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Title: Selective determination of poly(styrene) and polyolefin microplastics in sandy beach sediments by gel permeation chromatography coupled with fluorescence detection

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Keywords: Gel permeation chromatography; fluorescence; marine litter; microplastics; polystyrene; oxidized polyolefin

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Abstract: Microplastics generated by plastics waste degradation are ubiquitous in marine and freshwater basins, posing serious environmental concerns. Raman and FTIR spectroscopies, along with techniques such as pyrolysis-GC/MS, are typically used for their identification. We present a procedure based on gel permeation chromatography (GPC) coupled with fluorescence detection for semi-quantitative selective determination of the most common microplastics found in marine shoreline sediments: poly(styrene) (PS) and partially degraded polyolefins (LDPEox). By operating the detector at either 260/280 or 370/420 nm excitation/emission wavelengths PS can be distinguished from LDPEox upon GPC separation. Semi-quantitative determination of microplastics contents is also possible: dichloromethane extracts of PS and LDPEox yield linear plots of fluorescence peak area vs concentration (0-5.0 mg/mL range) and were used as reference materials for quantification of the plastics content in sand samples collected in the winter berm and dune sectors of a Tuscany beach in Italy



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Pisa, September 11th, 2018

Dear Editor,

the attached file is the revised version of the manuscript

“Selective determination of poly(styrene) and polyolefin microplastics in sandy beach sediments by gel permeation chromatography coupled with fluorescence detection”

by T. Biver, S. Bianchi, M. R. Carosi, A. Ceccarini, A. Corti, E. Manco and V. Castelvetro, Department of Chemistry and Industrial Chemistry of the University of Pisa, Pisa (Italy).

In this revised version the authors have taken in due consideration all the observations by the reviewers, as detailed in the attached “Response to the reviewers” file.

While we appreciate the positive comments of Reviewer #1, we are grateful to Reviewer #2 as well since it allowed us to improve the paper and to better focus it for a readership possibly less familiar with the subject of polymer degradation and characterization

Supplementary Material remains the same as previously provided for reviewing, since we did not deem it sufficiently self-consistent to be converted into a “Data in Brief” article as advised.

I can confirm again that the manuscript is unpublished and has not been submitted for publication elsewhere. Also, the TOC has been complied according to the journal’s TOC art policy, with the submitted image created by an author and never published.

With the additional information and more accurate and in depth discussion of the results, which is now included in a separate paragraph as suggested by the reviewer, we believe that the overall quality of the paper makes it suitable for publication in Marine Pollution bulletin.

Sincerely

Valter Castelvetro

Response to Reviewers

the reviewer's comments (or synopses) are marked in blue.

As mentioned by Reviewer #2, “the GPC-FLD method is well known method in polymer chemistry”. However, we would like to point out that it has not been explored as a method to selectively analyze a complex mix of polymeric materials characterized by a broad range of degradation levels. As it has been more clearly specified in the new “Discussion” section of the revised paper, the proposed method can only provide semi-quantitative data in the case of the oxidized polyolefin as it only allows analysis of the fraction soluble in the DCM solvent used to selectively extract microplastics from the environmental matrix. On the other hand, while the total amount of polyolefins in microplastics may include a fraction of less degraded macromolecules insoluble in DCM, the typical degradation pattern of polyolefins involves quite a thorough oxidative degradation before the onset of pulverization resulting in microparticle generation.

Concerning the statement: “semi-quantitative method for only soluble fraction of PS and oxidized polyolefin polymer such as LDPE, HDPE and PP is hardly applicable to field sample for real quantification of microplastic abundance, because the amount of soluble fraction is largely dependent on molecular size and oxidative status of polyolefin”, we wish to point out that, while the proposed method (being limited to the soluble fraction of polyolefins) may not include the whole pool of polyolefin microparticles, it is however suitable for providing indications on the extent of polyolefin microplastics pollution because the mechanism of polyolefin fragmentation is likely to result in quite a thorough oxidation level of the polyolefin once the micrometric size is achieved upon degradation. In fact, as mentioned in the cited previous paper (now ref. # 8, in the previously submitted version ref. #3), when the sand samples already extracted with dichloromethane were further extracted with boiling xylenes to collect the less degraded polyolefin fraction only very low amounts (typically 1-3 % of the total polymer extracts) of polyolefin material could be recovered.

Finally, we are presenting as a real case example “only two field samples for validation of this method” because our goal was to present a methodology that we consider suitable for improving our knowledge on the complex nature and composition of the microplastics pool in environmental samples, while we are presently performing more extensive field analyses on different sites and we are planning to submit soon new papers on additional methodologies for a broader range of microplastics.

The text has been thoroughly revised. The parts modified beyond those suggested by the reviewer (and specified in the point-by-point answers) concern either improvements in the form (for clarity) or more significant changes/addition; the locations of the latter are listed here:

- Line 45-48
- Additional text (lines 246-256) and the new figure 7 (further discussed in the following “Discussion” section) have been added to better highlight the significance and limitations of the proposed method for the semi-quantitative determination of the polyolefin microplastics content in sediment samples.

Below are reported the point-by-point answers to the Specific comments by Reviewer#2.

The line numbers must be given to facilitate review process.

- Manuscript modified as suggested

Abstract: Need to be more informative.

- Additional details have been added (lines 23-31)

P2 line3: "pollution of sediments by microplastics has been scarcely investigated". There has been considerable number of publications for microplastic abundance in beach and sub-tidal sediments. The authors should cite other studies.

- Additional references (1,2) have been added

P2 line 9: polystyrene density is generally higher than water density. Most of PS found on the beach is expanded polystyrene (Styrofoam). It should be mentioned with some references.

- Some specific reference has been added (in the reviewer's note there is clearly a refuse as the density of PS, and even more so that of Styrofoam, is lower than that of saltwater). Additional references (4,5,6) have been added to support the statements in the connected sentences.

P2 line 19:larger than 300 - 500 μm from.....There are increasing number of studies using a mesh size much smaller than 300 μm in seawater and freshwater studies. The authors should state those studies.

- Additional references have been added (now included in refs 7-10) and the sentence slightly updated

P4 lines 1-3: Where did you get LDPE? Provide information on LDPE used for aging. Recommend to provide photos and FTIR spectrums of before and after 120d aging of LDPE. What kind of commercial sample of expanded PS is used? Provided the more information on the field samples (sample size, amount/weight of sands extracted, etc).

- More detailed information on the PS and LDPEox reference samples are reported in the already cited reference (now ref. 8), along with the information on the two environmental samples. In particular, LDPEox was prepared from a commercial LDPE (IR spectra typical of polyethylene) that had been artificially oxidized (to have a well characterized reference sample) by thermal ageing in the presence of a Cobalt catalyst as those used in the formulation of oxo-degradable polyolefins.

P4 lines 11-12: How to make PS and LDPEox solution in different range and how to measure/know the concentrations?

