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Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues

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1 **Running Head:** Standard method for microplastics extraction from mussels

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9 **Title:** Development and optimisation of a standard method for extraction of microplastics in mussels
10 by enzyme digestion of soft tissues

11

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24 **Abstract:** We compared procedures for digestion of mussel soft tissues and extraction of

25 microplastics (MPs). Complete tissue digestion was achieved with 1M NaOH, 35% HNO₃ and by 0.1

26 UHb/mL protease, but use of HNO₃ caused unacceptable destruction of some MPs. Recovery of MPs

27 spiked into mussels was similar (93±10%) for NaOH and enzyme digestions. We recommend use of

28 industrial enzymes based on digestion efficiency, MP recovery and avoidance of caustic chemicals.

29

30 **Keywords:** Enzymatic digestion, FT-IR, Microplastics, Mussels, Emerging pollutants, Environmental

31 toxicology

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INTRODUCTION

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The presence and accumulation of plastic debris within marine environments has become an issue of high priority for environmental policy [1]. Microplastics [(MPs), particles between 5 mm [2] and 1 μm [3]], are reported as the most abundant pieces of plastics found in the marine environment [4] and these particles have accumulated at the sea surface on shorelines and in sediments [5]. They are also present in organisms [6] and are of toxicological concern [3]. Standardised methods for detection of MP accumulation in organisms are necessary to establish levels of exposure, facilitate comparison among studies, and to enable robust assessments of MPs risks in the environment.

Mussels are particularly good candidates for assessment of MP exposure, in the same way as they are used as indicator species for other environmental contaminants in monitoring programmes such as Mussel Watch (The National Oceanic and Atmospheric Administration, NOAA, USA). If standardised methods for extraction and quantification of MPs in tissues are established and based on good laboratory practice, mussels can act as sentinel species of MP contamination applicable over a wide range of geographical scales. For instance, *Mytilus* spp. are intertidal mussels with a large geographical distribution, they filter large volumes of water, are relatively immobile, and are easily accessible for collection throughout the year. Laboratory studies have demonstrated ingestion of MPs by *Mytilus* spp [7–9], and gut retention times for MPs can be above 72 h [8]. Microplastics have also been found in both wild and cultured *Mytilus* spp., but different soft tissue digestion and quantification methods make comparison of results challenging. For example, particles were found at concentrations between 5 - 75 particles per mussel in Nova Scotia [10], but in other studies the reported concentrations were 0.36 particles g^{-1} wet weight from North Sea coasts [11]) and up to 0.34 particles g^{-1} wet weight from various European specimens [12].

56 A standardised and specific method for extraction and quantification of MPs from mussels is
57 necessary to provide the data needed to assess levels of exposure of organisms to MPs, and to
58 provide support for environmental monitoring programmes and management decisions. Recently,
59 Vandermeersch et al. [12] reviewed and compared acid digestion procedures used for soft tissue
60 digestion of *Mytilus* spp., but their evaluation did not consider approaches for tissue digestions that
61 use strong bases or enzymes. Some methods used for extraction of MPs from mussels may not enable
62 accurate quantification of MP abundance. Techniques used for extraction of MPs can alter the shape
63 or destroy of particles present in samples. Extraction of MPs from bivalves in general, and *Mytilus* spp.
64 mussels in particular, has been accomplished by chemical digestion with simple and/or mixtures of
65 strong acids (HCl, HNO₃, HClO₄) [6,11,13] and bases (NaOH, KOH) [14–16]; however, some of these
66 methods can damage and/or destroy pH-sensitive polymers [11,13]. Hydrogen peroxide (H₂O₂) has
67 also been used to digest tissue prior to extraction of MPs, but limitations including incomplete soft
68 tissue digestion and production of foam was indicated to cause lower MP recovery from samples
69 [10,13]. Although not previously used to digest mussel tissues, enzyme digestion has been applied to
70 extract MPs from plankton-rich seawater samples, with reported high digestion rates (up to 97.7 %) and
71 no damage of particles [17]. Similarly, in forensic studies, enzyme digestion (industrial proteases
72 and lipases by Novozymes) has been used as a method for soft tissue digestion, and which
73 additionally does not cause bone damage [18], indicating the potential use of industrial enzymes
74 (used in washing powder and food industry, for instance) to digest soft tissue in other organisms.

