RESULTS OF MICROFIBERS AND MICROPLASTICS IN MADAGASCAR - NOSY BE –

Sand Analysis Protocol

The sand sample is transferred into aluminium trays (previously dried to a constant weight), to be dried in an oven at 105ºC for 24 hours. This temperature vaporises the water but does not affect the sand or microplastics. To remove organic matter remaining on the surface of the plastic and to aid identification, a 30% solution of H_2O_2 must be added. After filtering and drying, the sediment is added to the saturated high-density $CaCl_2$ solution (ρ≈1.6 g/mL, 37 g in 50 mL of water) under rapid stirring.

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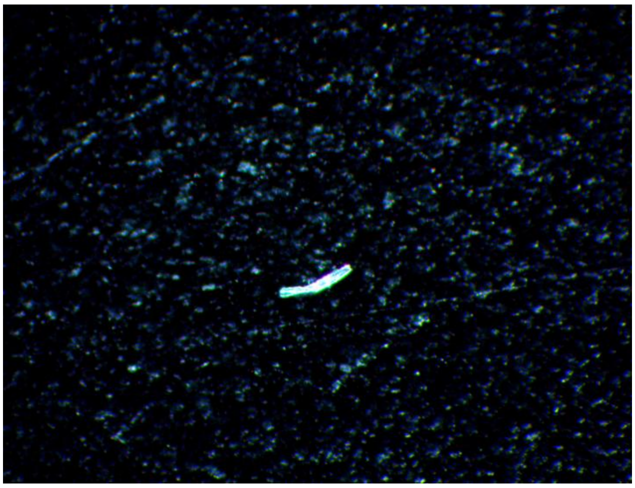
Polymers /fibers	Density (g/mL)	Appropriate Salt Solution
PETE	1.38	NaI, $ZnCl_2$, LMT
LDPE	0.92	All salt
HDPE	0.95	All salt
PS	1.05	All salt
PP	0.87-1.01	All salt
PC	1.2	NaI, $ZnCl_2$, LMT
PVC	1.3 -1.45	$ZnCl_2$, LMT
Polyester	1.3 -1.4	$ZnCl_2$, LMT
Nylon	1.02-1.15	NaI, $CaCl_2$, $ZnCl_2$, LMT

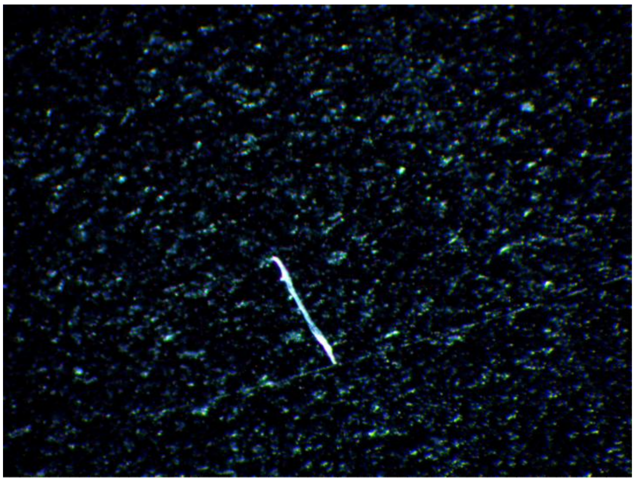
Note. polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), and polypropylene (PP). Sodium chloride (NaCl), sodium iodide (NaI), calcium chloride ($CaCl_2$), zinc chloride ($ZnCl_2$), and lithium metatungstate (LMT). (from different sources).

Table 1. Density of polymers and appropriate saturated salt for extraction.

The mixtures were shaken vigorously, then covered and allowed to stand. After 24 hours, the top portion of each solution was collected by decantation, ensuring that all floating materials, including microplastics, were recovered (but taking care not to disturb the sediment settling and non-floating microplastics). The decanted portion was then filtered through silicon filters using a vacuum filtration apparatus. The filters were previously dried at 60 °C to constant weight. In the end filters were observed with Optical Microscope by RAMOS 120 (Ostec, Milan, Italy).

