Title: New analytical technique for extraction and quantification of microplastics in marine sediments focused on easy implementation and repeatability.

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Standards preparation

The large pieces of plastics were converted into microplastics by using a conventional machining process, i.e., a drill with a sandpaper implement (Dremmel 300, 13 mm-60 grain size sandpaper). For this purpose, several sequential sessions of the machining processes were carried out in order to avoid changes in the physical properties of the plastics that would result from warming due to long exposure to friction.

After the preparation of the microplastics' pool, they were sieved using ethanol (96%) through two different mesh sizes until a final standard ranging from 0.850 mm to 0.1 mm size was achieved. Those upper and lower thresholds were selected as they were relevant for biota ingestion[S1] and were also appropriate for visual counting using microscopes. The entire standard used during these experiments was prepared together all at once and thoroughly mixed previously to each spiking in order to avoid differences in the percentage of presence from size groups. In addition, using a confocal microscope (Zeta Instruments, model Zeta 300) and object detection algorithms (Mathematica 10), the presence of each size group was determined (see Figure 1 and Section "Quantification").

To prepare standard solutions for both the developing calibration curves and for the spiking sediments, microplastics were suspended in ethanol 96% and shook using a magnetic stirrer. The selection of ethanol instead of water was decided because the microparticles of HDPE could not be homogeneously distributed in distilled water, not even under continuous shaking, due to the surface tension of water, its density and also due to the electrostatic forces as a result of the machining process. Using ethanol 96% and stirring at 300 rpm for two minutes solved all of these issues since its specific density of 0.95-0.97 g cm^{-3} was optimal for working with HDPE.

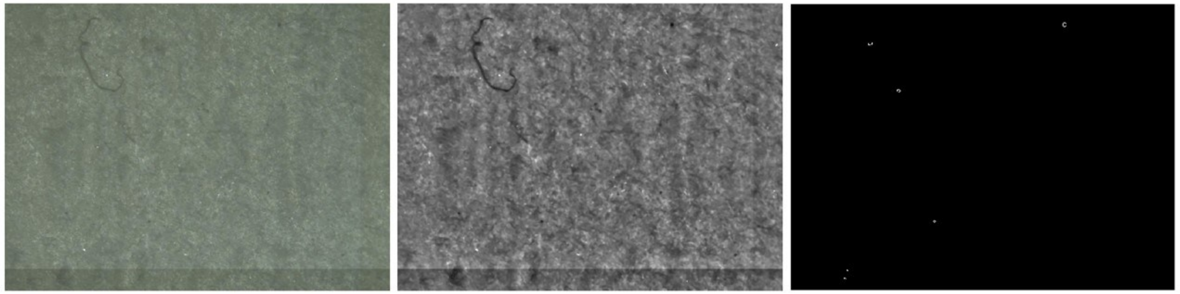
[S1]Carlo A.G, Stefania G., Francesco R., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. Marine Environmental Research, 111, 18–26.

Desarrollo

```
SetDirectory["F:\\Microplasticos\\Recta calibrado final\\"];
img = Import["E3_blancofinal.jpg"];
{pX, pY} = ImageDimensions[img];
δ = 50 000. / pY;
```

Estudio local

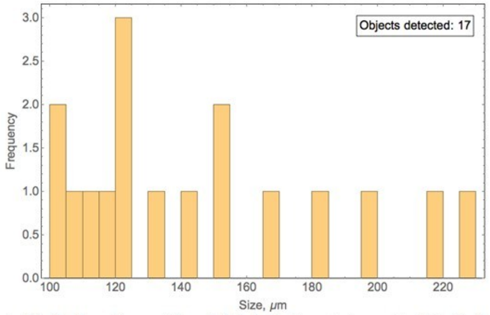
```
imgP = Partition[Flatten[ImagePartition[img, {pX / 10, pY / 14}]], 10];
i = 11;
j = 7;
b = Sharpen[ColorConvert[imgP[[i, j]], "Grayscale"], 100];
data = ImageData[GaussianFilter[b, 1]];
dataW = ImageData[GaussianFilter[b, 100]];
dataR = data - dataW;
imgR = CurvatureFlowFilter[ImageAdjust@Image[dataR], 1, 6];
GraphicsGrid[{{imgP[[i, j]], imgR, MorphologicalPerimeter[imgR, 0.15]}}, ImageSize → 1600, AspectRatio → 1 / 2.7, Spacings → 0]
```



```
Manipulate[Grid[{{ListPlot[{data[[i]], GaussianFilter[data[[i]], 20], data[[i]] - GaussianFilter[data[[i]], 20]}, Joined → True, AspectRatio → 1 / 5, ImageSize → pX / 10, Axes -> False, Frame → True, PlotRange → {-0.2, 1}]},
   {Show[{b, Graphics[{White, Line[{{0, pY / 14 - i + 1}, {pX / 10, pY / 14 - i + 1}}]}]}]}}], {i, 1, pY / 14, 1}]
DynamicModule[{p = {100, 100}}, {Show[{b, Graphics[{EdgeForm[{White, Thick}
      ], Opacity[0], Dynamic@Rectangle[p, p + {10, 10}], Locator[Dynamic[p], Appearance → Tiny]}]}], Dynamic[p], Dynamic@(Apply[Plus, Flatten[ImageData@ImageTake[b, pY / 14 - {p[[2]] + 10, p[[2]]}, {p[[1]], p[[1]] + 10}]]] / 100)}]
```

