016)

		Organic 226145000)	(17.34 mM)			
Protocol 5	Digestion	40 ml H_2O_2 (Panreac 121076)	30% H_2O_2 (w/v) (9.79 M)	75 ºC	30 min x3	Masura et al. (2015)
		40 ml Fe(II) $FeSO_4 \cdot 7H_2O$ (Panreac 131362) H_2SO_4 (VWR 20700.298)	0.05 M Fe(II) catalyst (7.5 g $FeSO_4 \cdot 7H_2O$; 500 ml H_2O; 3 ml H_2SO_4)			
Protocol 6	Density Separation	100 ml C_2H_6O (Scharlau ET00031000)	96% v/v EtOH (16.44 M)	20ºC	3 min	Present work

419

420 Table 3. Polymer types, colours, sizes (mm), original item and sources used for method validation
421 experiments.

Polymer	Colour	Size (mm)	Original product	Source
PE	Blue	1.50-3.48	Water bottle cap	Supermarket
PP	Green, orange	1.51-4.52	Plastic container	Supermarket
PS	White	1.8-4.55	Packaging	Supermarket
PA	Green, orange, blue	6.84-16.50	Fishing nets	Beach sample
Polyester	Dark blue	0.5-8	Textile fibers	Blanket
Resin pellets	White, transparent	4.42-6.12	Resin pellets	Beach sample

422

423 Table 4. Results of separation efficacy and recovery rates. Separation efficacy values (%Se) of the
424 protocols in algae and plant samples, displayed by means and standard deviation (mean ± SD). n/o= no
425 observed changes.

Procedure	Solution	Separation efficacy *(%Se)*	Impact on microplastics	Recovery rates (%)
Protocol 1	3% HCl	19.5 ± 6.4%	n/o	100% PE, PP, PS, PA, pellets
Protocol 2	40% NaOH	9.0 ± 5.4%	Damage to polyester fibers	100% PE, PP, PS, PA, pellets. Not recovery of polyester fibers
Protocol 3	10% KOH	24.0 ± 2.8%	n/o	100% PE, PP, PS, PA, pellets

Protocol 4	4% NaOH +SDS	40.9 ± 2.7%	n/o	100% PE, PP, PS, PA, pellets.
Protocol 5	30% H_2O_2	64.6 ± 7.1%	n/o	100% PE, PP, PS, PA, pellets.
Protocol 6	96% EtOH	97.3 ± 2.1%	n/o	100% PE, PP, PS, PA, pellets.

426

427 REFERENCES

428 Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605.
429 doi:10.1016/j.marpolbul.2011.05.030

430 Andrady, A.L., 2010. Proceedings of the Second Research Workshop on Microplastic Marine Debris.
431 NOAA Tech. Memo. 54.

432 Arthur, C., Baker, J., Bamford, H., 2009. Proceedings of the International Research Workshop on the
433 Occurrence, Effects, and Fate of Microplastic Marine Debris. Group 530.

434 Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and
435 characterization of microplastics in fish tissues: First observations in commercial species from
436 Adriatic Sea. Mar. Environ. Res. 111, 18–26. doi:10.1016/j.marenvres.2015.06.014

437 Barnes, D.K. a, Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of
438 plastic debris in global environments. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364, 1985–1998.
439 doi:10.1098/rstb.2008.0205

440 Besley, A., Vijver, M.G., Behrens, P., Bosker, T., 2017. A standardized method for sampling and
441 extraction methods for quantifying microplastics in beach sand. Mar. Pollut. Bull. 114, 77–83.
442 doi:10.1016/j.marpolbul.2016.08.055

443 Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested
444 microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.).
445 Environ. Sci. Technol. 42, 5026–5031. doi:10.1021/es800249a

446 Budimir, S., Lehtiniemi, M., Setälä, O., 2017. Microplastics Extraction Methods for Small Fishes, on
447 the Road to a Standard Monitoring Approach, in: Baztan, J., Jorgensen, B., Pahl, S., Thompson,
448 R.C., Jean-Paul Vanderlinden (Eds.), Fate and Impact of Microplastics in Marine Ecosystems.
449 Elsevier, pp. 58–59. doi:10.1016/B978-0-12-812271-6.00054-5

450 Bürkle GmbH, 2018. Chemical resistance of plastics [WWW Document]. URL
451 https://www.buerkle.de/files_pdf/wissenswertes/chemical_resistance_en.pdf (accessed 1.25.18).