NB4/2





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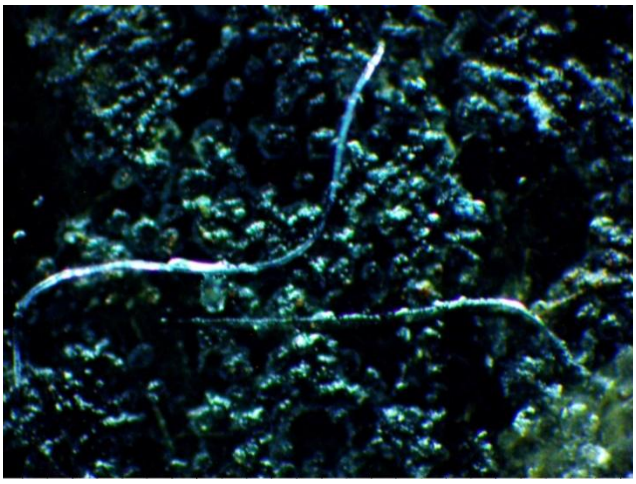
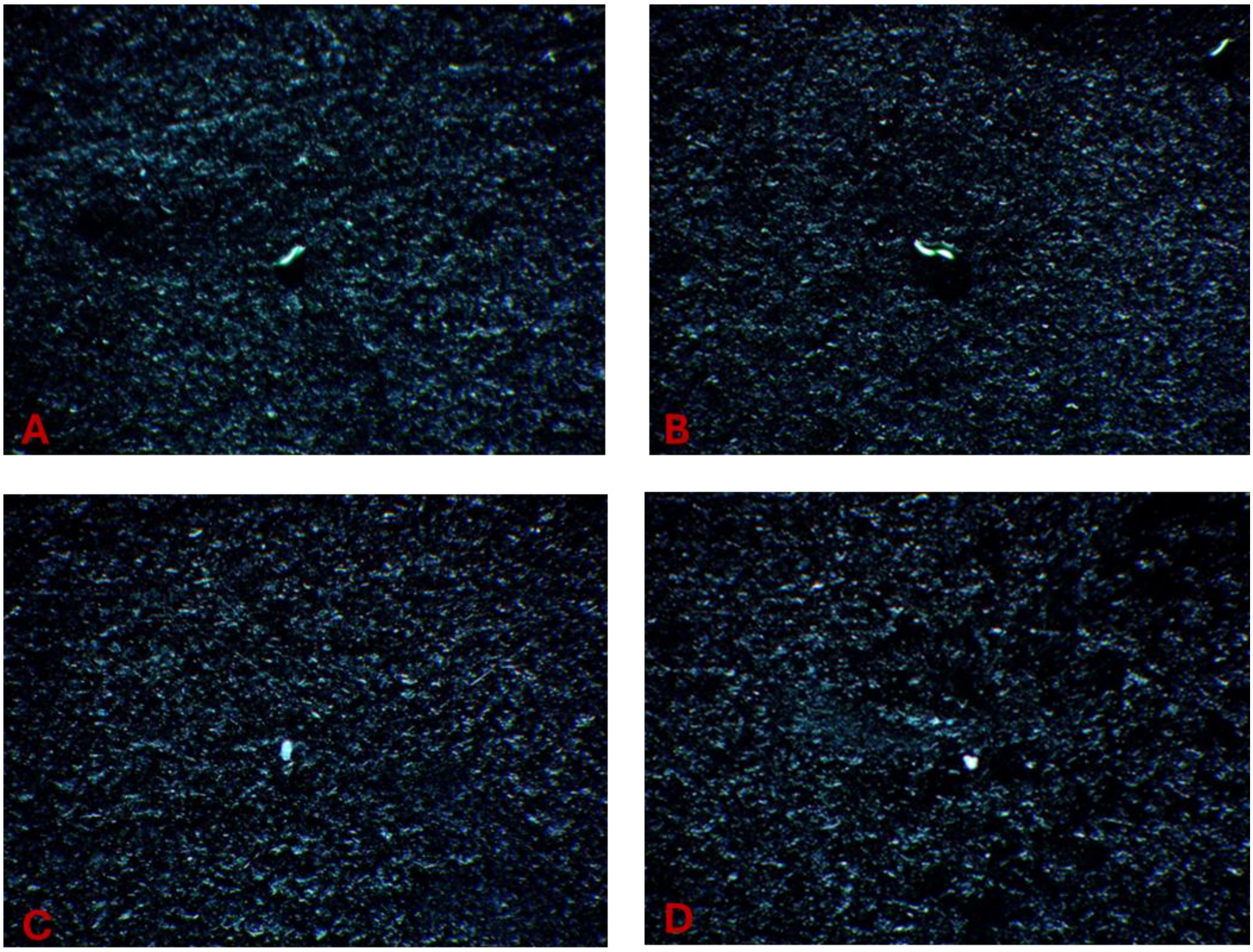