- Both PS and LDPEox used for calibration were the DCM soluble reference materials, thus the solutions were prepared simply by weighing solvent and polymer and by compensating for solvent evaporation before sealing the vials used to inject the solution in the HPLC apparatus.

Results: The content of this section is mostly results. It is strongly recommend to give a separate discussion section.

- A new Discussion section has been added, containing some of the text previously placed in the "Results" section (e.g. the present text at lines 271-284 and, with significant rewriting, at lines 300-310) and additional discussion (also with reference to the additional information from the new figure 7) to better explain the advantages and limitations of the proposed analytical procedure.

P9 3rd paragraph: This part should be given in Method section.

- Manuscript modified as suggested. The mentioned paragraph is now placed at the end of the "Materials and Methods" section

Table 1: It is not clear what are different from High MW PS (FLD260/280) in column 4 and Total PS (FLD 260/280) in column 5. The authors should explain clearly what is meaning of both the values. How are they calculated or determined (from the chromatogram)? These field samples should be tested with other conventional (FTIR or Raman) method for comparison.

- An additional explicatory note has been added to the table. No additional test was performed with the suggested FTIR or RAMAN techniques, the latter being more suitable for particle characterization than for quantification of the concentration of a polymer solution (from which the total amounts of extracted polymer from the environmental samples have been calculated once the dilution factors have been considered).

The limitation of GPC-FLD method for detection of microplastics should be given in discussion. For example: 1) no size information of microplastics, 2) no discrimination of polyolefin plastics, 3) change of the amount of DCM soluble fraction according to MW, oxidative status, etc, 4) overestimation with interfering natural organics, and so on

- The suggested points have been explicitly included in the new “Discussion” section; concerning point 3, additional information and a new figure 7 along with a thorough discussion of the significance and limitations of the method is now provided (in particular with reference to the polyolefin fraction in the text of lines 292-299 and 306-328). The conclusions have been updated accordingly.

Method validation should be done in the laboratory with varying condition of MW, oxidative stage of microplastics.

- While this is a very reasonable (and somehow expected) comment, we did not perform such additional experiments since we deemed them inadequate to actually achieve the validation of an analytical method presenting intrinsic limitations due to the extreme variability of the molecular features in degraded polyolefin samples from environmental matrices. No matter how expanded a chosen set of reference polymers with different oxidation degrees, it would never be strictly representative of a virtually endless combination of polyolefin types, ageing conditions and ageing stages. On the other hand, in the last paragraph of the “Discussion” section the rationale for the adopted criteria is further clarified and justified based on the current knowledge about the degradation pattern of polyolefins upon ageing.

More field samples were tested and their microplastic abundance and polymer composition should be compared with other well-known analytical method such as micro-FTIR.

- Again, this paper is not targeted at producing additional data on the extent of microplastics contamination of a specific site. Instead, it is meant to propose an analytical method for the selective identification and semi-quantitative determination of a limited number of polymer types, that are incidentally those more likely to be found in the seashore sediments due to their physico-chemical properties and to the main source of marine plastic pollution. The method must obviously be integrated in a more comprehensive procedure that allows tackling the various issues posed by the variability of environmental samples and of the microplastics resulting from progressive environmental degradation of plastic litter.

Supplementary Material remains the same as previously provided for reviewing, since we did not deem it sufficiently self-consistent to be converted into a “Data in Brief” article as advised.

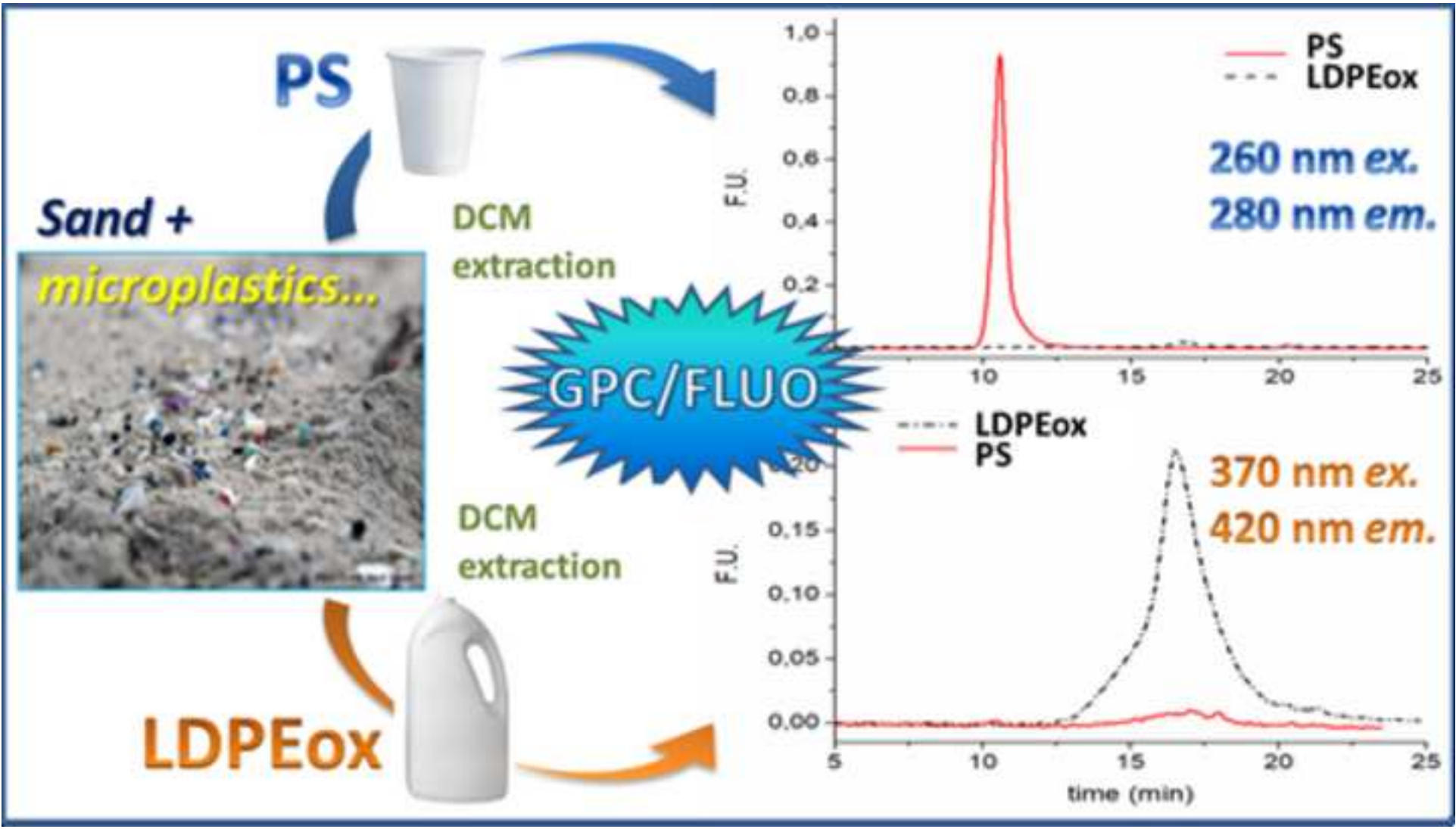
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Sincerely