452 Catarino, A.I., Thompson, R., Sanderson, W., Henry, T.B., 2017. Development and optimization of a
453 standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues.
454 Environ. Toxicol. Chem. 36, 947–951. doi:10.1002/etc.3608

455 Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for
456 the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull. 70,
457 227–233. doi:10.1016/j.marpolbul.2013.03.009

458 Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The Impact of Polystyrene
459 Microplastics on Feeding, Function and Fecundity in the Marine Copepod *Calanus helgolandicus*.
460 Environ. Sci. Technol. 49, 1130–1137. doi:10.1021/es504525u

461 Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of
462 microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 4528.
463 doi:10.1038/srep04528

464 Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M., Galloway, T.S., 2017. A small-scale, portable
465 method for extracting microplastics from marine sediments. Environ. Pollut. 230, 829–837.
466 doi:10.1016/j.envpol.2017.07.017

467 Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S.F., Narayanaswamy, B.E., 2017. Optimisation of
468 enzymatic digestion and validation of specimen preservation methods for the analysis of ingested
469 microplastics. Anal. Methods 9, 1437–1445. doi:10.1039/C6AY02343F

470 Crawford, C.B., Quinn, B., 2017. Microplastic separation techniques. Microplastic Pollut. 203–218.
471 doi:10.1016/B978-0-12-809406-8.00009-8

472 Curbell Plastics, 2013. Chemical Resistance Chart [WWW Document]. URL
473 https://www.curbellplastics.com/Research-Solutions/Technical-Resources/Technical-
474 Resources/Chemical-Resistance-Chart (accessed 1.25.18).

475 De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., Robbens, J.,
476 2014. Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial
477 and wild types. Mar. Pollut. Bull. 85. doi:10.1016/j.marpolbul.2014.06.006

478 Dehaut, A., Cassone, A.-L.L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G.,
479 Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood:
480 Benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233.
481 doi:10.1016/j.envpol.2016.05.018

482 Dubaish, F., Liebezeit, G., 2013. Suspended microplastics and black carbon particles in the Jade
483 system, southern North Sea. Water. Air. Soil Pollut. 224. doi:10.1007/s11270-012-1352-9

484 Dyachenko, A., Mitchell, J., Arsem, N., 2017. Extraction and identification of microplastic particles

485 from secondary wastewater treatment plant (WWTP) effluent. Anal. Methods 9, 1412–1418.
486 doi:10.1039/C6AY02397E

487 European Parliament, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17
488 June 2008 establishing a framework for community action in the field of marine environmental
489 policy (Marine Strategy Framework Directive. Off. J. Eur. Union 164, 19–40.

490 Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A. a, 2013.
491 Plastic in North Sea Fish. Environ. Sci. Technol. 47, 8818–8824. doi:10.1021/es400931b

492 Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High-levels
493 of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. 85, 156–163.
494 doi:10.1016/j.marpolbul.2014.06.001

495 Galgani, F., Hanke, G., Werner, S., De Vrees, L., 2013. Marine litter within the European Marine
496 Strategy Framework Directive. ICES J. Mar. Sci.

497 Galgani, Oosterbaan, L., Poitou, I., Hanke, G., Thompson, R., Amato, E., Janssen, C., Galgani, F.,
498 Fleet, D., Franeker, J. Van, Katsanevakis, S., Maes, T., 2010. Marine Strategy Framework
499 Directive: Task Group 10 Report Marine Litter., Group.

500 Gulmine, J. V., Janissek, P.R., Heise, H.M., Akcelrud, L., 2002. Polyethylene characterization by
501 FTIR. Polym. Test. 21, 557–563. doi:10.1016/S0142-9418(01)00124-6

502 Gutow, L., Eckerlebe, A., Gimenez, L., Saborowski, R., 2015. Experimental evaluation of seaweeds as
503 vector for microplastics into marine food webs. Environ. Sci. Technol. acs.est.5b02431.
504 doi:10.1021/acs.est.5b02431

505 Güven, O., Gökdağ, K., Jovanović, B., Kıdeyş, A.E., 2017. Microplastic litter composition of the

506 Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract
507 of fish. Environ. Pollut. 223, 286–294. doi:10.1016/j.envpol.2017.01.025

508 Hanvey, J.S., Lewis, P.J., Lavers, J.L., Crosbie, N.D., Pozo, K., Clarke, B.O., 2017. A review of
509 analytical techniques for quantifying microplastics in sediments. Anal. Methods 9, 1369–1383.
510 doi:10.1039/C6AY02707E

511 Herrera, A., Asensio, M., Martínez, I., Santana, A., Packard, T.T., Gómez, M., 2017. Microplastic and
512 tar pollution on three Canary Islands beaches: An annual study. Mar. Pollut. Bull. in press.