NB5/1



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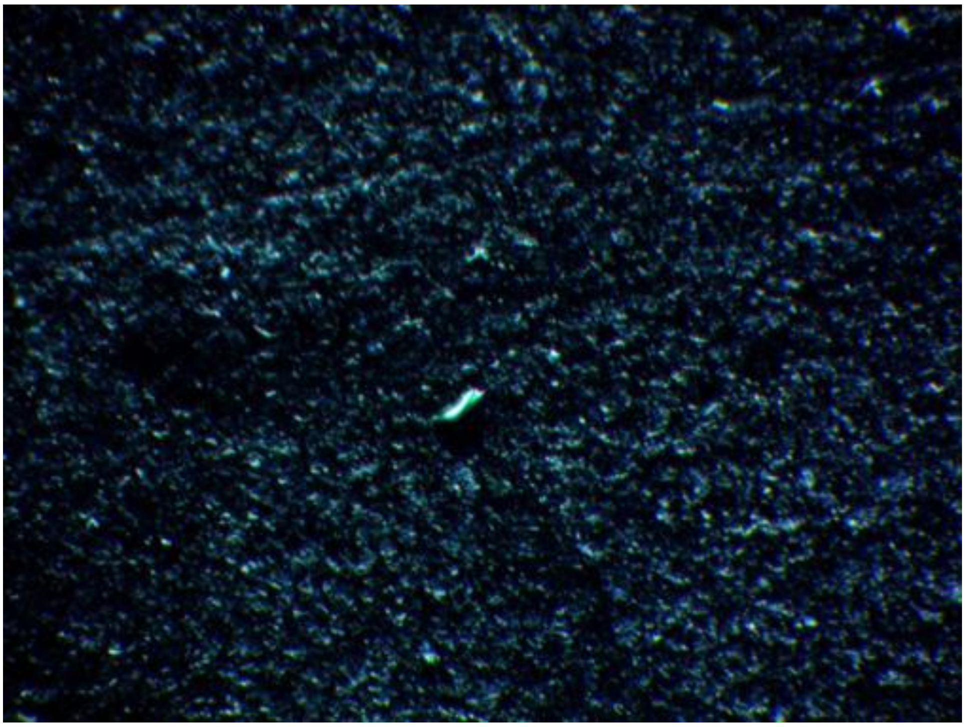
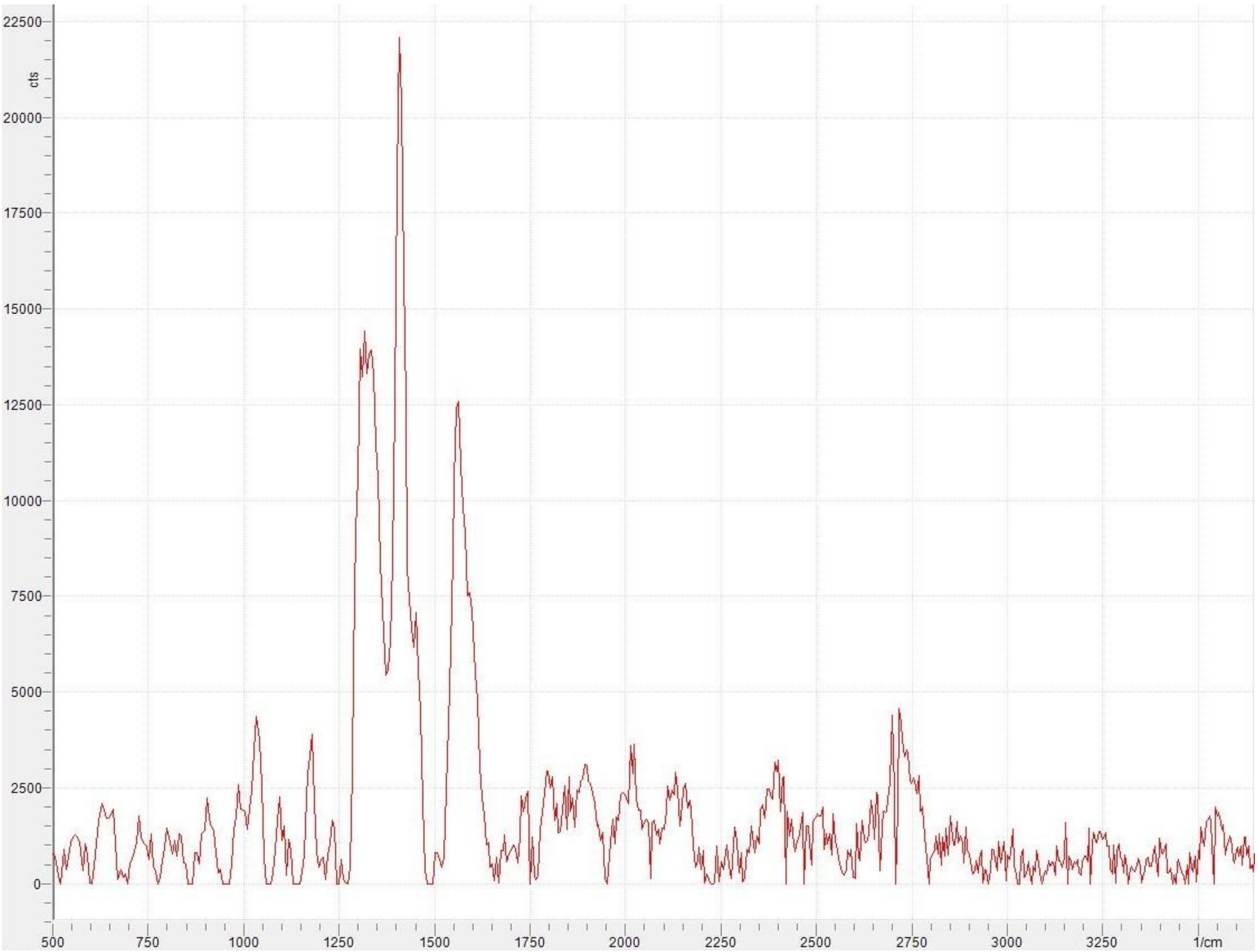
- **NB5/1_A**





