 doi:10.20944/preprints202102.0211.v1

Article

Polymer identification and specific analysis (PISA) of microplastic total mass in sediments of the protected marine area of the Meloria Shoals

Valter Castelvetro [1,2,*], Andrea Corti [1,2], Jacopo La Nasa [1], Francesca Modugno [1,2], Alessio Ceccarini [1], Stefania Giannarelli [1] and Virginia Vinciguerra [1,2]

[1] Department of Chemistry and Industrial Chemistry, University of Pisa, 56124 Pisa, Italy; valter.castelvetro@unipi.it
[2] CISUP - Center for the Integration of Scientific Instruments of the University of Pisa, University of Pisa, 56124 Pisa; valter.castelvetro@unipi.it
* Correspondence: valter.castelvetro@unipi.it; Tel.: +39-0502219256

Abstract: Microplastics (MPs) quantification in benthic marine sediments is typically performed by time-consuming and moderately accurate mechanical separation and microscopy detection. In this paper we describe the results of our innovative Polymer Identification and Specific Analysis (PISA) of microplastic total mass, previously tested on either less complex sandy beach sediment or less demanding (because of the high MPs content) wastewater treatment plant sludges, applied to the analysis of benthic sediments from a sublittoral area north-west of Leghorn (Tuscany, Italy). Samples were collected from two shallow sites characterized by coarse debris in a mixed seabed of *Posidonia oceanica*, and by a very fine silty-organogenic sediment, respectively. After sieving at <2 mm the sediment was sequentially extracted with selective organic solvents and the two polymer classes polystyrene (PS) and polyolefins (PE and PP) were quantified by pyrolysis-GC/MS. A contamination in the 8-65 ppm range by PS could be accurately detected. Acid hydrolysis on the extracted residue to achieve total depolymerization of all natural and synthetic polyamides, tagging of all aminated species in the hydrolyzate with a fluorophore, and reversed-phase HPLC (RP-HPLC) analysis, allowed to quantify within the 137-1523 ppm range the individual mass of contaminating Nylon 6 and Nylon 6,6, based on the detected amounts of the respective monomeric amines 6-aminohexanoic acid (AHA) and hexanediamine (HMDA). Finally, alkaline hydrolysis of the residue from acid hydrolysis followed by RP-HPLC analysis of the purified hydrolysate showed contamination by polyethylene terephthalate (PET) in the 12.1-2.7 ppm range, based on the content of its comonomer, terephthalic acid.

Keywords: microplastics; marine sediment; pet; nylon 6; nylon 6,6; reversed-phase HPLC; polyolefin; polystyrene; Pyr-GC/MS; polymer degradation.

1. Introduction

Plastic microparticles, commonly referred to as microplastics (MPs), either deriving from the environmental degradation of larger plastic waste items [1-3] or directly released as primary microparticles (microbeads, textile microfibers) in wastewaters, are a class of pollutants detected in virtually all natural environments, from oceans to inland waters [4], soils and even as airborne material [5], reaching such remote areas as the Arctic and Antarctica [6,7]. The ubiquitous presence of MPs, and likely so also of their ultimate products of further degradation into sub-micrometer sized particles (nanoplastics) [8], along with incipient evidence of their adverse interaction with living organisms [9], has stimulated increasing research efforts aimed at understanding their transport, distribution and fate [10-12]. Due to their small size microplastic can be ingested by various organisms at all trophic levels, and increasing scientific evidences highlight the

 doi:10.20944/preprints202102.0211.v1

possibility of their transfer into animal tissue and up the food chain reaching humans [13,14].

The most common synthetic polymers in plastic waste are polyolefins (homo- and copolymers of ethene and propene) and polystyrene, widely used in packaging and single use disposable items such as tableware; polyester (mainly polyethylene terephthalate, PET, used for beverage bottles, packaging and as staple textile fiber) and polyamides (often referred to according to the tradename nylons) represent an additional significant fraction of MPs pollution. In the case of polyolefins, the environmental degradation processes are mainly ascribed to photo-oxidation, resulting in oxygen pickup due to free radical reactions with cascade effects eventually leading to polymer chain fragmentation and insertion of oxidized functional groups (carbonyls, carboxyl, hydroxyl, etc.) [15]. Such chemical transformations bear several consequences: i) the initially high molecular weight is reduced and the polymeric material becomes more brittle, promoting progressive fragmentation into increasingly smaller particles; ii) its density and hydrophilicity increase along with surface polarity and reactivity, enhancing adsorption/absorption of low molecular weight organic (including toxic polycyclic aromatic hydrocarbons, PAHs, and polychlorinated biphenyls, PCBs) and inorganic (heavy metals) environmental pollutants; iii) increased wettability and specific surface area facilitate bio-fouling and adhesion inorganic particulate, all of the above promoting sinking down the water column and deposition in both shore and benthic sediments [16-19]. It has been estimated that less than 1% of the 5-12 million tons per year of plastics entering the oceans stays afloat for a long time, the remaining fraction reaching the seabed either in a very short time (this is the case of larger items of higher density plastics such as e.g. PET or low density polymers with inorganic fillers) or over longer periods regardless of the initial density because of the abovementioned degradation and fouling phenomena [12,20-22].

Here we report the results of the first investigation in which our recently developed analytical protocol for the quantitative determination of the total mass content of a well-defined set of microplastics [23], hereafter Polymer Identification and Specific Analysis (PISA), was employed for benthic marine sediments. The protocol allows the accurate quantification of the total mass of individual microplastic types, regardless of their size and morphology, of the following polymers: polyolefins (high density polyethylene, HDPE, low density polyethylene, LDPE, and polypropylene, PP), polystyrene (PS), polyethylene terephthalate (PET), and the two polyamides nylon 6 (polycaprolactame, the homopolymer of 6-aminohexanoic acid, AHA) and nylon 6,6 (copolymer of 1,6-hexanediamine, HMDA, with adipic acid) [24]. These are also the main commodity polymers and, not incidentally, represent the main macro- and microplastic marine pollutants. The benthic sediment samples were collected in two close locations of the Ligurian Sea within the shoals (Meloria protected marine area) and in shallow coastal waters, respectively, close to the harbor of Leghorn and the estuary of the Arno river, Italy. The sediments were sieved at 2 mm mesh and submitted to a sequence of fractional solvent extractions with refluxing dichloromethane (DCM) and xylene (Xy) as selective solvents for PS and polyolefins, respectively [25], followed by sequential hydrolytic depolymerization of polyamides, under acidic conditions, and of PET, under alkaline conditions. The total content of nylon 6 and nylon 6,6 are then calculated from the quantitative analysis, by reversed-phase HPLC, of the monomeric amines suitably tagged with a fluorophore [26], and of the dicarboxylic acid TPA [27], respectively.

2. Materials and Methods

2.1. Sediment sampling

Benthic (bottom) marine sediment samples were collected in four sites of relatively shallow waters of the continental shelf in the Ligurian sea along the northern coastline of Tuscany, Italy (Table 1). The sampling was performed on july 3rd, 2018, using a single corer 10 cm in diameter to collect the top ~5 cm of the benthic sediment.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 February 2021 doi:10.20944/preprints202102.0211.v1

Table 1. Sample acronyms and relevant sampling sites coordinates and depth.

Sample	Acronym	Geolocalization		Depth (m)
Meloria 1	MEL1	43°32'50.0"N 10°13'08.2"E	43.547219 lat. 10.218944 lon.	3
Meloria 2	MEL2	43°33'1,02" N 10°13'4,03" E	43.5502778 lat. 10.2177778 lon.	4
Calambrone 1	CAL1	43°35'5,21" N 10°17'2,34" E	43.5847778 lat. 10.2839722 lon.	20
Tirrenia-Calambrone 2	CAL2	43°36'9,67" N 10°16'7,80" E	43.6026944 lat. 10.2688333 lon.	17

The sedimentologic features are representative of two distinct benthic zones: the MEL samples were collected in the shoals of the marine Protected Area "Secche della Meloria", about 3 miles west of the harbour of Leghorn, with the bottom sediments consisting mainly of fragmented organogenic shells and carbonatic sand; the CAL samples were collected northeast of the MEL area, about 0.5-1 mile off the shore of intensive seasonal touristic sandy beaches and 5 miles south of the Arno river estuary, with the bottom sediments consisting of very fine sandy to silty material (Figure 1).