Estudio general

```
DiscoFiltro = Binarize[FillingTransform@SelectComponents[MorphologicalPerimeter[img, 0.3], "Area", # > 10 000 &]];
{pt0, pt1} = ComponentMeasurements[DiscoFiltro, "BoundingBox"][[1, 2]];
imgRecortada = ImageTrim[img, {pt0 + 900 {1, 1}, pt1 - 900 {1, 1}}];
mask =
  With[{WH = ImageDimensions[imgRecortada]}, Binarize[Graphics[{Black, EdgeForm[{Thick, Black}], Rectangle[{0, 0}, WH], White, Disk[{WH[[1]] / 2, WH[[2]] / 2}, WH[[1]] / 2 - 100]}, PlotRangePadding → None, ImagePadding → -10, ImageSize → WH]]];
imgBW = Sharpen[ColorConvert[imgRecortada, "GrayScale"], 100];
data = ImageData[GaussianFilter[imgBW, 1]];
dataW = ImageData[GaussianFilter[imgBW, 100]];
dataR = data - dataW;
imgR = CurvatureFlowFilter[ImageAdjust@Image[dataR], 1, 6];
imgProcesadaMascara = ImageMultiply[imgR, mask];
ObjetosEncontrados = SelectComponents[MorphologicalPerimeter[imgProcesadaMascara, 0.22], "BoundingDiskRadius", 100 < # 2 δ < 850 &];
Histograma = With[{rst = ComponentMeasurements[ObjetosEncontrados, "BoundingDiskRadius"][[All, 2]]},
  Show[{Histogram[2 δ rst, 30, Axes → None, Frame → True, FrameLabel → {"Size, μm", "Frequency"}, FrameStyle → 20],
    Graphics[Inset[Framed@Text[Style["Objects detected: " <> ToString@Length[rst], FontFamily → "Arial", 20]], {Right, Top}, {1.2, 2}]]}, ImageSize → 800]]
```




```
imgFinal = ImageCompose[ImageMultiply[imgRecortada, mask], ColorReplace[ObjetosEncontrados, Black]];
imgSalida = With[{m = ComponentMeasurements[ObjetosEncontrados, {"BoundingDiskCenter", "BoundingDiskRadius"}]}, Show[{imgFinal, Graphics[{Cyan, MapThread[Circle[#1, #2] &, {m[[All, 2, 1]], m[[All, 2, 2]]}]}]}]]
```