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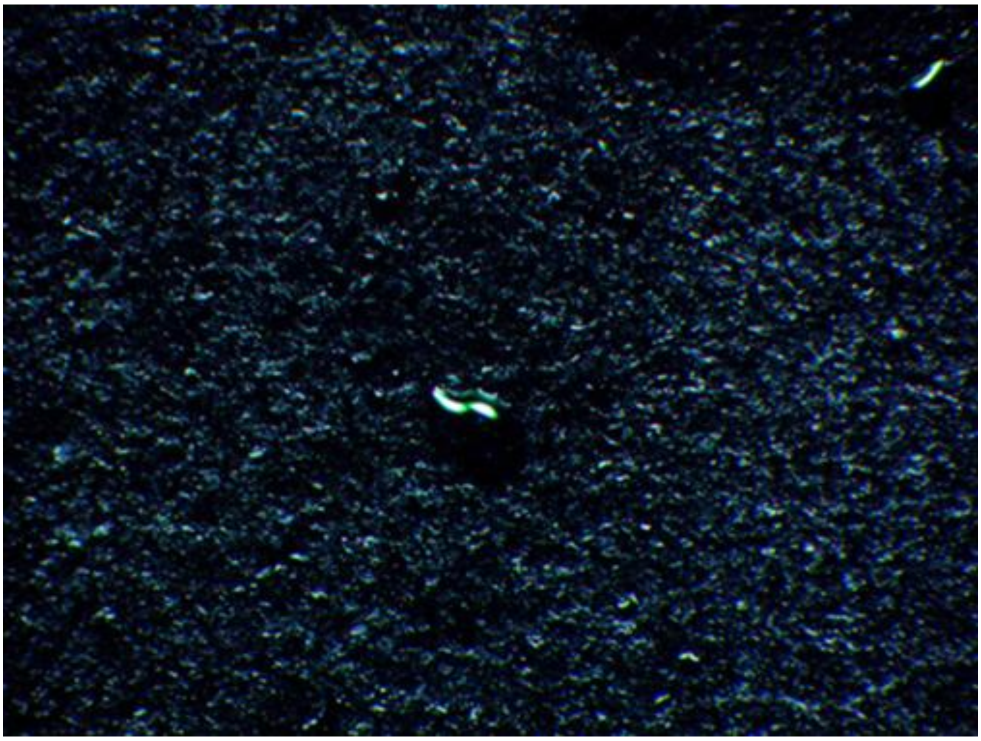