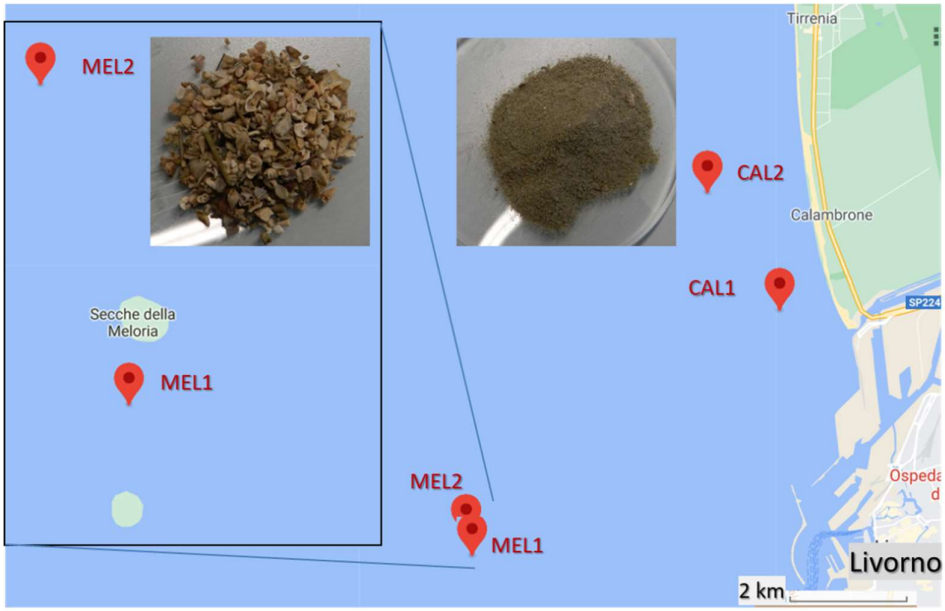


Figure 1. Sampling sites and morphology of the two types of sediment sample: CAL and MEL

2.2. Chemicals

Dichloromethane (DCM, 99.9%, stabilized with amylene, Romil-SpS, Romil Ltd., Cambridge, UK), xylene (Xy, 98.5%, Sigma-Aldrich, Merck Life Science S.r.l., Milano, Italy) methanol (99.9%, Sigma-Aldrich), acetic acid (99.85% Sigma-Aldrich), sulfuric acid (95-98%, Merck), hydrogen peroxide (30% w/v, Panreac, Nova Chimica Srl, Cinisello Balsamo, Italy), 6 N aqueous hydrochloric acid (prepared from 37 % HCl, Sigma-Aldrich), 1.9 N aqueous sodium hydroxide (from NaOH pellets, 98.0%, Sigma-Aldrich), hexadecyl-tributyl-phosphonium bromide (TBHDPB, 97%), HPLC grade

 doi:10.20944/preprints202102.0211.v1

water (Sigma-Aldrich), and reversed-phase SPE cartridges (Chromabond® C18ec loaded with 500 mg stationary phase, Macherey-Nagel GmbH & Co., Düren, Germany) were used for sediment extractions, extracts purifications, and for the acid and alkaline depolymerizations of the hydrolyzates. Dansyl chloride (DNS-Cl, 96%, Alfa Aesar), n-butylamine (99.5%, Fluka) and potassium carbonate (K_2CO_3, Carlo Erba) were used in the dansylation of PA's monomers. Chloroform (HPLC grade stabilized with ethanol, Sigma-Aldrich) was used as mobile phase in SEC analysis. Acetonitrile (HPLC grade, ≥ 99.9%, Sigma-Aldrich), HPLC grade water (Sigma-Aldrich), triethylamine (≥ 99.9%, Fluka), acetic acid (99,85%, Sigma-Aldrich), methanol (99.9%, Sigma-Aldrich), and phosphoric acid (Sigma-Aldrich) were used in the preparation of the HPLC eluents for determination of dansylated amine monomers in the acid hydrolyzates for the quantitative analysis of terephthalic acid in the alkaline hydrolyzates.

2.3 Analytical techniques

Size exclusion chromatography (SEC) analyses were performed with an instrument consisting of a Jasco (Jasco Europe S.R.L., Cremella, LC, Italy) PU-2089 Plus four-channel pump, a PL gel (Polymer Laboratories) pre-column packed with polystyrene/divinylbenzene, two in series PL gel MIXED D columns placed in a Jasco CO_2063 column oven, a Jasco RI 2031 Plus refractive index detector, and a Jasco UV-2077 Plus multi-channel UV spectrometer. Chloroform was used as the eluent at 1 mL/min flow rate.

Pyrolysis-Gas Chromatography/Mass spectrometry (Py-GC/MS). Analyses were performed using a multi-shot pyrolyzer EGA/PY-3030D (Frontier Lab, Japan) coupled with a 6890N gas chromatographic system with a split/splitless injection port and combined with a 5973-mass selective single quadrupole mass spectrometer (Agilent Technologies, USA). For the analysis of the extracts, 100-150 μL of solution were placed in stainless steel cups together with 2.0 μL of a solution of dibutyl phthalate-3,4,5,6-d_4 (500 ppm), and dried under nitrogen steam prior to the analyses [28]. The temperatures used for the double shot pyrolysis were 350 °C and 600°C while the interface was set at 280°C. The GC injection port temperature was 280 °C. The GC injection was operated in split mode with a split ratio of 1:10. For the analysis of particles, the pyrolysis was performed in a single shot at 600 °C with the interface set at 280°C and split ratio 1:10 [29]. The chromatographic and mass spectrometric conditions were as follows: 5 min isotherm at 50 °C, heating up to 180 °C at 12 °C/min, 2 min isotherm, heating up to 300 °C at 8 °C/min, and 20 min isotherm; 1.2 mL/min He (99.9995%) carrier gas; GC/MS interface temperature 280 °C and MS electron ionization voltage 70 eV. Perfluorotributylamine (PFTBA) was used for mass spectrometer tuning. MSD ChemStation (Agilent Technologies) software was used for data analysis and the peak assignment was based on a comparison with libraries of mass spectra (NIST 8.0).

Micro-ATR FT-IR analyses were performed with a Perkin Elmer Spectrum Autoimage System microscope equipped with a germanium ATR crystal operated in the mid-IR region (700–4000 cm^{-1}) using 64 scans at 4 cm^{-1} spectral resolution. The lateral spatial resolution corresponds to the contact area with the germanium crystal tip (30–40 micron).

Two instrumental setups were used for High Performance Liquid Chromatography (HPLC). Quantitative determination of TPA was performed using a Jasco PU-1580 isocratic pump connected with a Jones-Genesis Aq 120 reversed-phase column (150 mm x 4.6 mm, 4 μm particle size) operating at room temperature and a Jasco 1575 UV-Vis detector (UVD) set at 242 nm wavelength. Analyses were carried out on 20 μL of the solutions at 0.8 mL/min flow rate of an isocratic 30/70 vol/vol methanol/HPLC-grade water (acidulated with 0.1 wt% phosphoric acid) eluent. The DNSCl derivatives of AHA and HMDA were analysed with an Agilent 1260 Infinity Binary LC instrument equipped with pre-column, a reversed-phase Phenomenex-Aqua C18 column (250 mm x 4.6 mm, 5 μm particle size) and diode array (DAD VL+ 1260/G1315C, set at 335 nm wavelength) plus

 doi:10.20944/preprints202102.0211.v1

fluorescence (FLD 1260/G1321B, set at 335/522 nm excitation/emission wavelengths) double detector. Elution was performed at 1.0 mL/min flow rate in gradient mode by combining an aqueous solution of 2.5 % acetic acid and 0.83 % triethylamine (phase A) with acetonitrile (phase B) according to the program reported in Table 2.

Table 2. Elution program adopted for the analysis of dansylated amine derivatives.

Elution time (min)	Mobile phase (A) %	Mobile phase (B) %	Elution mode
0 → 20	60	40	isocratic
20 → 25	60 → 30	40 → 70	gradient
25 → 35	30	70	isocratic
35 → 37	30 → 60	70 → 40	gradient
37 → 50	60	40	isocratic

2.4 Synthetyc polymer recovery by sequential extractions with selective solvents

Sediment samples were submitted to a first step of sequential extractions with refluxing dichlormethane (DCM) followed by a second step in refluxing xylene. Approximately 100 g of sediment from each sample that had been previously sieved at 2 mm and air dried to nearly constant weight in equilibrium with the atmospheric lab conditions, was loaded in the thimble of a kumagawa apparatus and extracted for 2 h with 250 mL refluxing DCM. Before each extraction the apparatus was conditioned by refluxing 100 mL DCM for 3 h to remove any contaminant. The extract was reduced to 1-2 mL in a rotatory evaporator, then transferred into a 5 mL glass-vial conditioned to constant weight in an oven at 60 °C and weighed. The residues from the DCM extraction were then further extracted in the same apparatus with 250 mL refluxing xylene. The obtained xylene solution was transferred into a two-necked flask fitted with distillation head and condenser, reduced to 5 mL by distilling off the excess solvent, and then added with 20 mL of 1.6 M KOH in methanol; the obtained precipitate, mainly consisting of polyolefins (PP, LDPE, HDPE), was then collected by vacuum filtration through a 0.22 μm Durapore PVDF membrane (without PP backing).