513 Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the Marine
514 Environment: A Review of the Methods Used for Identification and Quantification.

515 Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P., Laforsch, C., 2012. A novel, highly efficient
516 method for the separation and quantification of plastic particles in sediments of aquatic
517 environments. Limnol. Oceanogr. Methods 10, 524–537. doi:10.4319/lom.2012.10.524

518 Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., van Franeker, J.A.,
519 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics
520 ingested by marine organisms. Mar. Pollut. Bull. 115, 86–90.
521 doi:10.1016/j.marpolbul.2016.11.034

522 Liebezeit, G., Dubaish, F., 2012. Microplastics in beaches of the East Frisian Islands Spiekeroog and
523 Kachelotplate. Bull. Environ. Contam. Toxicol. 89, 213–217. doi:10.1007/s00128-012-0642-7

524 Lusher, A.L., O'Donnell, C., Officer, R., O'Connor, I., 2016. Microplastic interactions with North
525 Atlantic mesopelagic fish 73, 1214–1225. doi:doi:10.1093/icesjms/fsv241

526 Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying

527 microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360.
528 doi:10.1039/C6AY02415G

529 Majewsky, M., Bitter, H., Eiche, E., Horn, H., 2016. Determination of microplastic polyethylene (PE)
530 and polypropylene (PP) in environmental samples using thermal analysis (TGA-DSC). Sci. Total
531 Environ. 568, 507–511. doi:10.1016/j.scitotenv.2016.06.017

532 Masura, J., Baker, J., Foster, G., Arthur, C., 2015. Laboratory methods for the analysis of microplastics
533 in the marine environment: recommendations for quantifying synthetic particles in waters and
534 sediments. NOAA Tech. Memo. NOS-OR&R-48.

535 Mathalon, A., Hill, P., 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax
536 Harbor, Nova Scotia. Mar. Pollut. Bull. 81. doi:10.1016/j.marpolbul.2014.02.018

537 McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an abundant
538 and distinct microbial habitat in an urban river. Environ. Sci. Technol. 48, 11863–11871.
539 doi:10.1021/es503610r

540 Miller, M.E., Kroon, F.J., Motti, C.A., 2017. Recovering microplastics from marine samples: A review
541 of current practices. Mar. Pollut. Bull. 1–13. doi:10.1016/j.marpolbul.2017.08.058

542 Mintenig, S.M., Int-Veen, I., Löder, M.G.J., Primpke, S., Gerdts, G., 2017. Identification of
543 microplastic in effluents of waste water treatment plants using focal plane array-based micro-
544 Fourier-transform infrared imaging. Water Res. 108, 365–372. doi:10.1016/j.watres.2016.11.015

545 Morent, R., De Geyter, N., Leys, C., Gengembre, L., Payen, E., 2008. Comparison between XPS- And
546 FTIR-analysis of plasma-treated polypropylene film surfaces. Surf. Interface Anal. 40, 597–600.
547 doi:10.1002/sia.2619

548 MSDF Technical Subgroup on Marine Litter, 2013. Guidance on Monitoring of Marine Litter in
549 European Seas. doi:10.2788/99475

550 Nuelle, M.-T., Dekiff, J.H., Remy, D., Fries, E., 2014. A new analytical approach for monitoring
551 microplastics in marine sediments. Environ. Pollut. 184, 161–9. doi:10.1016/j.envpol.2013.07.027

552 PlasticsEurope - Association of Plastics Manufacturers., 2015. Plastics - the Facts 2015 An analysis of
553 European plastics production, demand and waste data.