75 The need for a standardised method to assess MPs from organisms, including mussels, has been
76 highlighted by the International Council for the Exploration of the Sea (ICES) advice provided to the
77 OSPAR Commission on plastic monitoring in organisms [19] and more recently by Vandermeersch et
78 al. [12]. Our goal was to describe a procedure for extraction and quantification of MPs in marine

79 mussels. This method was developed specifically for *Mytilus* species, as the digestion of soft tissue
80 from other organisms will differ in methodological requirements (e.g. chitinous tissues in crustaceans
81 and gut content analysis in large fish [20]). Our approach was first to optimise the digestion efficiency
82 of *Mytilus edulis* soft tissues by 3 different methods: strong acid, strong base, and a new enzyme
83 procedure. This new enzyme procedure uses industrial enzymes that are less expensive than other
84 enzymes used for tissue digestion in laboratory research. Rates of soft tissue digestion were
85 compared and the effects of each digestion method on polymer integrity were assessed by FT-IR
86 analysis of extracted MPs from spiked samples. In addition, spike recovery rates of MPs were
87 determined, airborne fibre contamination assessed, and the industrial enzyme digestion procedure
88 was applied to quantify MPs in *M. edulis* exposed in the field to waters containing MPs. To enable
89 reproducibility, a more detailed standard operating procedure (SOP) based on Good Laboratory
90 Practice (GLP) guidelines [21], is provided in the supplement section.

91

METHOD DEVELOPMENT

92

93

Development of soft tissue digestion of mussels

95 Three tissue digestion agents were tested under the same conditions to determine the method that
96 provided the most complete digestion of soft tissue with the least damage to plastics. The 1st method
97 used a strong acid (HNO₃: 0, 9, 18, 35, 50 % (v/v); # 10050270 Fisher Scientific) and the 2nd method a
98 strong base (NaOH: 0.25, 0.5, 1.0, 2.5, 5.0 M; # 10142590 Fisher Scientific). Both methods were based
99 on previous procedures used for digestion of mussels for MP quantification using strong acids
100 [6,11,19] or strong acids or bases[13,17]. Selection of NaOH was because it is a strong base and its
101 base dissociation constant (pK_b) is representative of other strong bases (e.g. KOH). The 3rd method,
102 enzymatic digestion, used an industrial protease, Corolase 7089 (AB Enzymes), obtained from *Bacillus*
103 *subtilis* cultures (activity of 840 UHb) at volumes of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 1 mL to 100 mL of Milli-Q
104 water (Millipore, filtered at 0.22 µm). This enzyme is available commercially and is considerably less
105 expensive than enzymes offered by scientific supply companies. Corolase 7089 is active at pH 6 – 9,
106 allowing its use in water without addition of a buffer, as required by other proteases.

107 With the exception of the agent used, all conditions for tissue digestion were constant. Blue mussels
108 *Mytilus edulis* were obtained from local consumer fish markets (September and December 2014), and
109 specimens were frozen at -20 °C prior to digestion. Mussels were defrosted at room temperature (up
110 to 2 h); all soft tissues were removed from the shell, weighed (wet weight to 0.01 g), and placed in a
111 250-mL glass Erlenmeyer flask for digestion (one mussel per flask). A 100 mL volume of the digestion
112 agent at the indicated concentrations (see above, current section) was added to each Erlenmeyer
113 flask, which were covered with aluminium foil and placed on a magnetic multi-stirrer plate (up to 10
114 flasks simultaneously), and stirred for approximately 1 h at 60 °C. The 60 °C temperature was selected