2.5 Hydrolytic depolymerization procedures

The total content of nylon 6, nylon 6,6, and PET contaminating MFs in the sediment samples was calculated from the content of the corresponding monomers 6-aminocaproic acid (AHA), 1,6-hexanediamine (hexamethylenediamine, HMDA), and terephthalic acid (TPA), respectively, in the hydrolyzates obtained from the acid and alkaline depolymerizations for the polyamides and the polyester, respectively. The dry solid residue from the organic solvents extraction of each sample was transferred into a 100 mL round-bottomed flask equipped with a reflux condenser and magnetic stirring bar. After addition of approximately 80 mL 6 N HCl the stirred mixture was heated to the reflux temperature of about at 105 °C for 24 hours. At the end of the hydrolysis, the reaction mixture was vacuum-filtered on a 0.22 μm PVDF membrane to separate the solid residue from the acid solution. The filter membrane with the solid residue was carefully rinsed with small amounts of HPLC grade water for the subsequent treatments, while the acid solution was transferred in a 100 mL volumetric flask and taken to volume with 6 N HCl. A given volume (5 mL) of the obtained solution was weighed and neutralized to pH 6.5-7.5 with 5 N NaOH. To enable a highly sensitive quantification of the amino monomers AHA and HMDA, the solutions were treated with 5-dimethylaminonaphtalene-1-sulfonyl chloride (dansyl chloride, DNSCl), a derivatizing fluorophore commonly used in protein sequencing (Figure 2).

 doi:10.20944/preprints202102.0211.v1

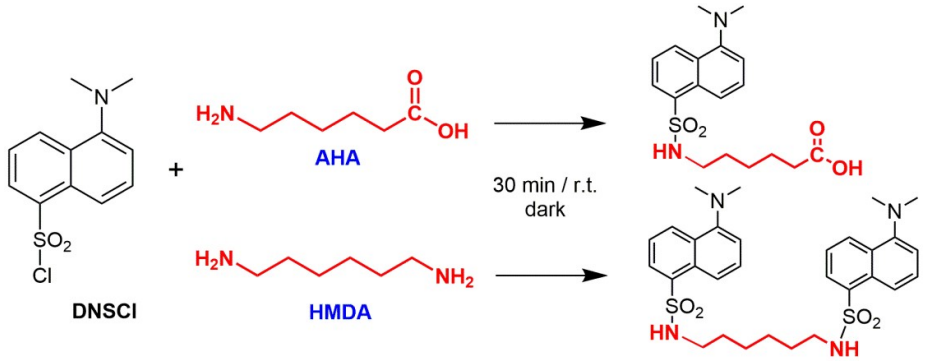


Figure 2. Dansylation of the amino-monomers from the depolymerization of nylon 6 and nylon 6,6.

For this purpose, 1 mL of the neutralized product of acid hydrolysis was loaded in a 5 mL glass vial, added with 1.0 mL aqueous K_2CO_3 solution (80 g/L), so as to favor the precipitation of calcium carbonate if present in the neutralized solution. After allowing the obtained mixture to settle, 1 ml was taken and added with a further 1.0 mL aqueous K_2CO_3 solution and 1.0 mL of a 5 g/L solution of DNSCl in acetone (18.5 µmol). After 30 min stirring at room temperature in the dark, an excess of n-butylamine (5.0 µL, 51·µmol) was added to quantitatively convert the unreacted DNSCl. The solution containing the derivatized amines (including those from the hydrolysis of both natural and synthetic polyamides) was then transferred into a 10 mL volumetric flask and taken to volume with a 1:1 (v/v) water/acetone mixture before HPLC analysis.

For the determination of the PET content, the solid residues collected at the end of the acid hydrolysis were treated under alkaline hydrolytic conditions to achieve the complete PET depolymerization. For this purpose each residue was rinsed with de-ionized water on the same PVDF membrane used for filtration, then transferred into a 100 mL round bottomed flask equipped with a reflux condenser and magnetic stirring bar, added with 40 mL 1.9 N NaOH and TBHDPB as a phase transfer catalyst, then the mixture was stirred at 85 °C for 6 hours. The final solution was vacuum-filtered on a 0.22 µm PVDF membrane, then transferred into a 50 mL volumetric flasks and taken to volume with 1.9 N NaOH. For the removal of most of the residual biogenic contaminants before HPLC analysis, 1 mL of hydrolyzate was weighed at 0.1 mg accuracy, transferred into a 10 mL glass-vial with 1–2 mL of 30 vol% H_2O_2 until complete discoloration and/or end of visible bubble formation, then added with 1 mL 1.9 M H_2SO_4. The resulting acidic solution was eluted through a reversed-phase SPE cartridge, the adsorbate was then desorbed with 0.8 mL MeOH and the recovered roughly 0.8 mL solution in methanol was weighed at 0.1 mg accuracy. Finally, 0.5 mL of the solution was taken up with a micropipette, placed in a vial and weighed again at 0.1 mg accuracy, then added with 0.75 mL aqueous CH_3COOH (1 wt% in HPLC-grade water) to obtain a 40/60 vol% methanol/water mixture.

The amounts of contaminating polymers in each sample (given in ppm, or mg polymer/kg dry sludge) was calculated from the corresponding monomer concentration C_{AHA}, C_{HMDA}, and C TPA (in ppm) as determined by HPLC, based on the calibrated response of both UV and fluorescence detectors (see Figure 4), according to equations 1-3:

$$\text{Nylon 6 (ppm)} = C_{AHA} \cdot \frac{MW_{PA6}}{MW_{AHA}}\ , \quad (1)$$

$$\text{Nylon 6,6 (ppm)} = C_{HMDA} \cdot \frac{MW_{PA6,6}}{MW_{HMDA}}\ , \quad (2)$$

$$\text{PET (ppm)} = C_{TPA} \cdot \frac{MW_{PET}}{MW_{TPA}}\ , \quad (3)$$

 doi:10.20944/preprints202102.0211.v1

where MW_{PA6}=113.16 g/mol, $MW_{PA6,6}$=226.32, and MW_{PET}=192.2 are the molecular weights of the repeat units in the corresponding polymer (Figure 3), and MW_{AHA}=131.17 g/mol, MW_{HMDA}=116.21 g/mol, and MW_{TPA}=166.13 g/mol those of the analytes.

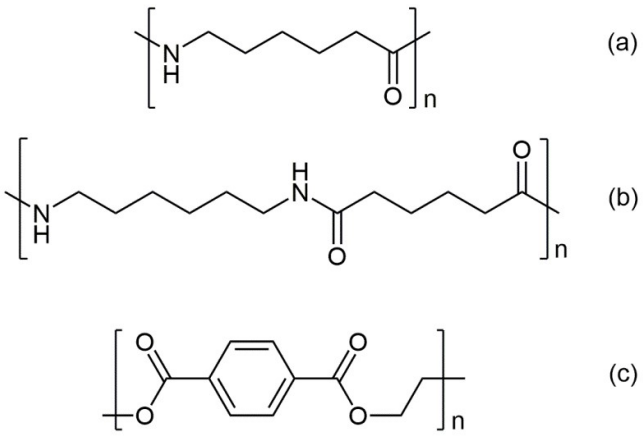


Figure 3. Polymer repeating units: (**a**) Nylon 6 (polycaprolactame, the homopolymer of 6-aminohexanoic acid); (**b**) Nylon 6,6 (copolymer of adipic acid-and 1,6-hexanediamine); (**c**) PET (polyethylene terephthalate).

2.6 Calibrations

Calibration of the response of the pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) system used for PS quantification in the DCM extract was performed by analysing a set of PS solutions prepared starting from a 100 ppm solution of PS in DCM, then weighed amounts of calibration solution with a total PS content in the 20−150 μg range were loaded in the crucible and dried. The quantification was based on the response (GC/MS peak) for the fragment corresponding to the styrene dimer [29], which gave a linear regression of the experimental calibration (r^2=0.9998), from which a limit of detection LOD=0.10 μg and a limit of quantification LOQ=0.35 μg (where LOD=3·*SD*/*m* and LOQ 10·*SD*/*m*, with *SD* standard deviation of the blank areas, and *m* slope of the calibration curve) could be calculated.