554 R Core Team, 2017. R: A Language and Environment for Statistical Computing.

555 Rochman, C.M., Regan, F., Thompson, R.C., 2017. On the harmonization of methods for measuring
556 the occurrence, fate and effects of microplastics. Anal. Methods 9, 1324–1325.
557 doi:10.1039/C7AY90014G

558 Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D. V., Lam, R., Miller, J.T., Teh, F.-C., Werorilangi,
559 S., Teh, S.J., 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish
560 and bivalves sold for human consumption. Sci. Rep. 5, 14340. doi:10.1038/srep14340

561 Scientific and Technical Advisory Panel, 2011. Marine debris as a global environmental problem:
562 Introducing a solutions based framework focused on plastic. Washington, DC.

563 Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the
564 planktonic food web. Environ. Pollut. 185, 77–83. doi:10.1016/j.envpol.2013.10.013

565 Song, Y.K., Hong, S.H., Jang, M., Kang, J.H., Kwon, O.Y., Han, G.M., Shim, W.J., 2014. Large
566 accumulation of micro-sized synthetic polymer particles in the sea surface microlayer. Environ.
567 Sci. Technol. 48, 9014–9021. doi:10.1021/es501757s

568 Štindlová, A., Garrido, P., Herrera, A., Gómez, M., 2017. Microplastic Ingestion by Planktivorous
569 Fishes in the Canary Current, in: Baztan, J., Jorgensen, B., Pahl, S., Thompson, R.C., Jean-Paul
570 Vanderlinden (Eds.), Fate and Impact of Microplastics in Marine Ecosystems. Elsevier, p. 157.
571 doi:10.1016/B978-0-12-812271-6.00156-3

572 Sutton, R., Mason, S.A., Stanek, S.K., Willis-Norton, E., Wren, I.F., Box, C., 2016. Microplastic
573 contamination in the San Francisco Bay, California, USA. Mar. Pollut. Bull. 109, 230–235.
574 doi:10.1016/j.marpolbul.2016.05.077

575 Tagg, A.S., Sapp, M., Harrison, J.P., Ojeda, J.J., 2015. Identification and Quantification of
576 Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging.
577 Anal. Chem. 87, 6032–6040. doi:10.1021/acs.analchem.5b00495

578 Tanaka, K., Takada, H., 2016. Microplastic fragments and microbeads in digestive tracts of
579 planktivorous fish from urban coastal waters. Sci. Rep. 6, 34351. doi:10.1038/srep34351

580 Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M. a, Jonsson, S., Björn, A., Rowland, S.J.,
581 Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H.,
582 Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai,
583 H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and
584 release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. Lond.
585 B. Biol. Sci. 364, 2027–2045. doi:10.1098/rstb.2008.0284

586 Thermo Scientific Nalgene, 2018. Chemical Compatibility Guide [WWW Document]. URL
587 http://sevierlab.vet.cornell.edu/resources/Chemical-Resistance-Chart-Detail.pdf (accessed
588 1.25.18).

589 Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D.,
590 Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838.
591 doi:10.1126/science.1094559

592 Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015. Microplastics in
593 sediments: A review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17.
594 doi:10.1016/j.marenvres.2015.06.007

595 Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human
596 consumption. Environ. Pollut. 193. doi:10.1016/j.envpol.2014.06.010

597 Vandermeersch, G., Van Cauwenberghe, L., Janssen, C.R., Marques, A., Granby, K., Fait, G.,
598 Kotterman, M.J.J., Diogène, J., Bekaert, K., Robbens, J., Devriese, L., 2015. A critical view on
599 microplastic quantification in aquatic organisms. Environ. Res. doi:10.1016/j.envres.2015.07.016

600 Von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and Effects of Microplastics on Cells
601 and Tissue of the Blue Mussel *Mytilus edulis* L. after an Experimental Exposure. Environ. Sci.
602 Technol. 46 (20), 11327–11335. doi:10.1021/es302332w

603 Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine
604 organisms: A review. Environ. Pollut. doi:10.1016/j.envpol.2013.02.031

605 Yamashita, R., Tanimura, A., 2007. Floating plastic in the Kuroshio Current area, western North
606 Pacific Ocean. Mar. Pollut. Bull. 54, 480–485. doi:10.1016/j.marpolbul.2006.11.016

607 Zettler, E.R., Mincer, T.J., Amaral-zettler, L.A., 2013. Life in the “Plastisphere”: Microbial
608 communities on plastic marine debris. Environ. Sci. Technol. 47, 7137–7146.
609 doi:10.1021/es401288x