Figure S1: Details of the program optimised for image-processing counting

Reference	Vol. Solution	Vol. or weight sediment	N. Extractions	Additives	Settling time	Filtering	Sieve opening	Microplastics abundance	% recovery	Plastics extracted	Agitation
Environmental Pollution (2015) 204, 17-25	250 mL	-	1	Distilled wáter $3Na_2WO_4.9WO_3.H_2O$ (1.5 Kg/L)	10min(sand beach)/5 min (lake sediment)		-		-	Pellets, fragments,intact (mayority 6 and 4)	Stirring 1 min
Marine Pollution Bulletin, (2015) 101, 274-279	5 L	1200 mL	5	Saline solution			65 µm	689- 3308 particles per m^2			
Marine Pollution Bulletin (2013) 70, 227-233	-	500 mL	2-3	NaI (1.6 Kg/L)		Sieve/membrane filter	35 µm/ 5 µm		100 % PVC particles. 98% fibres	Polyethilene and 3, fibers and granules.	Flow rate 300 L/h for 15 min. Aireation, Centrifugation 5 min-3500g
Marine Pollution Bulletin (2015) 98 (1-2), 274-280	200 mL	50 g	3	NaCl(300 gr/L)	1 h	Celulllose filter paper	-	251-436 particles per 50 g d.w.		1, 2 and 4	Manually shaken for two minutes
Marine Pollution Bulletin (2015) 99 (1-2) 216-229	900-1100 mL	-	1	$CaCl_2$	12 h	Zooplancton filter	55 µm	0-7 particles per kg d.w. and 2-11 fibres por Kg d.w.		1, 2, 4, 5 and 6. Size microplastic: 0.5-3mm	Air-vented 4h
Limnol. Oceanogr.: Methods (2012) 10, 524–537	68mL separation fluid										
Froth flotation experiment				Deionized water with pine oil and surfactants		Cellulose filter paper.	L-MPP		55.0 ± 28.8%	Polyamide (PA), Polycarbonate (PC), 1, 2, 3, 4, 5 and 6	Aireation 24h
Density separation using the classic setup			1 extraction. 3 rinses.	$ZnCl_2$ (1.6–1.7 kg/L)	1-2h	Quartz filter paper	L-MPP and S-MPP. 0.3 µm.		L-MPP (5–1 mm) 99.12 ±3.98% and S-MPP (<1 mm) 39.8% ± 16.6%	Polycarbonate (PC), 1, 2, 3, 4, 6 Polyamide (PA-66), 5 (PP-Homo), Polyoxymethylene (POM), Polyamide (PA),	Aireation 12h
Munich Plastic Sediment Separator		50 mL		saturated NaCl solution					100% for large microplastic particles (L-MPP, 1–5 mm) and of 95.5 ± 1.8% for S-MPP.		Stirring 15' to 12h
Environmental Science and Technology (2011), 45, 9175–9179		50mL	3 sequential extractions	saturated NaCl solution.		Glass microfiber filters: Whatman GF/A	1.6 µm				
Environmental Science & Technology (2010) 340, 4-9		50mL		saturated NaCl solution.				8 fragments per 50 mL sediment		mainly composed of denser plastics such as 3 (26%), polyester (35%), and polyamide (18%).	
Science (2004) 304- 5672, 838		250mL		NaCl (1.2kg/L)	2 min	Glass microfiber filters: Whatman GF/A	1.6 µm	8 particles per Kg in sediment		Acrylic,Alkyd,Polyamide,Polyester,Polypropilene	Stirring for 30 seconds
Marine Pollution Bulletin 89 (2014) 356–366	500 mL	150g		NaCl (360 g/L)	30 min	Decantation and inverse filtration	250 µm	Medium MP 133-155 particles per Kg/ MP 44 particles per Kg		Fibres, Fragments, Films	Manually shaken twice for two minutes
Marine Pollution Bulletin 81 (2014) 69–79	100 mL	10g	2 to 5	NaCl (250 g/L)	3-6 min	nitrocellulose filters	0.8 µm	20 to 80 microplastics per 10 g sediment		2, 4, 5 and 6-E	Stirring 1-2 min
ICES Annual Science Conference, 23 – 27 September 2013, Reykjavík, Iceland		100g		saturated NaCl solution.						Particles of 5, acrylate, and polyester/alkyd	
Estuarine, Coastal and Shelf Science 130 (2013) 54-61	until 1 L in total	250 g	3	NaCl (120 g/L)	1 h	fiberglass filters (Whatman GF-F)	0.7 µm	2175 to 672 SMPPs per Kg d.w.		1 mm or less (S-MPPs): 2, 3, 4, 5, 6, poly(ethylene-propylene) (PEP), y crylonitrile (PAN), alkyd resin (Alkyd), polyvinyl alcohol (PVOH) and nylon (Polyamide). 2, 4 and 5 accounted for more than 82% of the total S-MPPs detected	Shaken 1.5 min
Environmental Pollution 182 (2013) 495-499	3 L, as described in MPB 62, 2199–2204 (see below)		2-3	NaI (1.6 Kg/L)		Membrane filter (Germanscience)	0.8 µm	Average abundance of 0.5 microplastics per 25 cm^2			
Marine Pollution Bulletin 62 (2011) 2199–2204	3 L	1 kg (wet)	2	NaCl (1.2kg/L)	1 h		supernatant was poured through a 38 µm mesh sieve.	up to 390 particles per kg dry sediment	68.8 to 97.5% for the different sediment and particle types	Fibres, granules, plastic films and spherules. Fibres :7 (nylon), polyvinyl alcohol and 5. Granular particles: 6, 2, 4 and 5. Plastic film fragments: 7 (nylon). Spherules: 6.	Stirred 2 min
Environmental Pollution 186 (2014) 248-256	1.5 L and 60% (w/w), as described in Env. Poll. 184, 161-169 (see below)	1 Kg	2	preextraction: NaCl (1.2 g/cm3). Extraction: NaI (1.8 Kg/L).				1.7, 1.3 and 2.3 particles per Kg dry sediment		1, 2, 3, 4, 5, 6 and polyamide	
Marine Pollution Bulletin 60 (2010) 1988–1992				NaCl (140 g/L)						Microplastics were mostly 2 or 4 and 5	
Environmental Pollution 184, (2014) 161-169										1, 3, 5, 6, 6-E and PUR (polyurethane)	
Fluidisation in a lower density salt (first extraction step)	1.5 L (until 6.5 liters)	1 kg	Numerous rinsing steps	NaCl (26% w/w) (1.2 Kg/L)		stainless-steel sieve	25 mm				Air-induced overflow
Flotation of microplastics in a higher density salt (second extraction step)	60% (weight/weight)	Filter residue obtained	5	NaI (1.8 Kg/L)	10 min	nitrocellulose filter	0.45 µm		1, 3, 5 and PUR Mean recovery rates ranged between 91% and 99%. Only the extremely low dense 6-E exhibited a lower recovery rate of 68%		Shaken by hand for about 20 s

Table S1: Review selection of some of most detailed studies regarding extraction and quantification of microplastics in sediments. Polymers indentification number: 1, PETE; 2, HDPE; 3, PVC; 4, LDPE; 5, PP; 6, PS; 6-E, EPS and 7, Others