For the HPLC analyses of the dansylated amines the FLD detector response was calibrated against the concentration of dansylated AHA by recording a 4-point calibration using solutions in the 17.25-172.5 μg/L range plus a blank sample, all in triplicate; the same procedure was followed for dansylated HMDA, in the 13.25-132.5 μg/L range plus the blank sample. From the linear regression (Figure 4a,b) obtained for dansylated AHA ($A=1.246\cdot10^{-2}\cdot C_{AHA}+5.4\cdot10^{-2}$; r^2=0.99784) and dansylated HMDA ($A=1.682\cdot10^{-2}\cdot C_{HMDA}-8.195\cdot10^{-2}$; r^2=0.99791) the following values were calculated: LOD_{AHA}=0.903 μg/L; LOQ_{AHA}: 3.910 μg/L; LOD_{HMDA}=0.301 μg/L; LOQ_{HMDA}: 0.758 μg/L. The LOD and LOQ values are given as:

$$LOD = \frac{standard\ deviation\ of\ most\ diluited\ solution}{slope\ of\ linear\ regression} \cdot 3;$$

$$LOQ = \frac{standard\ deviation\ of\ most\ diluited\ solution}{slope\ of\ linear\ regression} \cdot 10.$$

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 February 2021 doi:10.20944/preprints202102.0211.v1

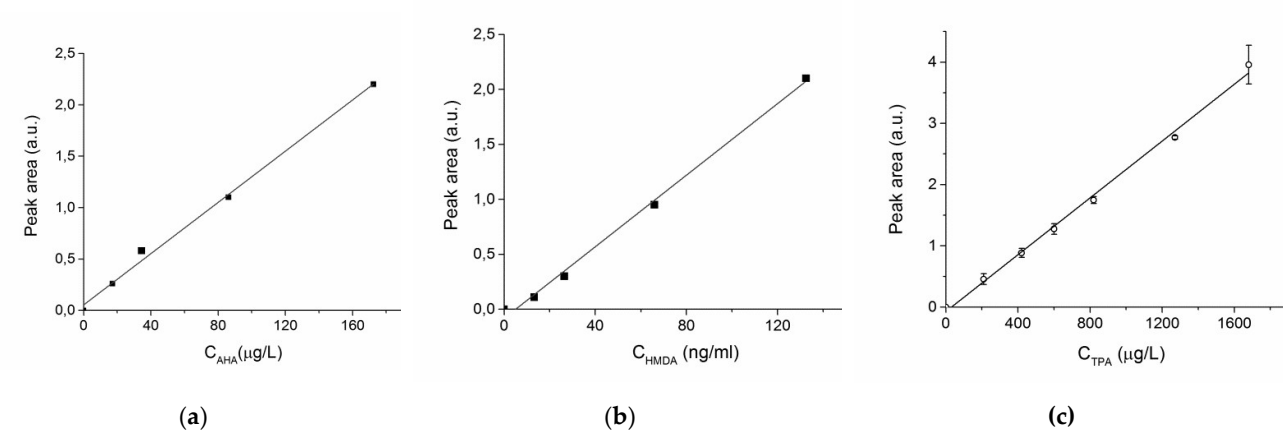


Figure 4. Linear fits of the calibration dataset for the quantitative determination of the monomeric units: **(a)** AHA, with FLD; **(b)** HMDA, with FLD; (c) TPA, with UV detector.

For the HPLC quantification of TPA a linear calibration of the UV detector response was obtained by recording a 6-point calibration based on standard TPA solutions in 2N NaOH in the 0.21-1.68 mg/L range plus a blank, all in triplicate [23]. From the linear regression (Figure 4c, $A=2.32\cdot10^5\cdot C_{TPA}-7237$; $r^2>0.995$) the following values were calculated: LOD=0.117 mg/L; LOQ: 0.391 mg/L. (calculated in this case as the ratio between the concentration, or the calibrated peak area, and the signal-to-noise ratio, times 3 for LOD and times 10 for LOQ; the blank sample gave no detectable peak).

3. Results

The overall procedure for the determination of the total mass of individual polymer types that are present as microparticles and fragments in the sediment involves a preliminary step of sieving to recover the fraction below 2 mm in size and air drying, followed by a first sequence of extractions with boiling solvents that are selective for the hydrocarbon polymers. In particular, extraction with DCM (boiling point b.p.=39.6 °C) allows to recover the amorphous polystyrene, along with most low MW organic compounds (both biogenic, such as fats, and synthetic, such as plasticizers and surfactants) and the oligomeric fraction deriving from the extensive photo- and thermal oxidation of polyolefins (PE, PP, and olefin copolymers). In addition, most vinyl polymers such as e.g. acrylics, polyvinyl chloride (PVC) and polyvinyl acetate may be co-extracted in boiling DCM, but their presence as microplastic contaminants in marine sediments is likely to be negligible because they are not (or no longer in the case of PVC) commonly used in the disposable items and packaging materials that are by far the main contributors to the plastic waste reaching the marine environment. The second extraction of the residue from the DCM extraction is performed in boiling xylene (b.p.=139 °C), to recover the less oxidized and high molecular weight polyolefins, possibly along with some proteins that may be co-extracted. The extractable fraction may then be further purified to remove the biogenic fraction, and analyzed by one or more techniques for the quantification of the total mass of each polymer type.

The subsequent steps, consisting in a sequence of hydrolytic treatments performed under acid and then alkaline conditions, are performed under optimized conditions to selectively and sequentially achieve the complete depolymerization of all aliphatic polyamides (both synthetic and natural) and all polyesters, respectively. The resulting hydrolyzates may then be submitted to further purification (different environmental matrices may require different purification procedures) before performing reversed-phase HPLC analysis that allows the accurate and sensitive quantification of the monomers and to calculate the corresponding amount of the original polymer. In the case of the polyamides, an additional tagging of the amino-monomers with a fluorophore is performed

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 February 2021 doi:10.20944/preprints202102.0211.v1

prior to the HPLC analysis to increase of orders of magnitude the sensitivity of the measurement.

3.1. MPs fractionation by polymer type through selective solvent extraction

3.1.1. Polystyrene and highly degraded hydrocarbon polymers

The DCM extracts are expected to contain not only PS, but also highly oxidized and degraded polyolefin oligomers and other vinyl polymers less frequently found as microplastic pollutants (e.g. polyacrylates, PVC, etc.), in addition to biogenic low molecular weight species. The total amounts of DCM extractable fraction in the four samples are reported in Table 3. Further extractions with refluxing xylene to collect the DCM-insoluble, less degraded polyolefin fraction gave in most cases very small amounts of dry matter that could be neither weighed with sufficient accuracy nor further purified, and were therefore not further analyzed; the only exception was the xylene extract from CAL2, from which a sizable solid particulate could be recovered (see section 3.1.2).

Table 3. Extractable fraction in DCM from the sediment samples.

Sediment sample	Extracted sediment (g)	Extractables (mg)	Total extract [1] (ppm)	PS content [1,2] (ppm)
MEL1	82.85	6.2	75	8
MEL2	107.96	12.8	119	11
CAL1	100.91	9.1	90	65
CAL2	112.57	10.7	95	16

[1] Total concentration expressed as mg of dry extractable matter per kg dry sediment.
[2] Determined by double shot-Py-GC/MS measurements, from the styrene dimer peak and the corresponding instrumental calibration.

The dried DCM extracts were picked up with chloroform to about 5 mg/mL and the obtained solutions were microfiltered (to remove any contamination by inorganic particles) and analyzed by SEC. The chromatographic profiles obtained with UV detectors set at 260 nm and 340 nm always show the presence of a main very broad and structured peak at retention times r.t.>15 min due to low molecular weight (MW) polymers and oligomers along with other low MW species; an additional weak peak roughly centred at r.t.≈12.5 min could be detected for samples MEL1, MEL2 and CAL1, corresponding to higher MW polymers (Figure 5 and Table 4).