610 Zhao, S., Danley, M., Ward, J.E., Li, D., Mincer, T.J., 2017. An approach for extraction,
611 characterization and quantitation of microplastic in natural marine snow using Raman microscopy.
612 Anal. Methods 9, 1470–1478. doi:10.1039/C6AY02302A

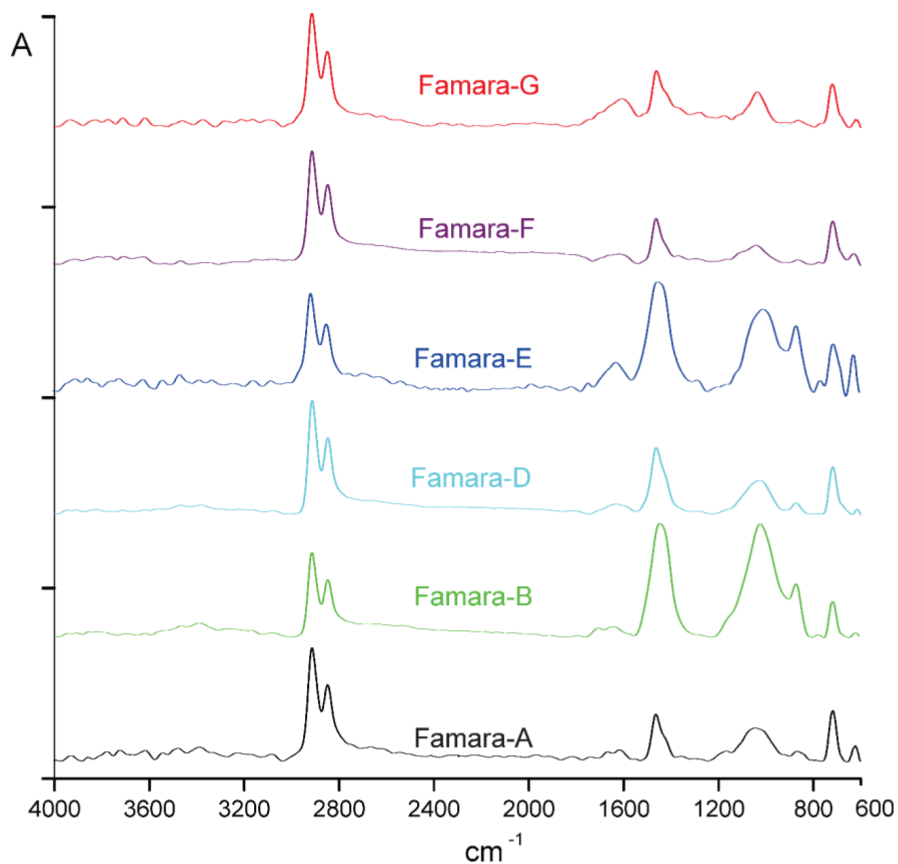
613

614 # Appendix A

615

616 Figure 1. Selection of pre-production pellets collected at Famara beach, Lanzarote, Canary Islands, that

617 were analyzed by Fourier transform infrared spectroscopy (FTIR).