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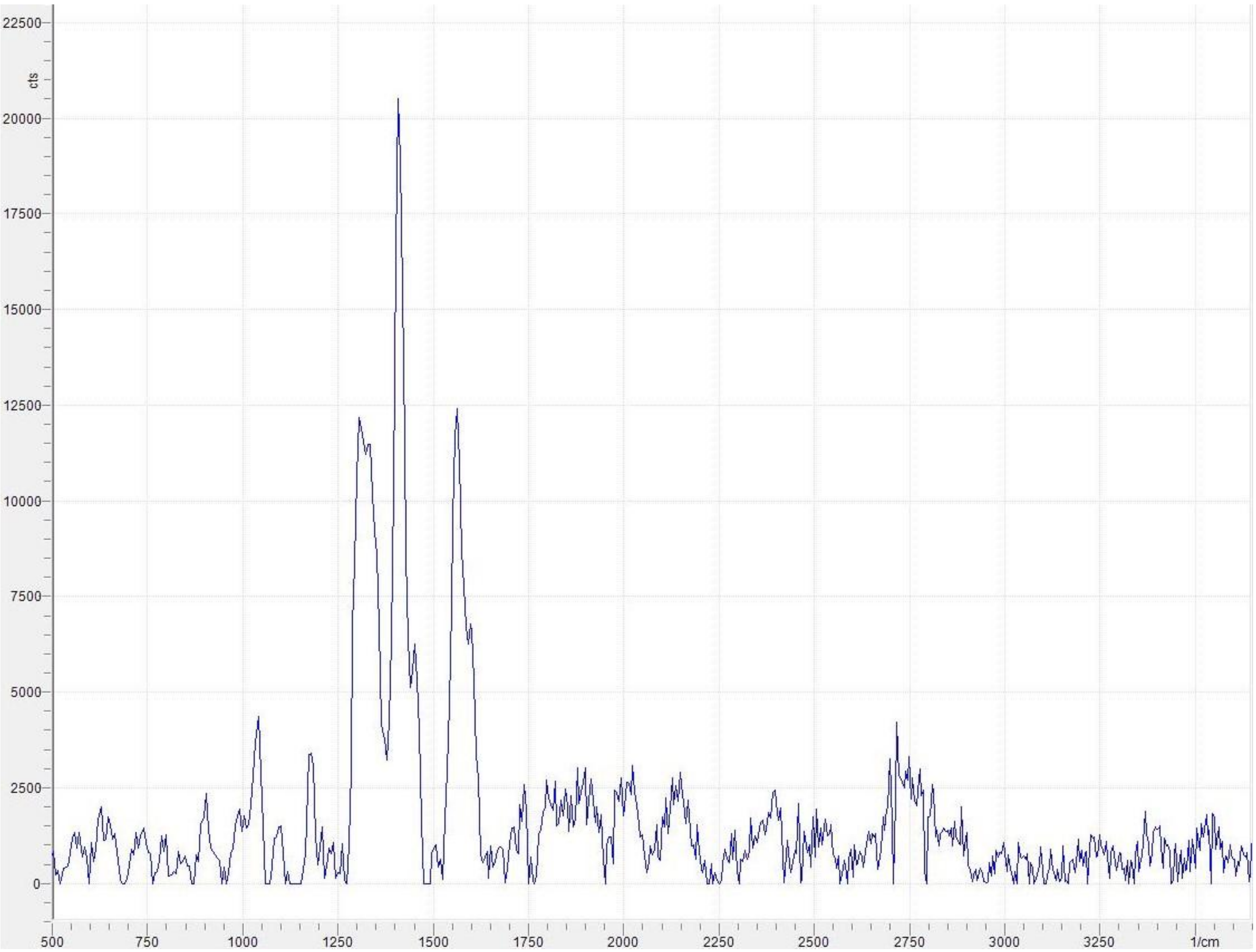