Prof. Valter Castelvetto





Highlights

- Microplastics accumulation ashore is largely based on low density hydrocarbon polymers
- Quantitative determination of microplastics from marine sediments requires suitable analytical protocols
- By combining solvent extraction and chromatographic separation with spectrofluorometric detection poly(styrene) and degraded polyolefin can be separately analyzed
- Unprecedented semi-quantitative and selective determination of microplastics from shoreline sand sediments is achieved

1 **Selective determination of poly(styrene) and polyolefin microplastics in sandy beach**
2 **sediments by gel permeation chromatography coupled with fluorescence detection**

3

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6

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10

11 *T. Biver and A. Corti proposed and participated to the planning, experimentation and writing; S.*
12 *Bianchi, M. R. Carosi and E. Manco carried out experimental work, A. Ceccarini supervised the*
13 *experimental work and V. Castelvetro coordinated the research, provided funding and revised the*
14 *paper.*

15

16 **Abstract**

17 Microplastics generated by plastics waste degradation are ubiquitous in marine and freshwater
18 basins, posing serious environmental concerns. Raman and FTIR spectroscopies, along with
19 techniques such as pyrolysis-GC/MS, are typically used for their identification. We present a
20 procedure based on gel permeation chromatography (GPC) coupled with fluorescence detection for
21 semi-quantitative selective determination of the most common microplastics found in marine
22 shoreline sediments: poly(styrene) (PS) and partially degraded polyolefins (LDPEox). By operating
23 the detector at either 260/280 or 370/420 nm excitation/emission wavelengths PS can be
24 distinguished from LDPEox upon GPC separation. Semi-quantitative determination of
25 microplastics contents is also possible: dichloromethane extracts of PS and LDPEox yield linear
26 plots of fluorescence peak area vs concentration (0-5.0 mg/mL range) and were used as reference
27 materials for quantification of the plastics content in sand samples collected in the winter berm and
28 dune sectors of a Tuscany beach in Italy.

29

30 **Keywords**

31 Gel permeation chromatography, fluorescence, marine litter, microplastics, polystyrene, oxidized
32 polyolefin

33

34 **Introduction**

35

36 The increasing attention towards the plastic pollution in water basins and their sediment systems is
37 stimulating efforts aimed at improving sampling and characterization procedures. In this context,
38 the pollution of sediments by microplastics has been investigated less extensively than that of the
39 water column, with analytical methods still being developed and requiring standardisation and
40 harmonisation.^{1,2} Microplastics are minute plastics debris and particles with size ranging from few
41 microns to a higher threshold varying, according to different researchers, from 500 μm up to 5 mm.³
42 Their dispersion in the environment is a consequence of their release into wastewater as primary
43 particles (e.g. synthetic fibers, microbeads), mainly from textile and personal care products, and of
44 the fragmentation of larger plastic items caused by environmental degradation (photo-oxidative,
45 hydrolytic) resulting in the generation of secondary particles. Commodity hydrocarbon polymers
46 such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) are those more likely to end
47 up in shoreline rather than sea bottom sediments because of their low density.⁴ For these polymers
48 environmental degradation occurs mainly through a photochemically assisted generation of
49 oxidized functional groups (carbonyl, carboxyl, hydroxyl) followed by chain scissions and
50 consequent reduction of the average molecular weight.⁵ These processes weaken the integrity of
51 plastic items that become brittle up to their powdery disintegration.⁶ It has been suggested that these
52 polymers may produce a substantial contribution in the pollution of coastal sediments by
53 microplastics since the processes of degradation and embrittlement of larger items proceed as they
54 float at the sea/freshwater surface, and are accelerated once they are deposited ashore, where photo-
55 oxidative, thermal and mechanical stresses are greater.⁷⁻¹⁰ While mesh sizes smaller than 300 μm
56 have also been used,¹¹⁻¹² sampling based on filtration of seawater or sieving of sediments has been
57 mostly limited to the sampling to microplastics larger than 300-500 μm , although in the case of
58 sediments sieving followed by density separation and filtration steps may allow collection and
59 identification of microplastics down to 1-2 μm .¹³ However, isolation and characterization of
60 individual small particles is not only impractical but also poorly representative of the diversity of
61 this kind of pollution, also as a consequence of the contamination of microplastics with organic
62 compounds and inorganic particles captured from the environment.¹⁴ Chemical and enzymatic pre-
63 treatments, including 30-35% hydrogen peroxide,¹⁵ 30% HCl, and concentrated alkaline (e.g.
64 NaOH) solutions,^{14,16} have been employed to remove organic contaminants from the microplastics
65 in coastal sediment samples. Such aggressive chemical agents, however, may cause significant
66 degradation or further alteration of the microplastics present in treated samples.¹⁷
67 Among the techniques used for the chemical identification of microplastics separated from sediment
68 samples the most common are Fourier transformed infrared and Raman spectroscopies^{13,18} and, for
69 smaller particles, micro-Raman and micro-FT-IR,¹⁹⁻²¹ the latter also associated with molecular
70 imaging or focusing tools allowing the collection of spectra during visual inspection of the samples.

71 Less practical for routine analysis but very effective for the chemical identification of plastics debris
72 and their degradation products is pyrolysis coupled with gas-chromatography-mass spectrometry
73 (Py-GC/MS).²² Fabbri et al²³ used this technique to assess the contamination with poly(vinyl
74 chloride) and other polymers in bottom sediments of a coastal lagoon, after isolation of the
75 polymeric fractions by solvent extraction and re-precipitation in n-hexane. Direct analysis without
76 polymer isolation suffers from the interference of inorganic components of the sediments affecting
77 the degradation patterns of the polymeric materials during the analysis,²⁴ and from natural organic
78 matter such as humic compounds whose thermal degradation produces the same congeners of
79 synthetic polymers, particularly styrenic ones.^{25,26} A two-step procedure based on thermal
80 decomposition followed by absorption into a solid-phase device and subsequent GC/MS
81 identification of the pyrolysis products has been proposed for the analysis of microplastics from
82 environmental samples of complex composition.^{27,28} However, the same factors influencing the Py-
83 GC/MS response may reduce the accuracy and reliability of the latter procedure. Several interfering
84 factors may also affect the results of FT-IR and Raman spectroscopic analyses, including the
85 morphology of single microplastics fragments,²⁰ the presence of surface contaminants such as
86 natural compounds, persistent organic pollutants²⁹ or biofilms.^{30,31} Many of the cited methodologies
87 comprise a separation step or the isolation and analysis of single microplastics fragments, limiting
88 the minimum size of the fragment that can be reliably characterized and possibly excluding the
89 extensively degraded ones, which may be more difficult to separate from the inorganic sediment or
90 from biogenic debris.