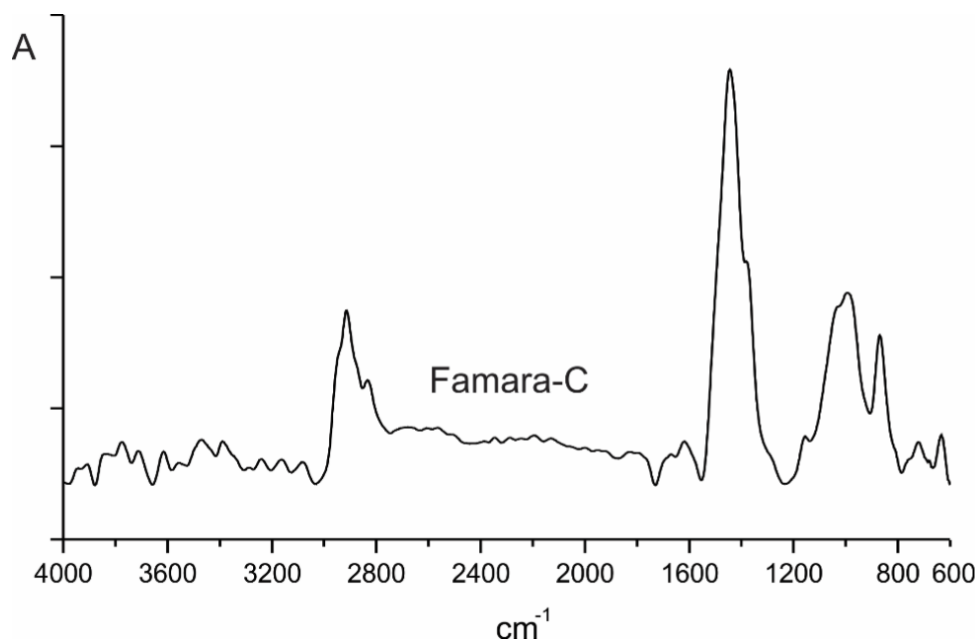


618

619 Figure 2. Fourier transform infrared spectroscopy (FTIR) spectra of pre-production pellets collected at

620 Famara beach corresponding to polyethylene (PE).

621



622 Figure 3. Fourier transform infrared spectroscopy (FTIR) spectra of pre-production pellets collected at
623 Famara beach corresponding to polypropylene (PP).

624

625 Table 1. Results of melting temperature (T_m) and degradation temperature (T_d) determined by
626 differential scanning calorimetry (DSC); and comparative coincidence with FTIR database.

Sample	**T_m (ºC)**	**T_d (ºC)**	**Coincidence FTIR (%)**	**Polymer**
FAMARA-A	133,5	226,6	82	PE
FAMARA-B	122,8	240,9	57	PE
FAMARA-C	160,8	195,1	52	PP
FAMARA-D	130,9	241,6	83	PE
FAMARA-E	134,2	227,2	56	PE
FAMARA-F	111,4	251,7	87	PE
FAMARA-G	112,9	207,4	83	PE

Results of pre-production pellets polymer identification

FTIR can provide valuable information about the identification of the polymer due to its characteristic absorption in the infrared region. The IR energy is related to the vibrational energy of different bonds found within different functional groups in a polymer. Figures 2 and 3 shows the FTIR absorbance spectra of pre-production pellets collected from Famara beach. As shown in Figure 2, all sprectra are similar and display characteristics of PE spectra. The most distinctive bands of PE spectra are found at: 2920 cm^{-1}, indicating CH_2 asymmetric stretching; 2850 cm^{-1}, also indicating CH_2 symmetric stretching; 1460 cm^{-1}, indicating bending deformation; and 720 cm^{-1}, indicating rocking deformation (Gulmine et al., 2002). The band observed at 1035 cm^{-1} is likely due to pellet degradation, since they have been exposed to the marine environment and solar light for a long time. Figure 3 shows the spectrum of the Famara-C sample. It is different from those observed in Figure 2 and it is similar to a spectrum of PP, due to the peaks between 3000 and 2800 cm^{-1}. These peaks are attributed to asymmetric and symmetrical stretching vibration in the CH_2 and CH_3 groups. The two intense peaks, at 1460 cm^{-1} and 1378 cm^{-1}, are caused by CH_3 asymmetric deformation vibrations or CH_2 scissor-vibrations and by CH_3 symmetric deformation, respectively (Morent et al., 2008). The difference between the spectrum of the Famara-C sample and the spectrum of a PP is due to the degradation caused by exposure to the sun in a marine environment.

The identification of Famara beach pellets by FTIR was reinforced by differential scanning calorimetry. Table 1 shows the melting temperature (T_m) and the degradation temperature (T_d) of the different samples, as well as the percentage of coincidence of their spectrum compared to the most similar spectrum material of the FTIR database used in this study to identify polymers. It can be seen that the Famara-A, Famara-B, Famara-D, Famara-E, Famara-F and Famara-G samples had a melting temperature between 111 and 135 ºC, corresponding to the melting temperature of the low and high

650 polyethylene density, respectively. Their degradation temperature was between 207 and 250 °C which
651 also corresponds to the degradation temperature of this type of materials. On the other hand, the
652 melting temperature of the Famara-C sample was 160.8 °C, similar to the melting point of
653 polypropylene. However, the degradation temperature was low, 195.1 °C, indicating that this material
654 has suffered heavy degradation.