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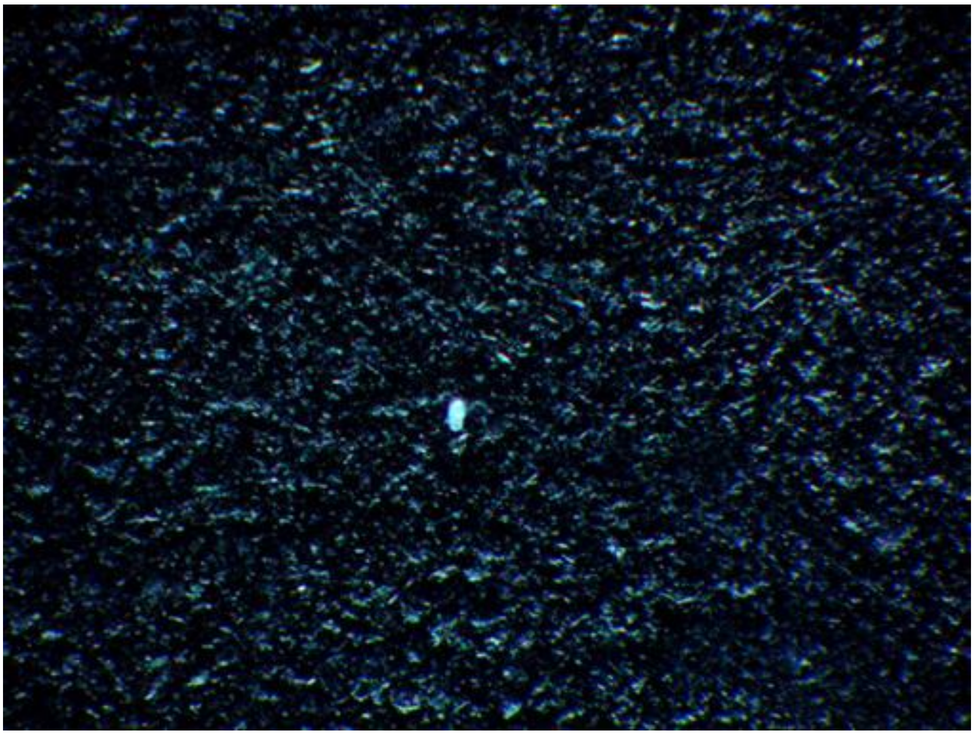