91 The present article is intended as a contribution to the improvement of the methodologies and
92 techniques that, if used in a synergistic way, may provide more accurate information about level of
93 contamination and fate of very small size microplastics and relevant degradation products in coastal
94 sediments. In particular, gel permeation chromatography (GPC) equipped with refractive index, UV
95 diode array and spectrofluorometric detectors has been used for qualitative and quantitative analysis
96 of the polymer content in solvent extracts from marine sediments sampled in a sandy beach of
97 northern Tuscany (Italy), which had been found to contain mainly low density polymers such as
98 expanded polystyrene, polyethylene and polypropylene along with their partial degradation
99 polymeric and oligomeric species.⁸

100

101 **Materials and Methods**

102

103 Two polymeric materials representative of degraded poly(ethylene) and poly(styrene) were used as
104 reference compounds: i) the dichloromethane (DCM) soluble fraction of a partially oxidized low
105 density poly(ethylene) (LDPEox) obtained after catalytically enhanced oxidation (120 days thermal

106 aging in air ventilated oven a 70 °C); ii) a commercial sample of expanded poly(styrene). As the
107 environmental materials, DCM extracts previously collected from shoreline sand samples were
108 analyzed.⁸

109 *Gel permeation chromatography (GPC)* analyses were performed with an Agilent 1260 Infinity
110 Binary LC instrument equipped with diode array (DAD VL+ 1260/G1315C) plus fluorescence
111 (FLD 1260/G1321B) double detector, and two in series PLgel MIXED-E Mesopore columns
112 (Polymer Laboratories) thermostated at 30°C, using chloroform at 1.0 mL/min flow rate as the
113 eluent. Ten polystyrene standards with molecular weights ranging from 800 to 300,000 g/mol⁻¹
114 (Polymer Laboratories Ltd. and Varian, Inc.) were used for calibration. Both GPC detectors were
115 calibrated for quantitative determination of the polymeric materials. For this purpose, five DCM
116 solutions of PS (concentration range 0.02-4.15 mg/mL) and three DCM solutions of LDPEox
117 (concentration range 0.54-5.61 mg/mL) were analyzed in triplicate by using the DAD detector at
118 261 nm and 243 nm, respectively, recording the average peak areas and relevant confidence interval.
119 The same PS and LDPEox solutions were also used to calibrate the FLD detector once checked the
120 most selective excitation and emission wavelengths for each polymeric material.

121 Fluorescence spectra were separately recorded with a Perkin Elmer LS55 instrument, using
122 reference materials and environmental samples in DCM, previously filtered on 0.2 µm PTFE
123 membrane. Both excitation (wavelengths in the 200 - 775 nm range) and emission (200 - 800 nm
124 range) spectra were recorded at 120 nm/min scan rate, adjusting the slits at 4 nm. It is worth
125 pointing out that, although at a given wavelength the intensity of fluorescence emission $F = \phi_F(I - I_0)$
126 (where ϕ_F is the quantum yield, I_0 and I the incident and transmitted radiation, respectively) scales
127 exponentially with the concentration C of the fluorophore along with equation (1),

128
$$\frac{I}{I_0} = e^{-2.3 \epsilon b C} \quad (1)$$

129 (where ϵ and b are the molar absorption coefficient and the optical path, respectively), the linear
130 approximation $I = I_0(1 - 2.3\epsilon b C)$ that holds for equation (1) under conditions of negligible inner filter
131 effect (low absorption coefficient at the excitation wavelength and low concentration) result in
132 linearity of the response also for the fluorescence intensity, $F = 2.3\phi_F I_0 \epsilon b C$. These are the conditions
133 normally used for analytical applications and that are found to apply in the systems investigated
134 here.

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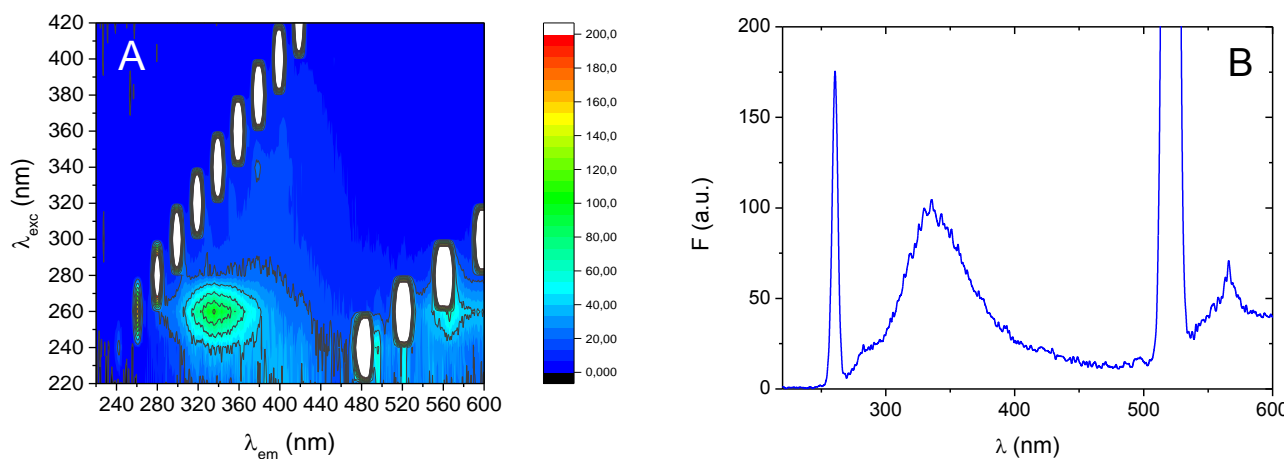
136 **Results**

137

138 *Definition and optimization of instrumental parameters with reference materials*

139 In order to optimize the operating parameters for the spectrofluorometric detector to be used in the
140 GPC analyses, 3D fluorescence spectra (x-axis: emission wavelength; y-axis: excitation