655 **References**

656 Gulmine, J. V., Janissek, P.R., Heise, H.M., Akcelrud, L., 2002. Polyethylene characterization by
657 FTIR. Polym. Test. 21, 557–563. doi:10.1016/S0142-9418(01)00124-6

658 Morent, R., De Geyter, N., Leys, C., Gengembre, L., Payen, E., 2008. Comparison between XPS- And
659 FTIR-analysis of plasma-treated polypropylene film surfaces. Surf. Interface Anal. 40, 597–600.
660 doi:10.1002/sia.2619

661

662

663

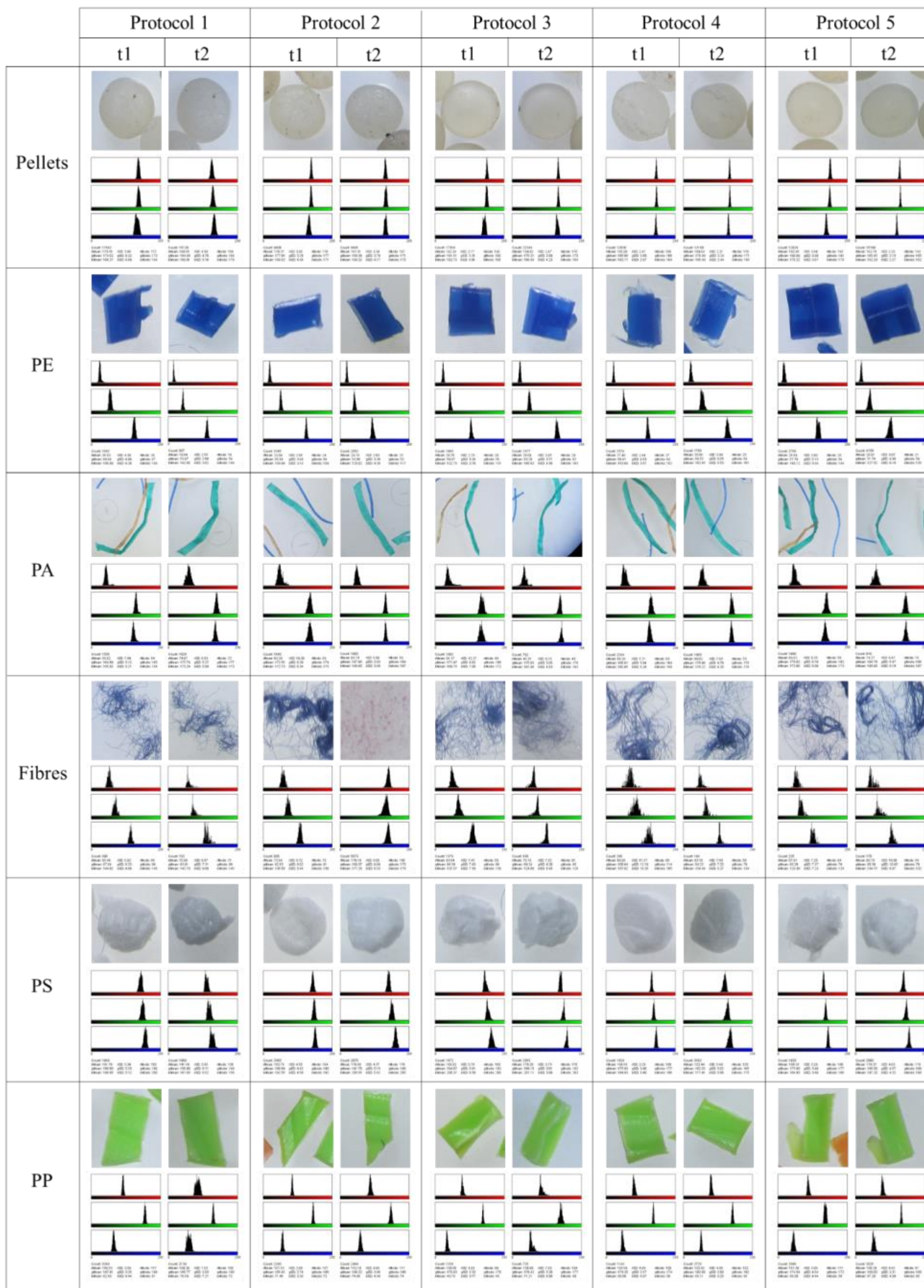


Figure 4. Photographs and colour histograms of each type of plastic polymer before (t1) and after (t2) treatments.