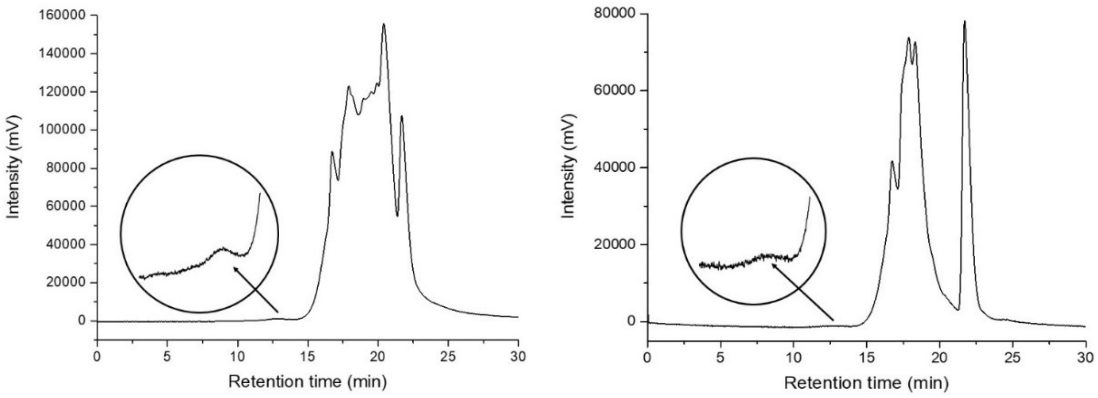


Figure 5. Representative examples of the SEC traces (the curves displayed are those recorded with UV detector set at 260 nm) for the DCM extractable fractions of: **(a)** MEL1; **(b)** MEL2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 February 2021 doi:10.20944/preprints202102.0211.v1

Table 4. SEC analysis of the DCM extractable fractions in the sediment samples.

Sample	Retention time [1] (min)	$\bar{M}_n$ (g·mol^{-1})	$\bar{M}_w$ (g·mol^{-1})	PDI [2]
MEL1	12.83	35883	42245	1.18
	20.41	92	367	4.0
MEL2	12.22	36855	48336	1.31
	17.89	254	585	2.30
CAL1	12.71	29653	46250	1.56
	17.88	270	529	1.96
CAL2	n.d. [3]	n.a. [3]	n.a. [3]	n.a. [3]
	18.04	196	475	2.43

[1] peak value reported here only as an aid for better visualization
[2] Polidispersity Index PDI=$\bar{M}_w/\bar{M}_n$
[3] n.d.= not detectable (below the limit of detection, LOD); n.a. = not applicable

The SEC-UV detector response for the high MW fractions in MEL1, MEL2, and CAL1 is characterized by a strong absorption at 260 nm that becomes negligible at 340 nm, as one would expect from polystyrene [30], but differently from most nonaromatic polymers.

The overall PS content in the sediment samples (including the low MW oligomeric fraction) was determined by double shot-Py-GC/MS analysis performed on the DCM extracts, according to a calibration based on the MS count corresponding to the PS dimer fragment; the results are reported in Table 3. The double shot technique also allows to separately detect different species; in the first shot at lower temperature (here 350 °C) low MW compounds such as hydrocarbon species deriving from highly degraded polyolefins or plasticizers and other common plastics additives are typically observed, along with some polystyrene oligomers, while the presence of other synthetic polymers could be detected in the second shot (here 600 °C). The main species identified in the DCM extracts of the four samples are listed in Table 5, while the Py-GC/MS chromatograms recorded after each shot are shown in Figure 6 and Figure 7.

Table 5. Most abundant species identified by double shot-Py-GC/MS in the DCM extratcs.

Acronym	First shot (350 °C)	Second shot (600 °C)
MEL1	TBP, DBP	PS, siloxane
MEL2	TBP, DBP, BEHP, fatty acids	PS, PE, branched hydrocarbons, sterols
CAL1	TBP, DBP, fatty acids	PS, sterols, branched hydrocarbons
CAL2	TBP, DBP, DOA	PS, PE, sterols, branched hydrocarbons

[1] TBP=tributyl phosphate; DBP=dibutyl phthalate; BEHP=bis(2-ethylhexyl) phthalate; DOA=diisooctyl adipate

The Py-GC/MS chromatogram obtained at 350 °C from the MEL1 extract was mainly characterized by the presence of tributyl phosphate (TBP) and dibutyl phthalate (DBP), two nearly ubiquitous environmental pollutants largely used as plasticizers in many applications. The pyrolysis products from the high temperature shot were mainly the typical markers of PS (styrene and its low oligomers) and of polysiloxane.

Similarly, in all the other MEL and CAL extracts the 350 °C shot resulted in the release of various plasticizers such as TBP, DBP, bis(2-ethylhexyl) phthalate (BEHP) and diisooctyl adipate (DOA), along with naturally occurring fatty acids. At 600 °C the py-

 doi:10.20944/preprints202102.0211.v1

rolysis markers of PS were always detected, along with those of PE (only in the case of the extracts from MEL2 and CAL2); finally, various sterols of likely natural origin, and branched hydrocarbons possibly originating from the degradation of synthetic surfactants could also be detected.

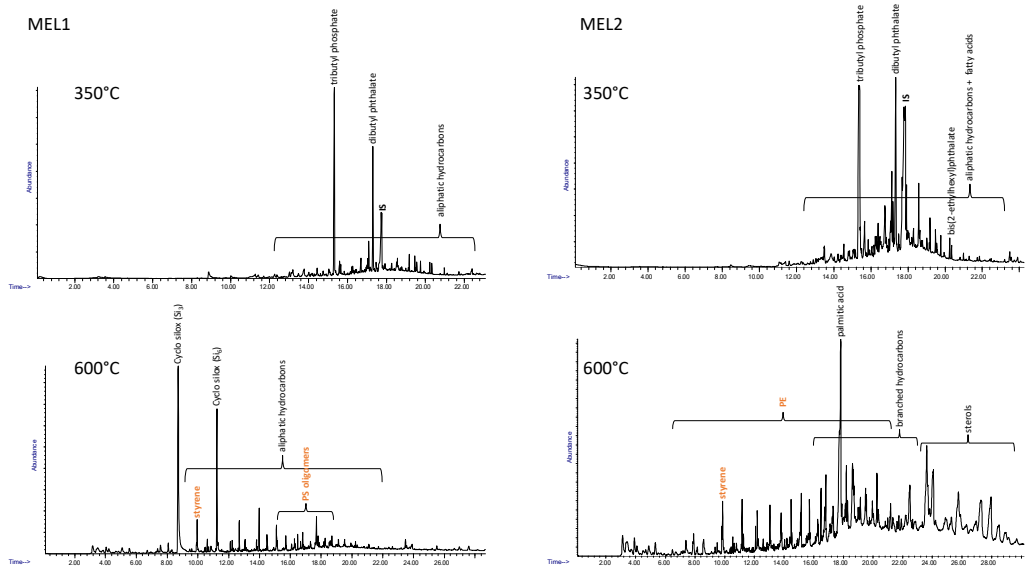


Figure 6. Py-GC-MS chromatograms of sediment DCM extracts: **(a)** MEL1; **(b)** MEL2.

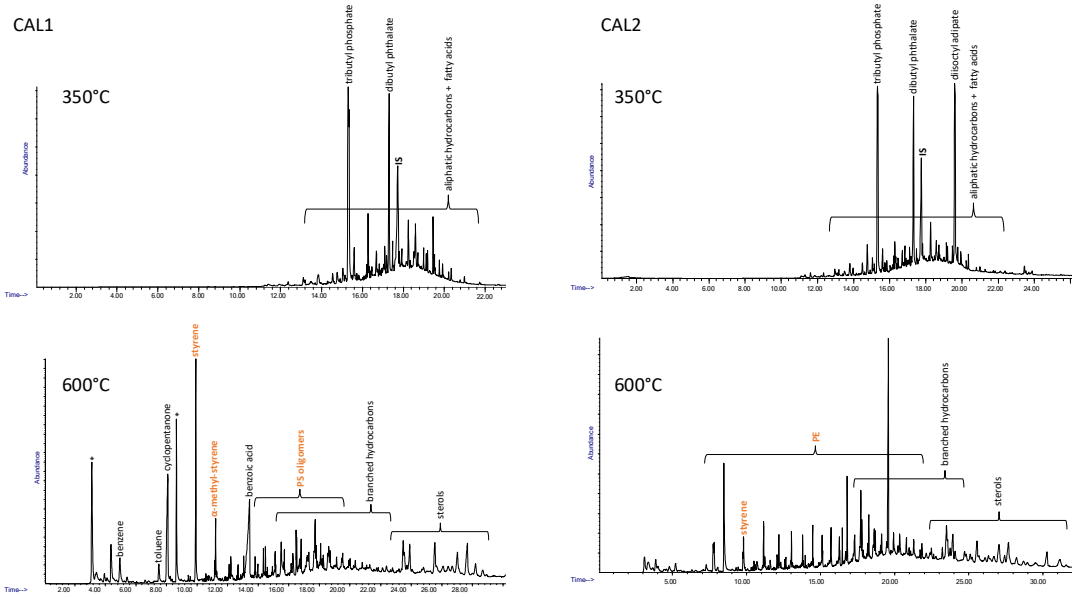


Figure 7. Py-GC-MS chromatograms of sediment DCM extracts: **(a)** CAL1; **(b)** CAL2.