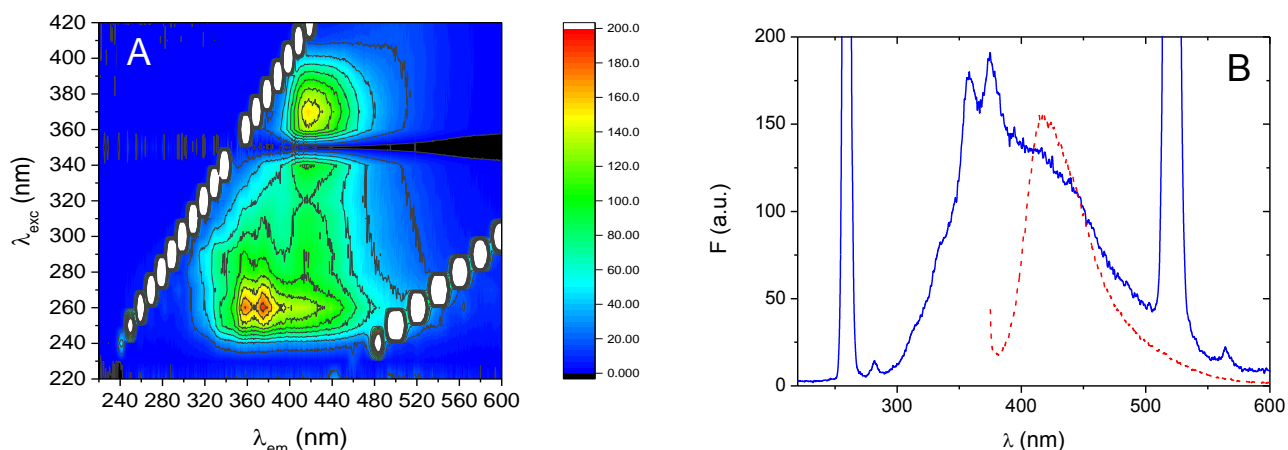
141 wavelength; z-axis; intensity) of the two selected reference materials were recorded. The obtained
142 fluorescence spectral map of polystyrene shows a maximum excitation at $\lambda_{\text{ex}}=260$ nm with a
143 corresponding maximum emission centered at 335 nm (figure 1). These features of the fluorescence
144 spectrum have been attributed to the emission from excimers formed between nearest neighbors
145 pendant phenyl groups,³²⁻³⁵ the fluorescence intensity being dependent on the molecular weight
146 (MW).³⁶
147



148
149 **Figure 1.** (A) 3D fluorescence spectral map the DCM extract of PS (x-axis = emission wavelength,
150 y-axis = excitation wavelength, z-axis = intensity); (B) emission spectrum of a solution
151 of PS in DCM with $\lambda_{\text{exc}} = 260$ nm.

152
153 In the case of the LDPEox reference material the maximum emission can again be obtained at
154 $\lambda_{\text{ex}}=260$ nm, resulting in two main emission bands centered at 358 and 375 nm, together with a
155 bathochromically shifted, broad shoulder above 400 nm that appears as a single and more intense
156 band upon excitation at $\lambda_{\text{ex}}=370$ nm. However, the 3D spectral map shows a secondary main
157 emission centered at 420 nm upon excitation at 370 nm (figure 2).

158

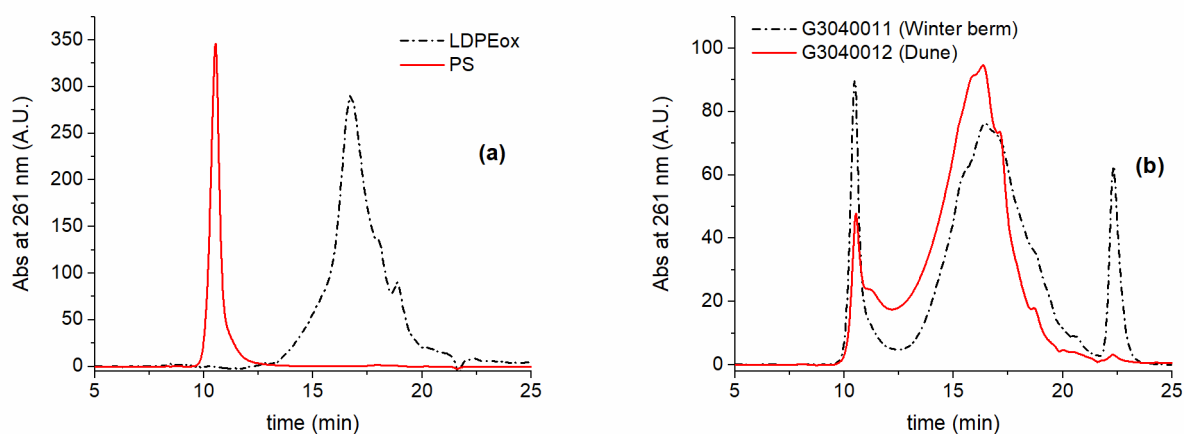


159

160 **Figure 2.** (A) 3D fluorescence spectral map of the DCM extract of LDPEox (x-axis = emission
 161 wavelength, y axis = excitation wavelength, z-axis = intensity); (B) emission spectra of
 162 a solution of LDPEox in DCM with $\lambda_{exc} = 260$ nm (solid line) and 370 nm (dashed line).

163

164 The presence of distinctive photoluminescence features in the PS and LDPEox samples allows to
 165 use them as the reference materials for setting up the operating conditions of a GPC apparatus
 166 equipped with fluorescence detector, with the aim of assessing the suitability and accuracy of the
 167 technique for the selective semi-quantitative determination of naturally oxidized PS and polyolefins.
 168 In figure 3 are reported the GPC traces recorded with a DAD detector at 261 nm from the analysis
 169 of the DCM-soluble fractions of the two reference materials and of the DCM extracts obtained from
 170 beach sand samples collected in the winter berm and dune sectors (samples G3040011 and
 171 G3040012, respectively) of a touristic seashore site in north Tuscany, Italy.⁸



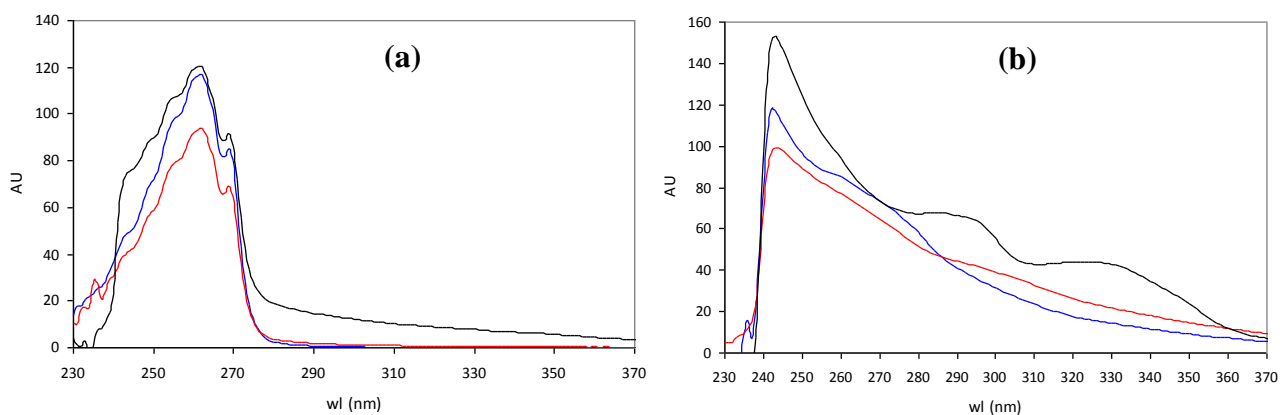
172

173 **Figure 3.** GPC traces recorded with DAD detector at $\lambda=261$ nm: (a) DCM-soluble fraction of
 174 LDPEox and from PS (whole sample) reference materials; (b) DCM extracts of the sand samples
 175 from the winter berm (sample G3040011) and dune (sample G3040012) sectors.