115 as it is within the range of action of the industrial enzymes (optimum at 55 °C, activity up to 65 °C)
116 and this temperature was unlikely to affect plastics [22]. After digestion, the final product was
117 vacuum filtered [Whatman filters of cellulose nitrate 0.8 µm or glass microfiber 1.6 µm, when MPs
118 were to be analysed by FTIR (see section *Effect of digestion procedure on polymers*)] and remaining
119 intact soft tissue on the filter membrane was weighed (< 0.01 g). Digestion efficiency (%) was
120 calculated as the percent of tissue that remained after digestion [i.e., (1 - final weight / initial weight)
121 × 100%]. Each digestion procedure was tested with 2 independent replicates per concentration. To
122 avoid contamination of samples by airborne fibres and other particles throughout all procedures, the
123 recommendations of ICES [19] and Woodall et al. [23] were followed. Samples were covered to avoid
124 air exposure, vials were capped with aluminium foil during digestion, personnel used protective
125 cotton lab coats, equipment was thoroughly rinsed using Milli-Q water, and glassware was acid-
126 washed prior to use. Procedural blanks, ie, positive controls, to account for airborne fibres
127 contamination, were conducted simultaneously during soft tissue digestions.

128

129 *Effect of digestion procedure on polymers*

130 To determine the effects of digestion on MPs, MPs of a single polymer type spiked into *M. edulis*
131 samples (one individual per flask) were evaluated after digestion of mussel tissue. Particles were
132 selected based on commonly found particles in marine litter [24] and include polyethylene
133 terephthalate (PET), PET flakes, high-density polyethylene (HDPE), polyvinyl chloride (PVC), all
134 between 500 - 125 µm, and Nylon, between 1,000 - 500 µm. The particles used were obtained from
135 Plastic Industry Development Center, Taiwan, (PET, PVC), Dow Chemical Co. (HDPE) and PET
136 Processors LLC (PET flakes). Nylon particles were cut under a dissection microscope from Nylon thread
137 (obtained from efco), and resulting particles were sorted by size ranges using stainless steel sieves.

138 Based on the previous observations, overnight (> 12 h) digestions of soft tissue were performed with
139 Corolase 7089 (1 mL to 100 mL Milli-Q water), 1 M NaOH and 35 % HNO₃. For each digestion agent, 2
140 replicates were used per type of polymer. Soft tissue was weighed to the nearest decimal (WW) and
141 digestion efficiency was calculated as a percentage of soft tissue digested. After digestion and
142 subsequent filtration, all filters were placed in covered plastic petri dishes and oven dried for
143 approximately 24 h at 60 °C. Filters were observed with a dissection microscope and particles were
144 stored in closed vials until further use (cork lid, to avoid crossed contamination by other polymers).

145 Transmittance FT-IR (Brüker IFS 66 Spectrometer with a Bruker Hyperion 1,000 microscope) was
146 used to determine if integrity of polymers was altered during the digestion procedures (i.e. if the
147 polymer would still be identifiable after digestion). Prior to the analysis, MP specimens were placed
148 into a Specac DC-2 diamond compression cell and flattened using manual pressure, reducing the
149 thickness to allow for suitable absorbance. For each particle, the type of polymer was identified by
150 generating a spectrum (after 32 scans) and comparing it against a spectral database of synthetic
151 polymers (Brüker I26933 Synthetic fibres ATR-library) [24]. An FT-IR analysis was also performed on
152 particles from the original stock of each polymer not subjected to any digestion procedure.

153

154 *Recovery rate of particles and assessment of airborne fibres contamination*

155 The recovery rates of MPs that were spiked into water samples prior to digestion were assessed to
156 determine the ability of the digestion method to quantify MPs in unknown samples. In each vial, 30
157 particles of a single type of MP [PET, HDPE, or Nylon (all particles < 500 µm)] were added and there
158 were 3 independent replicates for each type of MP. Two procedures, enzyme digestion (Corolase
159 7089, activity 840 UHb, dilution 1:100) and 1 M NaOH, were selected based on results of experiments
160 described in the section *Effect of digestion procedure on polymers* and tested separately, only in Milli-