3.1.2. High molecular weight polyethylenes (HDPE, LDPE) and polypropylene

For the semi-quantitative determination of semicrystalline polyolefin MPs (polyethylenes and polypropylene) the residues from DCM extraction were further extracted with refluxing xylene. After distilling off most of the xylene from the final extracts they

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 February 2021 doi:10.20944/preprints202102.0211.v1

were added with an excess of a solution of KOH in methanol and the precipitate was then collected by filtration, dried, and weighed. A quantifiable amount of precipitated particles (2.9 mg from 81.6 g of sediment) was only recovered from sample CAL2. The micro-ATR FTIR spectrum in Figure 8 clearly indicates that the precipitate mainly consists of oxidized polyethylene (methylene CH stretchings at 2917 and 2849 cm^{-1}, weak methyl CH stretchings at 2960 and 2866 cm^{-1}, methylene bendings at 1452, and 1375 cm^{-1}), with a high oxidation level shown by the intense and broad carbonyl absorption with main peaks at 1710 and 1660 cm^{-1} (isolated and conjugated aldehydes and ketones generated by photooxidation and subsequent chain cleavage reactions) and the broad absorption centered at 3400 cm^{-1} from hydroxyl groups. Further absorptions can be ascribed at least partially to polydimethylsiloxane (methyl deformation at 1260 cm^{-1}, symmetric and asymmetric Si-O-Si stretchings at 1088 and 1018 cm^{-1}, and Si-C stretching at 800 cm^{-1}, in addition to a small C-H stretching peak at 2950 cm^{-1}) possibly due to contamination by silicone grease during the lab operations.

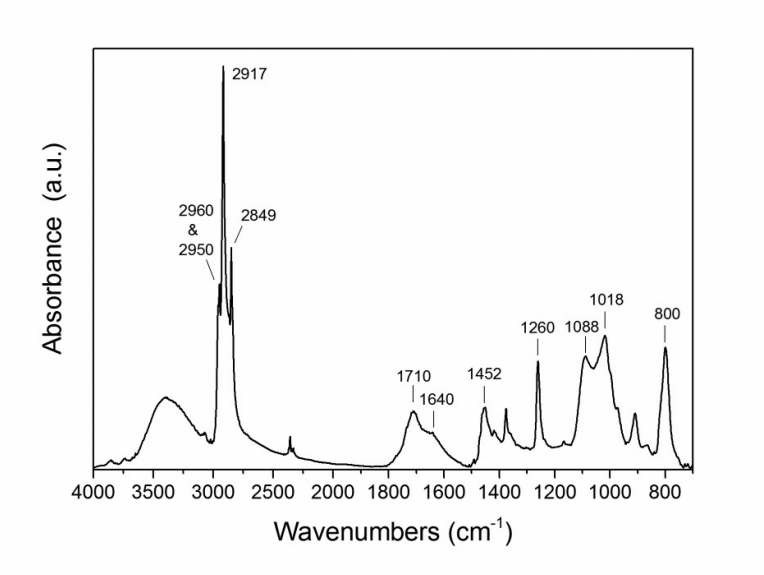


Figure 8. Micro-ATR-FTIR spectrum of the xylene-extractable fraction of CAL2.

3.2. Total mass content of polyamide (nylon 6 and nylon 6,6) and polyester (PET) MPs by depolymerization and quantitative analysis of the resulting comonomers

For the quantification of nylon 6 and nylon 6,6 polyamides the solid residues from the sequential extractions with DCM and Xy were treated with refluxing 6 N HCl to achieve the total depolymerization of both natural and synthetic polyamides. Due to the high carbonate content of the two MEL sediments samples a high volume of HCl had to be slowly added to allow complete evolution of CO_2 upon conversion of the carbonate mineral into the corresponding chlorides. The hydrolyzate solutions were separated from the residue by filtration on 0.22 μm PVDF membranes, neutralized and treated with the fluorescent tag dansyl chloride (DNS-Cl) before reversed-phase HPLC analysis, as described in detail in Section 2. The residues from the acid hydrolysis were then treated with 1.9 N NaOH to achieve quantitative depolymerization of PET MPs, followed by purification of the hydrolyzate and quantification of the TPA content by reversed-phase HPLC analysis, as described in detail in Section 2.

In Table 6 are reported the detected concentration of the dansylated AHA and HMDA and of TPA from which the concentration of nylon 6, and nylon 6,6, and PET MPs in the sediment samples (the air dried sediments were considered as a starting material) could be calculated.

 doi:10.20944/preprints202102.0211.v1

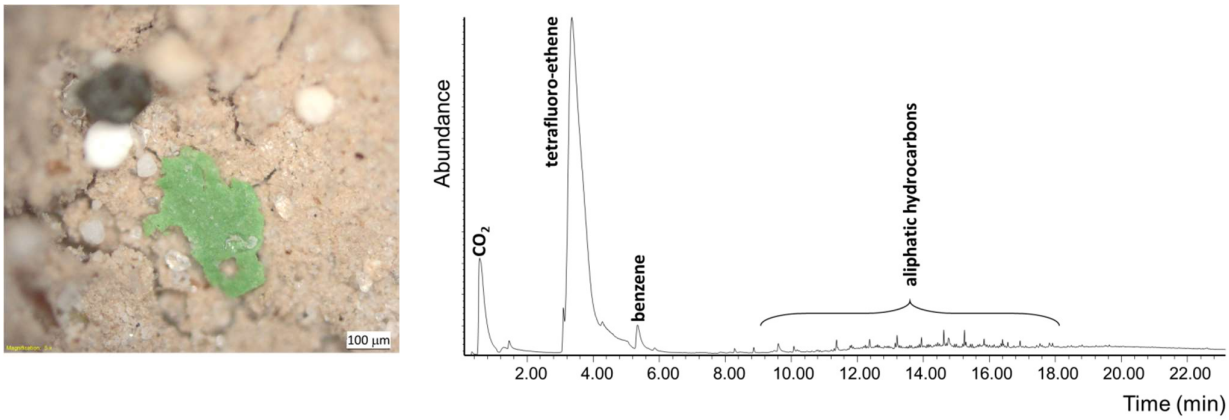
Table 6. Concentration of PA's monomers and relative polymers in sediment sample.

Acronym	AHA [1] (μg/L)	Nylon 6 [2] (ppm)	HMDA [1] (μg/L)	Nylon 6,6 [2] (ppm)	TPA [1] (mg/L) [1]	PET (ppm) [2]
MEL1	n.d. [3]	n.a. [3]	n.d.	n.a.	0.101	290
MEL2	n.d.	n.a.	n.d.	n.a.	0.0489	137
CAL1	36.6	11.2	8.97	2.7	0.633	1523
CAL2	35.0	12.1	n.d.	n.a.	0.061	174

[1] Concentration of the monomer (or its dansyl derivative in the case of the two amines) in the solution obtained after purification of the corresponding acid (for the two amines) or alkaline (for TPA) hydrolysate.
[2] Total concentration in ppm (mg polymer/kg sediment) as calculated from the detected amount of the corresponding monomers.
[3] n.d.= not detectable (below the limit of detection, LOD); n.a. = not applicable

3.3. Analysis of microplastic's particles detected on filter membrane

The final residue recovered from the filter in last step of the overall procedure was observed under an optical microscope to detect the presence of any microplastic particle resistant to all the extraction and hydrolysis processes. In the case of the MEL1 sample, a few sub-millimeter sized green plastic fragments weighing about 50 μg each could easily be detected in the brown-greyish inorganic residue (Figure 9). The fragments were identified as polytetrafluoroethylene (PTFE) from the presence of a tetrafluoroethene main peak in the Py-GC/MS chromatogram recorded from each individual particle.



(**a**) (**b**)

Figure 9. PTFE microparticles collected from the MEL1 sample: (**a**) micrograph of a green microparticle taken with a stereomicroscope; (**b**) pyrogram of the microparticle.

4. Discussion

The complete Polymer Identification and Specific Analysis (PISA) protocol for the separation, purification and quantification of the total mass contents of PS, polyolefins, PET, nylon 6 and nylon 6,6 MPs in environmental matrices, recently developed and previously applied in the analysis of the contamination level in sandy shore sediments and in wastewater treatment plant sludges, has been successfully applied for the first time to benthic marine sediments, considered to be the final sink of most of the plastic waste inflow in the oceans.

The selection of the polymer types to be investigated, which dictated the design of the overall separation, fractionation and analysis scheme, was based on the considerations, supported by an increasing number of scientific papers and technical reports, that

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 February 2021 doi:10.20944/preprints202102.0211.v1

the most abundant polymer types in benthic marine sediments correspond to those that are also produced globally in larger amounts. These are: polyolefins and polystyrene, largely used in short lifetime applications such as packaging and single use disposable items, and therefore likely to end up as unmanaged plastic waste; the two main synthetic polymer classes used as staple textile fibers or as materials of fishery and aquaculture activities, that is the polyester PET and the two polyamides nylon 6 and nylon 6,6, included among the target polymers because textile fibers released in laundering wastewaters and mismanaged fishing gears are well recognized threats for the marine ecosystems.