176

177 The GPC trace of LDPEox in figure 3a presents a single broad and structured peak at high retention
178 times (r.t.) associated with low-to-medium MW fractions (as expected, since high MW polyolefins
179 are insoluble in DCM); on the other hand the GPC trace of the reference PS is characterized by a
180 single peak at lower r.t. (its narrowness being most likely an artifact caused by a MW distribution
181 covering a range close to the exclusion limit of the GPC columns), indicating the almost exclusive
182 presence of high MW polymeric material. Indeed, the GPC traces from both sand DCM extracts
183 (figure 3b) show the presence of both a narrow peak at low r.t. and a broad peak at high r.t.,
184 indicating the presence of both high and low-to-medium MW fractions, respectively.

185 The UV spectra collected with the DAD detector in correspondence with the narrow peak at low r.t.
186 (structured absorption band with $\lambda_{\max}=262$ nm and a secondary characteristic peak at $\lambda=269$ nm)
187 and with the broad peak at high r.t. (broad absorption band with λ_{\max} around 242 nm and a long tail
188 extending up to nearly 400 nm) from the GPC fractionation of the two sand extracts matched quite
189 well those recorded under the same conditions from the reference PS and LDPEox, respectively
190 (figure 4).



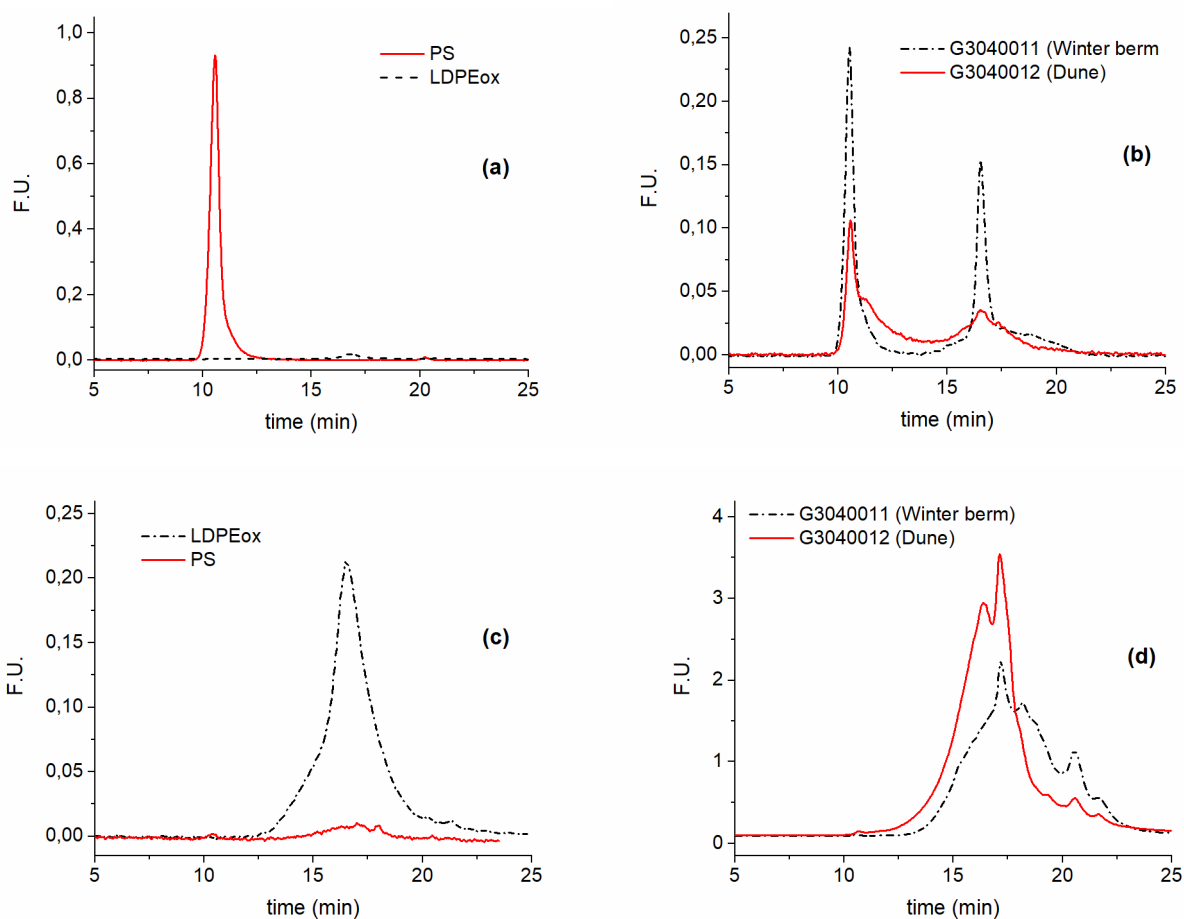
191
192 **Figure 4.** DAD UV spectra of GPC elution fractions of DCM extracts from test samples and
193 reference materials recorded at 10.5 min (a) and 16.5 min (b) retention time. Black lines: reference
194 PS (A) and LDPEox (B) polymeric material; red lines: winter berm sample; blue lines dune sample.
195

196 In order to check the accuracy of the DAD detector for the evaluation of the concentrations of the
197 two polymer types (PS and oxidized polyolefins) in real samples, two calibration curves were
198 obtained by running GPC analysis of PS and LDPEox DCM solutions at different concentrations.
199 The analyses, performed in triplicate for each concentration of the reference materials, gave a
200 strictly linear relationship between concentration and DAD peak area within the explored range of
201 25-5500 mg/L for PS and of 720-7400 mg/L for LDPEox.

202 The GPC runs with the same reference PS and LDPEox solutions and with the two beach sand
203 DCM extracts were then replicated in four subsequent experiments by setting up the fluorescence
204 detector (FLD) at four different excitation/emission wavelengths combinations: 260/280 nm,

205 260/335 nm, 370/395 nm, and 370/420 nm. Representative GPC traces obtained from the same
206 sample but with different FLD setup (excitation/emission wavelengths at 260/280 nm and 370/420
207 nm, respectively) are shown in figure 5.

208 In accordance with the fluorescence spectral map of the reference materials (figure 1 and figure 2),
209 when the fluorescence detector was set at 260/280 nm excitation/emission wavelengths the
210 fluorescence contribution of LDPEox (DCM-soluble fraction) was found to be negligible, as
211 opposed to the strong fluorescence response associated with PS materials (figure 5a-b). Such a
212 clear-cut discrimination of the two polymeric materials was obtained neither by recording the
213 emissions at 335 nm with the same excitation wavelength, nor with the FLD set at 370/385 nm
214 excitation/emission. On the other hand, with the FLD set at 370/420 nm excitation/emission the
215 fluorescent response of PS-like materials becomes negligible, while LDPEox and, more generally,
216 oxidized polyolefins exhibit a residual fluorescence sufficient for their selective quantification
217 (figure 5c-d).