161 Q water. The digestion procedure was as described in the section *Effect of digestion procedure on*
162 *polymers* (overnight digestion in 30 mL of Milli-Q water) and samples were filtered (0.8 µm filters)
163 before being oven dried at 60 °C for approximately 24 h. A stereomicroscope was used to count the
164 number of MPs on the dried filter, and particle recovery rate (%) is expressed as the number of MPs
165 counted divided by the number of MPs spiked into the sample (i.e., MPs counted / 30 × 100 %). As
166 selected particles were easily identifiable and distinct from possible contamination sources, the same
167 samples were further examined for the presence of airborne fibres, i.e. the number of fibres present
168 in the filters were quantified. Differences in recovery of MPs (i.e., percent recovery) were tested by
169 two-way ANOVA with particle type and digestion procedure as independent factors along with the
170 particle type × digestion procedure interaction term. The number of plastic particles (not spiked MPs)
171 that contaminated samples (i.e., the particles that entered during digestion method or by airborne
172 contamination) was compared between the 2 digestion methods by t-test. For all statistical tests,
173 normality and homoscedasticity were tested and a probability level of $p < 0.05$ was used to determine
174 if differences were statistically significant, and all analyses were done using the software Statistica
175 (StatSoft, Inc).

176
177 *Application of mussel digestion method to quantify MPs in wild mussels*

178 Live *M. edulis* (obtained from a commercial supplier) were held in the intertidal zone for 18 days
179 (January/February 2015) in cylindrical stainless steel mesh cages (10 x 8 cm, height and diameter
180 respectively) in the estuary of the Forth River, Edinburgh, UK, in Port Edgar (N 55°, 59'42", W
181 3°, 24'30"). Eight mussels from 2 cages (4 mussels per cage) were digested overnight (60 °C) with
182 Corolase 7089 enzyme (activity 840 UHb, dilution 1:100), and MPs quantified according the methods
183 described in the section *Effect of digestion procedure on polymers*. To assess airborne fibre

184 contamination during this procedure, 2 Milli-Q water control samples (100 mL) were submitted to the
185 same procedure, and 4 damp filters were held in plastic petri dishes under the same conditions as
186 other filters and oven dried (60 °C for ~ 24 h). All filters were examined with a stereomicroscope for
187 enumeration of particles. Differences in the number of MPs found in mussel samples were compared
188 between deployed cages by t-test and considered significant at a probability level of $P < 0.05$
189 (Statistica, StatSoft, Inc).

190

191

RESULTS AND DISCUSSION

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194 Each of the three procedures, acid, base and enzymatic digestion, digested all of the mussel soft
195 tissue present in the tested samples. The minimum concentrations required to achieve complete
196 digestion of soft tissue after 1 h at 60 °C, were 1 M for NaOH, 35 % (v/v) HNO₃, and 0.5 mL of Corolase
197 7089 to 100 mL of water. Although a 100 % digestion efficiency occurred, visual inspection of the
198 filters revealed the presence of tissue residues (less than 0.01 g) in all tested methods. These residues
199 were very small pieces of soft tissue that were below the range of conventional balance (< 0.01 g) and
200 considered not to interfere with MPs quantification. To guarantee complete digestion of soft tissue,
201 overnight (~12 h) digestion is recommended and was used in all subsequent digestions. An additional
202 1 mL of enzyme solution to 100 mL of water was used for mussels weighting between 2 - 5 g, to
203 ensure complete digestion. For larger mussel samples (e.g., 8 - 21 g WW of *Modiolus modiolus*) a
204 similar complete digestion was achieved by increasing the enzyme volume to 2 mL of Corolase in 100
205 mL of Milli-Q water (data not shown).

206 We obtained higher mussel soft tissue digestion efficiencies using lower concentrations of HNO₃
207 and NaOH after a shorter period of time, than those reported by other authors. For instance, mussel
208 samples were reported not to have been 100 % digested using 52.5 M NaOH (during 1 h at 60 °C plus
209 1 h at 100 °C) [13], while complete digestion occurred in the present study at 1 M NaOH after 1 h.
210 Similarly, for HNO₃ digestion, Claessens et al. [13] reported lower digestion efficiency for *M. edulis*
211 after use of 22.5 M HNO₃ (98.9 % at 1 h at 60 °C plus 1 h at 100 °C). Our samples were fully digested
212 at 35 % (v/v) HNO₃ after 1 h and it is possible that higher digestion efficiencies obtained in our
213 method can be explained by use of frozen samples (increased destruction of cells) and mild stirring

214 during the procedure (increased mechanical disaggregation of tissue and contact with chemical
215 agents).