The overall procedure, schematically shown in the flowchart of Figure 10, allows to tackle the two main challenges faced when such polymeric materials end up in sea bottom sediments, either because of their high density or as a result of photo-oxidation and/or biofouling promoting vertical transport down the water column. These are the lengthy (and possibly inaccurate) procedures for the density separation of the MPs from the sediment, and the size threshold for their detection by micro-spectroscopy techniques, a possibly critical issue in particular for the low density polyolefins and PS. Indeed the latter hydrocarbon polymers are likely to reach the benthic sediments only once they have undergone significant degradation, which may include fragmentation down to the sub-micrometer size range, well below the detection limit of a few micrometers and up to tens of micrometers typical of the micro-spectroscopy techniques commonly used for MPs in sediments.

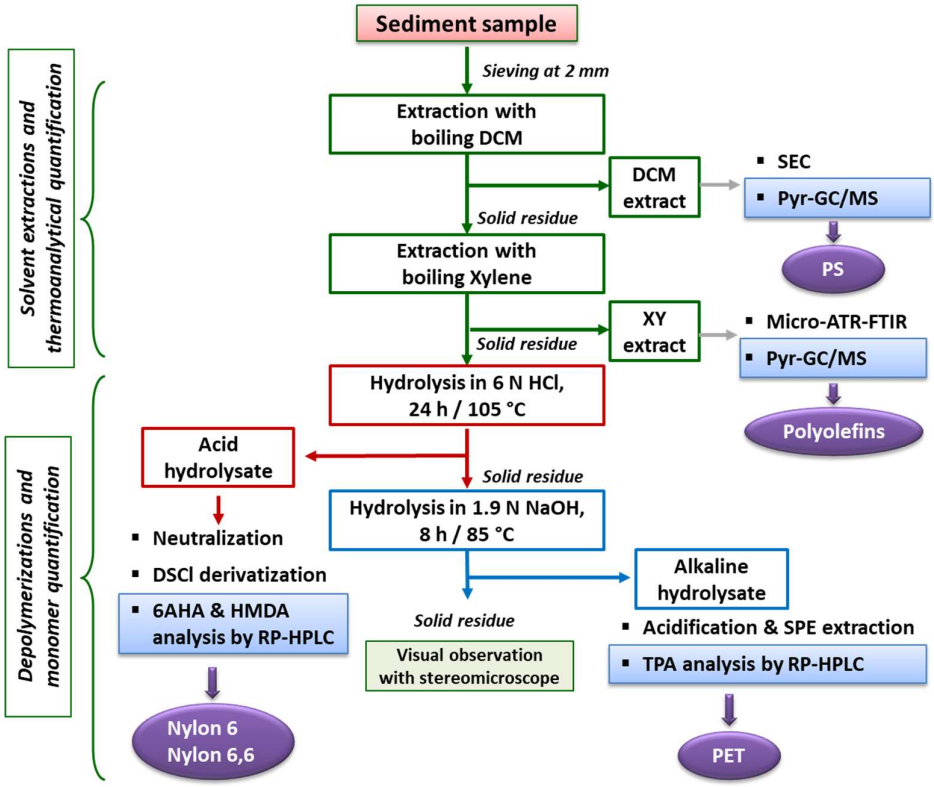


Figure 10. Flowchart of the entire analytical protocol for the separate quantification of the total mass of micro- and nanoparticles of different polymer types.

Among the most noteworthy results presented here are the successful implementation of an improved version of the procedure for the quantification of PS, and the results confirming the presence of both low molecular weight and high molecular weight low density polyolefins in benthic sediments. In particular, the Pyr-GC/MS technique

adopted for the quantification of PS allows to improve the accuracy with respect to the previously reported procedure based on FD-SEC [30], in which only the high MW fraction could be evaluated due to the interference by biogenic species and oxidized polyolefin molecular fragments in the low molecular weight fraction. The presence of PS in the 8-65 ppm concentration range could thus be determined with accuracy, while the molecular weight distribution determined by SEC analysis highlighted the presence of a significant fraction of degraded low molecular weight PS, in agreement with the expected presence in the bottom sediment of low density hydrocarbon polymers that have undergone significant photo-oxidation. The presence of highly oxidized high molecular weight polyolefin MPs, although in very small amount, clearly indicates that polymer oxidation contributes to their vertical transport and ultimate deposition due to their increased density and hydrophilicity.

The results obtained in this work also highlight the versatility of the procedure, which allowed to deal with sediments of very different compositions (silicatic silt and coarse organogenic carbonate debris).

Finally, the double shot Py-GC/MS technique allowed to separately detect in the DCM extracts of all samples various phthalates and other low molecular weight plasticizers (suspect endocrine disruptors), while the high molecular weight fraction was found to contain, in addition to PS, also polysiloxane (in the DCM extract of MEL1) possibly from silicones used in formulations of personal care products, and degraded fractions of polyethylene (in MEL2 and CAL2). Although not mentioned before, the likely presence of biodegradable (not necessarily so once in the marine sediment) aliphatic-aromatic polyesters could also be identified from the presence of some markers of poly(butylene terephthalate-co-adipate).

The hydrolytic depolymerization of the higher density heteropolymers (PET, nylon 6 and nylon 6,6) followed by HPLC analysis of the resulting monomers allowed to accurately quantify the contamination level presumably associated with the deposition of synthetic textile fibers carried by urban wastewaters. The somewhat surprising higher level of contamination by polyamides (2.7-12.1 ppm) compared to PET (1.5-0.2 ppm) may be the result of different sources of pollution (e.g. fishing gears), although the number of analyzed samples was too small to allow drawing general and accurate considerations. In fact, while PET was detected in all the analyzed samples, polyamides were only detected in some of them. Finally, the presence of PTFE particles isolated from the final residue could be the result of a point source or of a specialized source of pollution, as this material is widely used in technical fishing equipment.

While the methodology used in this work cannot provide information on important parameters such as the number, size and shape of the individual plastic particles, it provides important complementary and unique quantitative information allowing to gain an accurate picture on the transport, extent and distribution of MPs in the marine environment, thus clarifying the actual role of the sea bottom as MPs sink.

Author Contributions: Conceptualization, V.C. and A.Co.; methodology, A.Co. and J.L.N.; validation, A.Co. and J.L.N.; investigation, A.Co., J.L.N., S.G. and V.V.; resources, V.C., F.M. and A.C.; writing—original draft preparation, A.Co., J.L.N. and V.V.; writing—review and editing, V.C.; supervision, A.Co. and F.M.; funding acquisition, V.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fondazione Cassa di Risparmio di Lucca (Bando Ricerca 2019-21, CISUP project "Micro- e nano-plastiche: metodologie di quantificazione, valutazione dell'impatto in ecosistemi marini e lacustri, strategie di remediation ambientale") and University of Pisa PRA project 2020_27 on *Micro- and nanoplastics*.