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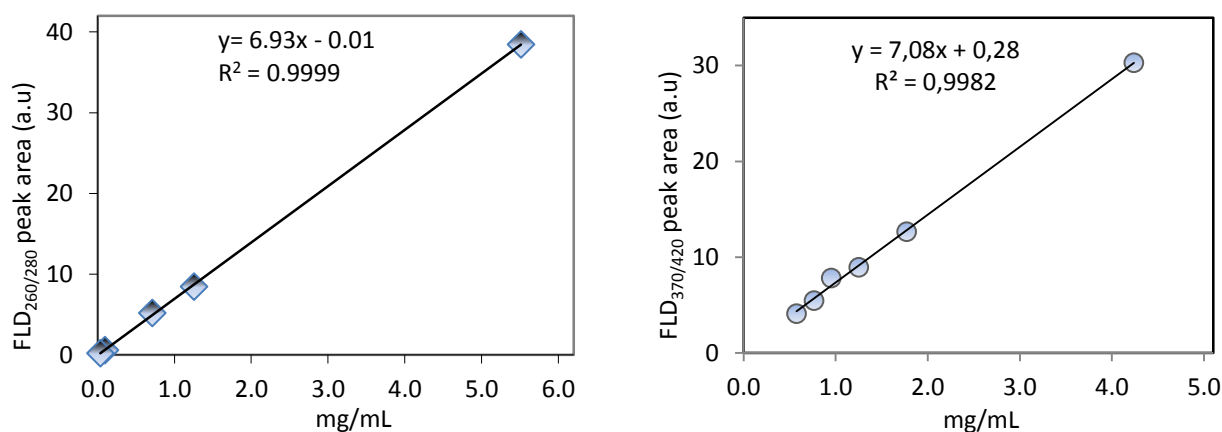
219

220 **Figure 5.** GPC traces recorded with different FLD set ups: with 260/280 nm excitation/emission
221 wavelengths from LDPEox and PS reference materials (a) and from the DCM extracts of the beach
222 sand samples from the winter berm and dune sectors (b); with FLD set up at 370/420 nm
223 excitation/emission wavelength from LDPEox and PS reference materials (c) and from the DCM
224 extracts of winter berm and dune samples (d).

225

226 Based on the above results, the 260/280 and 370/420 nm combinations of excitation/emission
227 wavelengths can be considered as suitable to distinguish and recognize polystyrene-like materials
228 from degraded and oxidized polyolefins within the same sample along with GPC separation.

229 To assess the accuracy of the detection also for the quantitative analysis of these two classes of
230 polymeric materials, and more specifically for their determination as environmental contaminants
231 extracted as DCM soluble fraction from coastal sand sediments, two distinct calibration curves were
232 obtained by analyzing DCM solutions of the reference PS (5.51 - 0.033 mg/mL range) and DCM
233 extracts of the reference LDPEox (4.33 - 0.57 mg/mL range) with the FLD set at 260/280 nm and
234 370/420 nm excitation/emission, respectively. In figure 6 are reported the linear fits of the
235 calibration curves obtained by considering the GPC peak areas from the GPC/FLD traces.



236

237 **Figure 6.** GPC calibration curves based on FLD detector. Left: reference PS ($\lambda_{exc}=260$ nm; $\lambda_{em}=280$
238 nm); right: reference LDPEox ($\lambda_{exc}=370$ nm; $\lambda_{em}=420$ nm).

239

240 The experimental (weighed extracts) and calculated data from the quantitative analysis of the two
241 environmental samples G3040011 (winter berm) and G3040012 (dune) are reported in table 1,
242 where the calculated data are based on DAD and FLD detection of the GPC eluates, respectively,
243 and POox indicates the DCM-soluble fraction of degraded polyolefins in general, including LDPE,
244 HDPE (high density polyethylene), PP, and similar semicrystalline olefin copolymers.

245 The main source of the mismatch between the weighed total amounts of extracted microplastic
246 material and the sum of PS and POox as calculated from the FLD peak areas based on the relevant
247 calibration curves (e.g. 18.7 g vs. 8.4+3.3=11.7 g, respectively, for the winter berm sample) could
248 be ascribed to the heterogeneous nature of the POox fraction originated from the environmental
249 pool. As a means to clarify this issue, as discussed later, in figure 7 are reported the GPC traces of
250 the reference LDPEox recorded with three different detectors, namely refractive index (RI),
251 providing a signal intensity scaling linearly with concentration by mass, UV and FLD, the intensity

252 of their response being proportional to the concentration of the absorbing/emitting
 253 chromophores/fluorophores. The peak intensities are normalized for easier comparison.

254

255 **Table 1.** Content and composition of the DCM extracts from environmental sand samples
 256 calculated using the DAD and FLD calibration curves based on PS and LDPEox reference materials.

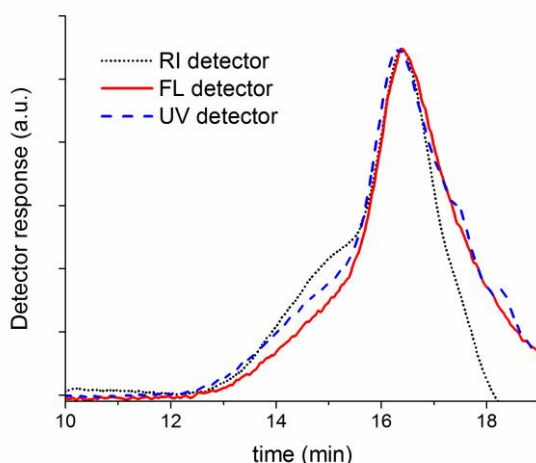
| Sampling sector | Sample | Total extracts ^(a) (mg/kg) | High MW PS ^(b) (mg/kg) | | Total PS ^(c) (mg/kg) | POox ^(d) (mg/kg) |
|-----------------|----------|--|--------------------------------------|------------------------|------------------------------------|--------------------------------|
| | | | DAD | FLD _{260/280} | | |
| | | | FLD _{260/280} | FLD _{370/420} | | |
| Dune | G3040012 | 327.0 | 57.0±7.6 | 66.1±4.1 | 104.3±7.0 | 76.9 |
| Winter berm | G3040011 | 18.7 | 6.1±1.5 | 5.7±0.5 | 8.4±1.0 | 3.3 |

257 ^(a) weighed amount of polymeric material extracted with DCM from 1 kg sand;

258 ^(b) calculated from the lower r.t. GPC peak area (see reference 3);

259 ^(c) calculated from the combined low r.t. (high MW fraction) and high r.t. (low MW fraction) GPC
 260 peak areas using the FLD_{260/280} detector and related PS calibration curve.