216 The integrity of plastics spiked into mussels was affected by digestion method. Visual inspection of
217 samples revealed that HNO₃ digestion induced melding (i.e. fusing and/or merging) of some PET and
218 HDPE particles, and that all Nylon fibres were no longer present at the end of overnight digestion.
219 Plastic polymers have viscoelastic properties that can be altered by temperature and chemical action,
220 but that will not necessarily affect their chemical composition/optical properties[25]. Despite possible
221 changes in particle morphology, all particles present were able to be identified using FT-IR (Figure 1).
222 However, loss of material and melding of particles done by chemical digestion may lead to erroneous
223 quantification of MPs. Loss of nylon fibres due to strong acid digestion of mussels has been reported
224 previously [13], and likely leads to underestimation of MPs in wild mussel samples [11]. Digestion of
225 tissues by strong acids has also been reported to meld and/or damage MPs in the digestion of soft
226 tissue from fish [26] and plankton [17], and was not recommended by these authors. Therefore, we
227 discontinued further use of acid digestion and agree that acid digestion should not be used for MPs
228 extraction and quantification.

229 The recovery of particles spiked in to mussel samples did not differ among particle types tested
230 [HDPE, PET and Nylon particles ($p = 0.06$)] or digestion method [NaOH, Coralase, ($p = 0.74$)]. Although
231 not statistically significant, the mean recovery of the Nylon particles was lower than for the other
232 polymers, as well as more variable: mean recovery of Nylon was 85 % \pm 13.2 SD ($n = 6$) compared to
233 97 % \pm 6.3 SD ($n = 6$) for PET and 98 % \pm 2.0 SD ($n = 6$) for HDPE. This was likely due to difficulties in
234 working with Nylon particles because they appeared to have higher static electricity and prevention of
235 particle loss was more difficult. The mean recovery rate for enzymatic digestion was 93 % \pm 10.8 SD (n
236 = 9) and for 1 M NaOH digestion was 94 % (\pm 10.0 SD, $n = 9$), with a total mean recovery rate of 93 % \pm

237 10.1 SD ($n = 18$). These spike recovery values are consistent with the 93.6 - 98.3 % recovery rates after
238 acid digestion of mussels reported by Claessens et al. [13].

239 The suitability of the enzymatic digestion protocol was verified in *M. edulis* live specimens placed in
240 the field, showing practical applicability of the method. Fibres, films and particles (spherules,
241 spongy and other particles) were extracted and quantified (Table 1). Specimens from 2 different
242 cages placed in the field did not present any difference in amount or type of particles extracted ($p >$
243 0.40). The number of particles is within the expected range with fibres reaching 10.4 per g mussel
244 WW \pm 3.42 SD), particles detected were $0.9 \pm 0.99 \text{ g}^{-1}$ mussel WW \pm while films was $1.3 \pm 2.38 \text{ g}^{-1}$
245 mussel WW. Mussels from the North Sea collected in Belgium were reported to have 0.36 particles g^{-1}
246 wet weight soft tissue [11].

247 Airborne fibres contamination did not differ between tested digestion methods NaOH and Corolase
248 ($p = 0.15$) and the mean number of fibres observed per sample was of 5 ± 6.4 SD fibres per sample (n
249 = 18). In the enzymatic digestion of field samples, the observed airborne fibre contamination within
250 the same range (3 and 6 fibres). This level of airborne fibre contamination is consistent with reported
251 contamination of the method used by De Witte et al. [6] for acid extraction of MPs of mussels (limit of
252 detection of airborne fibres between 1.5 - 4.7 fibres per analysis). Some earlier studies, eg [16] on
253 MPs quantification in mussels failed to report on airborne fibres contamination. Other studies, such
254 as Mathalon and Hill [10], reported up to 100 plastic fibres per digested sample that were attributed
255 to airborne contamination. The use of procedural blanks for systematic monitoring of fibre
256 contamination and the application of good laboratorial practices is essential for quality assurance for
257 quantification of MPs in tissues. Contamination from airborne fibres can occur at any time during the
258 digestion procedure, but samples are possibly more vulnerable during initial stages, such as open-air
259 dissection and weighting of samples. According to our results, in the filters only submitted to oven

260 drying ($n = 4$), when samples were covered, the mean airborne fibre contamination was low, 0.8 ± 1.5
261 SD fibres per filter. Therefore, special care should be taken during initial procedural stages (e.g.
262 dissection, sample digestion, and processing), and the manipulation of samples in a confined and
263 clean room and/or laboratorial hood is recommended. The use of procedural blanks during the entire
264 procedure is essential to monitor the presence of airborne fibres contamination and to assure a more
265 accurate quantification of observed MPs from field samples. Quality assurance and data reliability of
266 data are important considerations, and must be consistent with international recommendations for
267 good laboratory practice (GLP) as recommended by the EFSA Panel on Contaminants in the Food
268 Chain (CONTAM) [20].