Acknowledgments: The authors are thankful to the Harbour Masters Corps - Coast Guard of Leghorn (Capitaneria di Porto – Guardia Costiera di Livorno) for logistic support in the sampling.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Da Costa, J.P.; Nunes, A.R.; Santos, P.S.M.; Girão, A.V.; Duarte, A.C.; Rocha-Santos, T. Degradation of polyethylene microplastics in seawater: Insights into the environmental degradation of polymers. *J. Environ. Sci. Health A* **2018**, *53*, 866-875. Doi: 10.1080/10934529.2018.1455381
2. Andrady, A.L. Microplastics in the marine environment. *Mar. Poll. Bull.* **2011**, *62*, 1596-1605. Doi: 10.1016/j.marpolbul.2011.05.030
3. Alimi, O.; Farner Budarz, J.; Hernandez, L.M.; Tufenkji, N. Microplastics and nanoplastics in aquatics environments: aggregation, deposition, and enhanced contaminant transport. *Environ. Sci. Technol.* **2018**, *52*, 1704-1724. Doi: 10.1021/acs.est.7b05559
4. Eriksen, M.; Laurent, C.M.; Carson, H.S.; Thiel, M.; Moore, C.J.; Borerro, J.C.; Galgani, F.; Ryan, P.G.; Reisser, J. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *Plos One* **2014**, *9*, 111913. Doi: 10.1371/journal.pone.0111913
5. Evangeliou, N.; Grythe, H.; Klimont, Z.; Heyes, C.; Eckhardt, S.; Lopez-Aparicio, S.; Stohl A. Atmospheric transport is a major pathway of microplastics to remote regions *Nat. Commun.* **2020**, *11*, 1–11. Doi: 10.1038/s41467-020-17201-9
6. Waller, C.L.; Grifiths, H.J.; Waluda, C.M.; Thorpe, S.E.; Loaiza, I.; Moreno, B.; Pacherres, C.O.; Hughes, K.A. Microplastics in the Antarctic marine system: an emerging area of research. *Sci. Tot. Environ.* **2017**, *598*, 220-227. Doi: 10.1016/j.scitotenv.2017.03.283
7. Bergmann, M.; Wirzberger, V.; Krumpen, T.; Lorenz, C.; Primpke, S.; Tekman, M.B.; Gerdts, G. High quantities of microplastic in Arctic deep-sea sediments from the Hausgarten observatory. *Environ. Sci. Technol.* **2017**, *51*, 11000-11010. Doi: 10.1021/acs.est.7b03331
8. Schwaferts, C.; Niessner, R.; Elsner, M.; Ivleva, N.P. Methods for the analysis of submicrometer- and nanoplastic particles in the environment. *Trends Anal. Chem.* **2019**, *112*, 52-65. Doi: 10.1016/j.trac.2018.12.014
9. Coyle, R.; Hardiman, G.; O'Driscoll, K. Microplastics in the Marine Environment: A Review of their Sources, Distribution Processes and Uptake into Ecosystems. *Case Stud. Chem. Environ. Eng.* **2020,** *2*, 100010. Doi: 10.1016/j.cscee.2020.100010
10. Kihara, S.; Köper, I.; Mata, J.P.; McGillivray, D.J. Reviewing nanoplastic toxicology: It's an interface problem. Adv. Colloid Interface Sci. 2021, 288, 102337. Doi: 10.1016/j.cis.2020.102337
11. Choy, C.A.; Robison, B.H.; Gagne, T.O.; Erwin, B.; Firl, E.; Halden, R.U.; Hamilton, J.A.; Katija; K.; Lisin, S.E.; Roisky, C.; Van Houtan K.S. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. *Sci. Rep.* **2019**, *9*, 7843. Doi: 10.1038/s41598-019-44117-2
12. van Sebille, E. et al. The physical oceanography of the transport of floating marine debris. *Environ. Res. Lett.* **2020**, *15*, 023003. Doi: 10.1088/1748-9326/ab6d7d
13. Courtene-Jones, W.; Quinn, B.; Gary, S.F.; Mog, A.O.M.; Narayanaswamy, B.E. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. *Environ. Poll.* **2017**, *231*, 271-280. Doi: 10.1016/j.envpol.2017.08.026
14. Botterell, Z.L.R.; Beaumont, N.; Dorrington, T.; Steinke, M.; Thompson, R.C.; Lindeque, P.K. Bioavailability and effects of microplastics on marine zooplankton: a review. *Environ. Poll.* **2016**, *245*, 98-110. Doi: 10.1016/j.envpol.2018.10.065
15. Waldman W.R., Rillig M.C. Microplastic Research Should Embrace the Complexity of Secondary Particles. *Environ. Sci. Technol.* **2020**, *54*, 7751−7753. Doi: 10.1021/acs.est.0c02194
16. Kooi, M.; Van Nes, E.H.; Scheffer, M.; Koelmans, A.A. Ups and Downs in the Ocean: Effects of Biofouling on Vertical Transport of Microplastics. *Environ. Sci. Technol.* **2017**, *51*, 7963-7971. Doi: 10.1021/acs.est.6b04702
17. Martin, J.; Lusher, A.; Thompson, R.C.; Morley, A. The deposition and accumulation of microplastics in marine sediments and bottom water from the Irish continental shelf. *Sci. Rep.* **2017**, *7*, 10772. Doi: 10.1038/s41598-017-11079-2
18. Peng, X.; Chen, M.; Chen, S.; Dasgupta, S.; Xu, H.; Ta, K.; Du, M.; Li, J.; Guo, Z.; Bai, S.; Microplastics contaminate the deepest part of the world's oceans. *Geochem. Perspect. Lett.* **2018**, *9*, 1-5. Doi: 10.7185/geochemlet.1829
19. Singh, N.; Tiwari, E.; Khandelwala, N.; Darbha, G.K. Understanding the stability of nanoplastics in aqueous environments: effect of ionic strength, temperature, dissolved organic matter, clay, and heavy metals. ***Environ. Sci.: Nano*** **2019**, *6*, 2968-2976. Doi: 10.1039/C9EN00557A
20. Thompson, R.C.; Olsen, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; Jhon, A.W.G.; McGonigie, D.; Russell, A. Lost at sea: where is all the plastic? *Science* **2004**, *304*, 838. Doi: 10.1126/science.1094559
21. van Sebille, E.; Wilcox, C.; Lebreton, L.; Maximenko, N.; Hardesty, B.D.; van Franeker, J.A.; Eriksen, M.; Siegel, D.; Galgani, F.; Lavender Law K. A global inventory of small floating plastic debris. *Environ. Res. Lett.* **2015**, *10*, 124006. Doi:10.1088/1748-9326/10/12/124006
22. Van Cauwenberghe, L.; Devriese, L.; Galgani, F.; Robbens, J.; Janssen, C.R. Microplastics in sediments: A review of techniques, occurrence and effects. *Mar. Environ. Res.* **2015**, *111*, 5-17. Doi: 10.1016/j.marenvres.2015.06.007

23. Corti, A.; Vinciguerra, V.; Iannilli, V.; Pietrelli, L.; Manariti, A.; Bianchi, S.; Petri, A.; Cifelli, M.; Domenici, V.; Castelvetro, V. Thorough Multianalytical Characterization and Quantification of Micro- and Nanoplastics from Bracciano Lake's Sediments. *Sustainability* **2020**, *12*, 878. Doi: 10.3390/su12030878
24. Castelvetro, V.; Corti, A.; Biale, G.; Ceccarini, A.; Degano, I.; La Nasa, J.; Lomonaco, T.; Antonella Manariti, A.; Manco, E.; Modugno, F.; Vinciguerra, V. New methodologies for the detection, identification, and quantification of microplastics and their environmental degradation by-products. *Environ. Sci. Poll. Res.* **28** (2021). *doi: 10.1007/s11356-021-12466-z*
25. Ceccarini, A.; Corti, A.; Erba, F.; Modugno, F.; La Nasa, J.; Bianchi, S.; Castelvetro, V. The Hidden Microplastics: New Insights and Figures from the Thorough Separation and Characterization of Microplastics and of Their Degradation By-products in Coastal Sediments. *Environ. Sci. Technol.* **2018**, *52*, 5634−5643. Doi: 10.1021/acs.est.8b01487
26. Castelvetro, V.; Corti, A.; Ceccarini, A.; Petri, A.; Vinciguerra, V. Nylon 6 and nylon 6,6 micro- and nanoplastics: a first example of their accurate quantification, along with polyester (PET), in wastewater treatment plant sludges. *J. Hazard. Mater.* **2021**, *407*, 124364. *doi:10.1016/j.jhazmat.2020.124364*
27. Castelvetro, V.; Corti, A.; Bianchi, S.; Ceccarini, A.; Manariti, A.; Vinciguerra. V. Quantification of poly (ethylene terephthalate) micro- and nanoparticle contaminants in marine sediments and other environmental matrices. *J. Hazard. Mater.* **2020**, *385*, 121517. Doi: 10.1016/j.jhazmat.2019.121517
28. La Nasa, J.; Biale, G.; Mattonai, M.; Modugno, F. Microwave-assisted solvent extraction and double-shot analytical pyrolysis for the quali-quantitation of plasticizers and microplastics in beach samples. *J. Hazard. Mater.* **2021**, *401*, 123287. Doi: 10.1016/j.jhazmat.2020.123287
29. La Nasa, J.; Biale, G.; Fabbri, D.; Modugno, F. A review on challenges and developments of analytical pyrolysis and other thermoanalytical techniques for the quali-quantitative determination of microplastics. *J. Anal. Appl. Pyrol.* **2020**, *149*, 104841. Doi: 10.1016/j.jaap.2020.104841
30. Biver, T.; Bianchi, S.; Carosi, M.R.; Ceccarini, A.; Corti, A.; Manco, E.; Castelvetro, V. Selective determination of poly(styrene) and polyolefin microplastics in sandy beach sediments by gel permeation chromatography coupled with fluorescence detection. *Mar. Poll. Bull.* **2018**, *136*, 269–275. Doi: 10.1016/j.marpolbul.2018.09.024