261 ^(d) calculated from the calibration curve obtained using LDPEox as the reference material.



262

263 **Figure 7.** Normalized GPC traces of LDPEox as recorded with refractive index (RI), DAD (UV
 264 absorption at $\lambda=260$ nm) and FLD ($\lambda_{exc}=370$ nm; $\lambda_{em}=420$ nm) detectors.

265

266 Discussion

267

268 Photoluminescence of polyolefins is somewhat controversial,³⁷ having been associated with the
 269 presence of oxidized “impurities” such as carbonyl end groups,^{38,39} α,β -unsaturated carbonyl (enone
 270 and dione types)⁴⁰ and dicarbonyl species. Photoluminescence and chemiluminescence properties of
 271 oxidized polyolefins have already been exploited for quantitative analyses because of their higher
 272 sensitivity with respect to e.g. FT-IR spectroscopy; the kinetics of thermo- and photo-oxidative
 273 degradation of polyolefins could thus be studied from the very onset, when only few oxidized

274 groups are present.⁴¹⁻⁴⁴ In most cases, an initial decrease in the emission, ascribed to the loss of
275 fluorescent additives (e.g. antioxidants) has been observed, followed by a substantial increase
276 closely related with the evolution of degradation processes.⁴³ Such post-oxidative luminescence has
277 been generally attributed to the initial formation of α,β -unsaturated carbonyl and similarly
278 oxygenated groups, followed by the development of more extended conjugated systems such as
279 short polyene sequences that may or may not be conjugated to carbonyl groups;^{45,46} the latter are
280 typically excited at longer wavelengths (between 300 and 400 nm) with ensuing fluorescence at
281 wavelengths exceeding 500 nm.^{43,47}

282 Owing to these properties, selective detection of PS and LDPEox photoluminescence is possible,
283 because the former would not be excited (and thus would not exhibit any photoluminescence) upon
284 excitation in the visible range of wavelengths, as opposed to the latter. The complementary spectral
285 response for the two polymer species is highlighted in figure 5a,c, in which the GPC traces for the
286 DCM extracts of the two reference materials are recorded with the FLD detector set at either
287 260/280 (for the selective detection of PS) or 370/420 nm (for LDPEox) excitation/emission
288 wavelengths.

289 The GPC traces recorded from the DCM extracts of the beach samples, figure 5b,d, show in both
290 cases the presence of a high and a low-to-medium MW fraction. Despite the inherently complex
291 composition of the samples, the UV spectra collected with the DAD detector in correspondence
292 with the narrow GPC peak at low r.t. and with the broad peak at high r.t. were found to match with
293 those of the reference PS and LDPEox, respectively (figure 4). This suggests that the chosen
294 reference materials are appropriate to describe the DCM extracts from the sandy beach samples,
295 irrespective of the fact that LDPEox is actually a heterogeneous mixture of variously oxidized
296 medium-to-low MW LDPE degradation products of a single polyolefin type.

297 Concerning the PS content, both FLD and DAD detectors allow quantitative analysis for the high
298 MW fraction, as this is nearly pure PS since even partially oxidized polyolefins are not soluble
299 DCM at molecular weights exceeding a few thousand Daltons. On the other hand, while DAD does
300 not allow discrimination between PS and POox, by setting the FLD detector at 260/280 nm
301 excitation/emission it is possible to selectively perform quantitative analysis of the PS content in the
302 low MW fraction containing also POox, and thus the total amount of PS can be determined .

303 Instead, the figures calculated from the GPC peak area with FLD set for POox detection and based
304 on the calibration with LDPEox appear to largely underestimate the actual content in the
305 environmental extracts as determined by gravimetry (see table 1). Such a mismatch may be at least
306 partially ascribed to the presence of low MW hydrocarbon species such as natural waxes and heavy
307 aliphatic hydrocarbon pollutants (e.g. from oil spillage). However, an additional source of
308 inaccuracy may be ascribed to the intrinsic limitations of the spectroscopic detection, associated

309 with calibration based on a specific LDPEox reference material, for the quantitative determination
310 of the DCM-soluble fraction of the variable pool of POox from sampled microplastics. The GPC
311 traces in figure 7 clearly show that the two spectroscopic detectors, and FLD in particular,
312 underestimate the amount of higher MW fraction and overestimate the lower MW one as compared
313 to the RI detector. Such discrepancy can be ascribed to the differences in fluorophores
314 (photoluminescent moieties) molar concentrations per unit mass of POox as a function of MW, a
315 result of photooxidative chain scission occurring largely by beta elimination with formation of
316 carbonyl groups at the chain end, which may then further react to yield fluorescent conjugated
317 moieties.⁴¹ Thus, the shorter the residual chain fragment, the higher the molar concentration of
318 fluorophores per unit mass, in agreement with the observed mismatch between the response of FLD
319 and RI detectors.

320 Therefore, depending on the actual composition of a given environmental POox sample, the
321 calibration based on LDPEox may result in either underestimation (for highly degraded
322 microplastics with a larger low-MW fraction) or overestimation of the actual amount of POox.
323 However, since extensive degradation is required for polyolefin fragmentation and pulverization,
324 the structural features of different polyolefins once turned into microplastics are likely to be
325 similar.⁴⁸

326

327 **Conclusions**

328 GPC chromatography with fluorescence detection (FLD) allows selective determination of
329 hydrocarbon-based microplastics pollutants sampled from marine and freshwater coastal sediments.
330 Linear FLD response with concentration was found for both PS and LDPEox reference materials
331 used as calibration standards. Accurate direct determination of PS can be achieved, while for
332 polyolefins the analysis is semi-quantitative, being limited to the DCM-soluble, highly degraded
333 polyolefin fraction. In the latter case a lower accuracy may result from the intrinsic structural
334 variability of the POox pool and from the environmental contamination by hydrocarbons pollutants
335 (e.g. from oil spillage).

336 Compared to the most common analytical procedures based on particle counting and identification,
337 the proposed method based on solvent extraction and GPC-FLD analysis provides no information
338 on the size distribution of the microplastics; besides, different polyolefins cannot be distinguished.

339 Nevertheless, the simple and straightforward method proposed here allows to perform qualitative
340 and semi-quantitative determination of the microplastics from marine sediments, preferably in
341 combination with gravimetric determination of the whole DCM soluble fraction.

342 Finally, it is worth pointing out that the onset of pulverization of a macroscopic polyolefin item,
343 resulting in the generation of secondary microplastics, occurs when thorough oxidative degradation

344 has already been achieved. Thus the DCM-soluble fraction is likely to be the main one in polyolefin
345 microplastic particles and its fluorescence response is not expected to be significantly affected by
346 the type of original polyolefin material.

347

348

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351

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Supplementary Data

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