269 Based on soft tissue digestion efficiency, ability to maintain MP integrity during digestion, and high
270 MP spike recovery, the enzyme digestion method described in the present research is offered to
271 become a standard method for MP quantification in mussels (see supplemental data for Standard
272 Operating Procedure, SOP). We believe that our method provides not only a high MP recovery rate,
273 but it also enables recovery of sensitive MPs without damage, and increases the utility of this
274 extraction method for a more accurate estimation of the number of MPs present in bivalves.
275 Furthermore, the use of industrial enzymes is a safer procedure than use of NaOH, and can be
276 conducted without use of a fume cupboard. Compared to other enzymes used in laboratory
277 procedures, industrial proteases have the advantage that they are supplied in a liquid form that does
278 not need to be buffered, and they present lower hazard problems compared to powder forms. In
279 summary, the method advocated here consists of soft tissue digestion overnight (~ 12 h) at $60\text{ }^{\circ}\text{C}$, in a
280 stirred preparation of water and industrial proteases. After digestion, the preparation is filtered, the
281 filter paper is dried in a covered plastic petri dish at $60\text{ }^{\circ}\text{C}$ (~ 24 h), and quantification of MPs is
282 conducted by examination with a stereomicroscope ($60 - 310\times$ magnification). This method is

283 relatively inexpensive, can be applied in most laboratories, and if employed as described will enable
284 direct comparison of MPs quantification among future studies. The application of this method as a
285 standardised procedure will enable MP assessment to be integrated into existing environmental
286 monitoring programmes, such as the Mussel Watch (The National Oceanic and Atmospheric
287 Administration, NOAA, USA).

288

289

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291

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SUPPLEMENTAL DATA

367

368

369 Standard operating procedure for digestion of mussel soft tissues and extraction of microplastics:

370 1. Recommendations to avoid airborne sample contamination:

371 Samples should be covered to avoid air exposure, vials capped with aluminium foil during
372 digestion, personnel should use protective and clean cotton lab coats, equipment must be
373 thoroughly rinsed using Milli-Q water, and glassware should be acid-washed (nitric acid 2 %)
374 prior to use. The use of blanks during the whole procedure is highly recommended, to allow
375 for airborne fibre contamination to be quantified.

376 2. Samples processing:

- 377 a. Allow samples, ie mussels, to freeze (-20 °C) for at least 24 h as this process helps
378 breaking down fibres and softening tissues
- 379 b. Before processing the samples allow them to thaw (room temperature, < 2 h), then cut
380 adductor muscles using scalpel and open shell. Scrap all soft tissue and register wet
381 weight (to 0.01 g)

382 3. Soft tissue digestion:

- 383 a. Place tissue in a 250 mL glass Erlenmeyer (1 mussel per vial)
- 384 b. Add 100 ml of MilliQ water and 1 mL of Corolase 7089 enzyme aqueous solution (AB
385 Enzymes)
- 386 c. Add stirring magnet and cover the top of the vial with aluminium foil
- 387 d. Place vials on a magnetic multi-stirrer plate and allow samples to digest overnight at 60
388 °C

389 e. Blank should be submitted to the same procedure but no sample should be placed in
390 the dedicated vial

391 4. Filtration and drying:

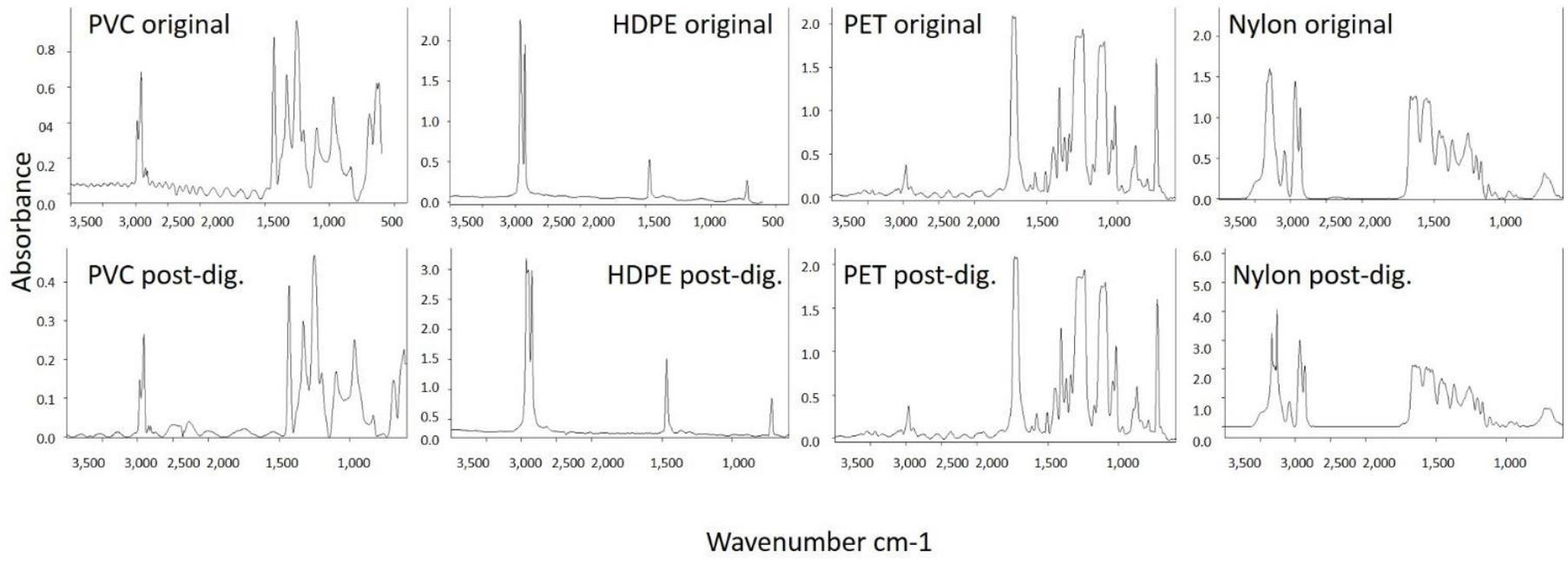
- 392 a. When present in the samples, microplastics are recovered through vacuum filtration
393 using a glass Whatman filter holder device
- 394 b. Filter pore size is a trade of between the minimum size of the particles to be recovered
395 and how fast/efficient the filtration procedure is – for small particles recovery use
396 filters of 0.8 - 1.6 μm
- 397 c. It is advisable for filters to be of cellulose nitrate, but glass microfiber material can be
398 more suitable for particles to be analysed using FT-IR
- 399 d. After vial and sample cool down, pour mixture into Whatman filter funnel, rinsing
400 digestion vial thoroughly with MilliQ water and active the filter pump
- 401 e. Once filtration ends, collect the filter carefully, place it in a plastic petri dish, and allow
402 filter to oven dry (covered) at 60 °C for 24 h
- 403 f. Filters can be stored for later observation under a stereomicroscope (60 – 310 x
404 magnification)

405

406 **Figure 1. FT-IR spectra of tested polymers (PVC, HDPE, PET and Nylon) spiked in mussel samples: 1)**
407 **not submitted to digestion (original particles) and 2) after Corolase digestion (post dig.)**
408

Table 1. The number (mean \pm SD, $n = 18$) of microplastics (fibres, particles and films) per mussel and per g wet weight (WW) of mussel

	Fibres	Particles	Films
MPs / mussel	10.4 \pm 3.42	0.9 \pm 0.99	1.3 \pm 2.38
MPs / g (ww)	2.0 \pm 0.42	0.2 \pm 0.21	0.3 \pm 0.59



Wavenumber cm-1