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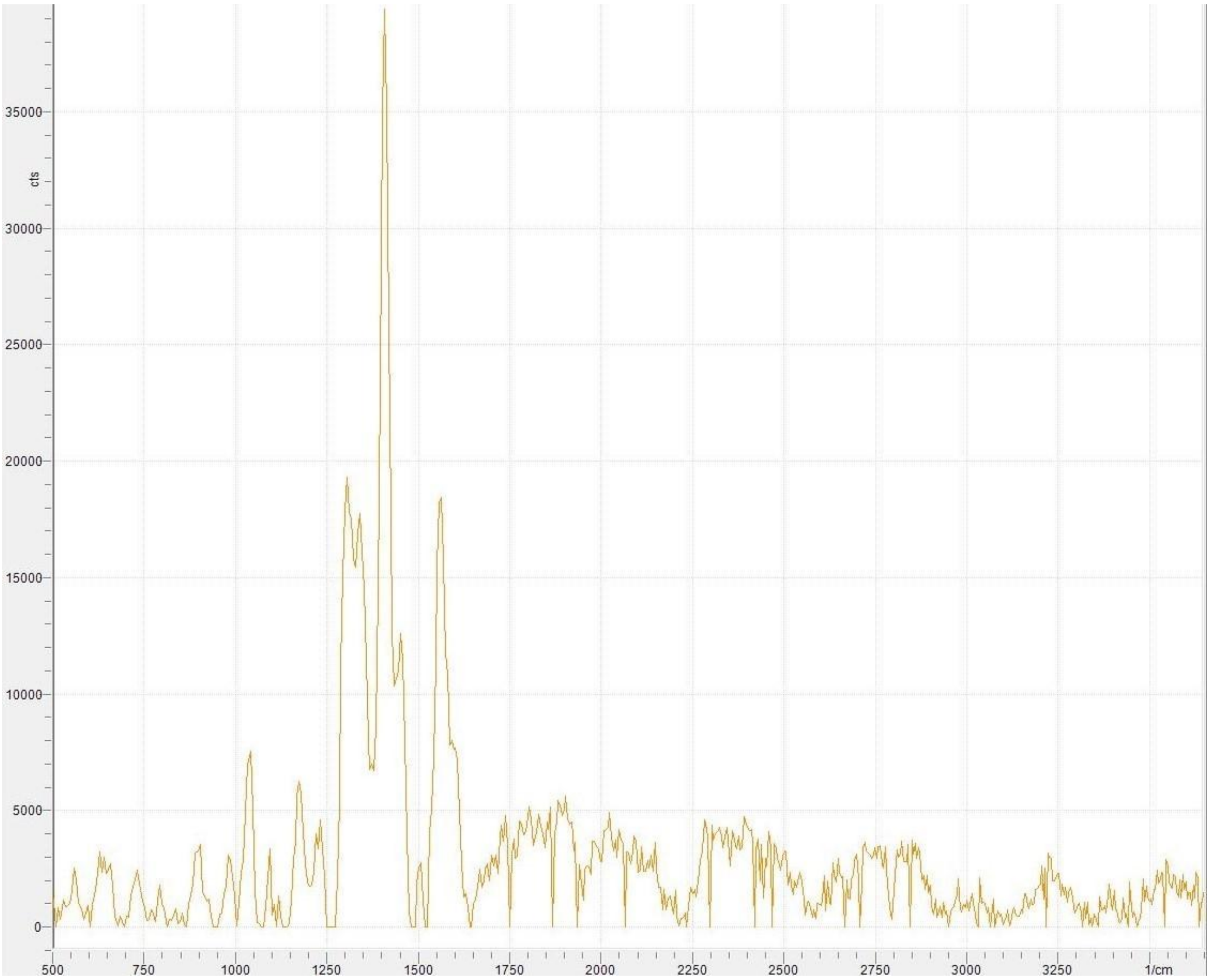

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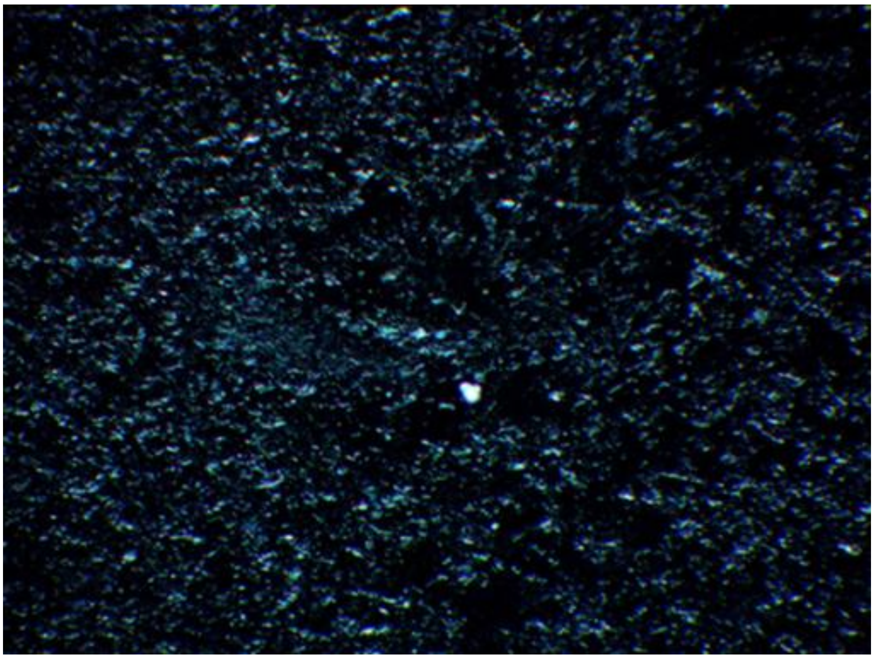

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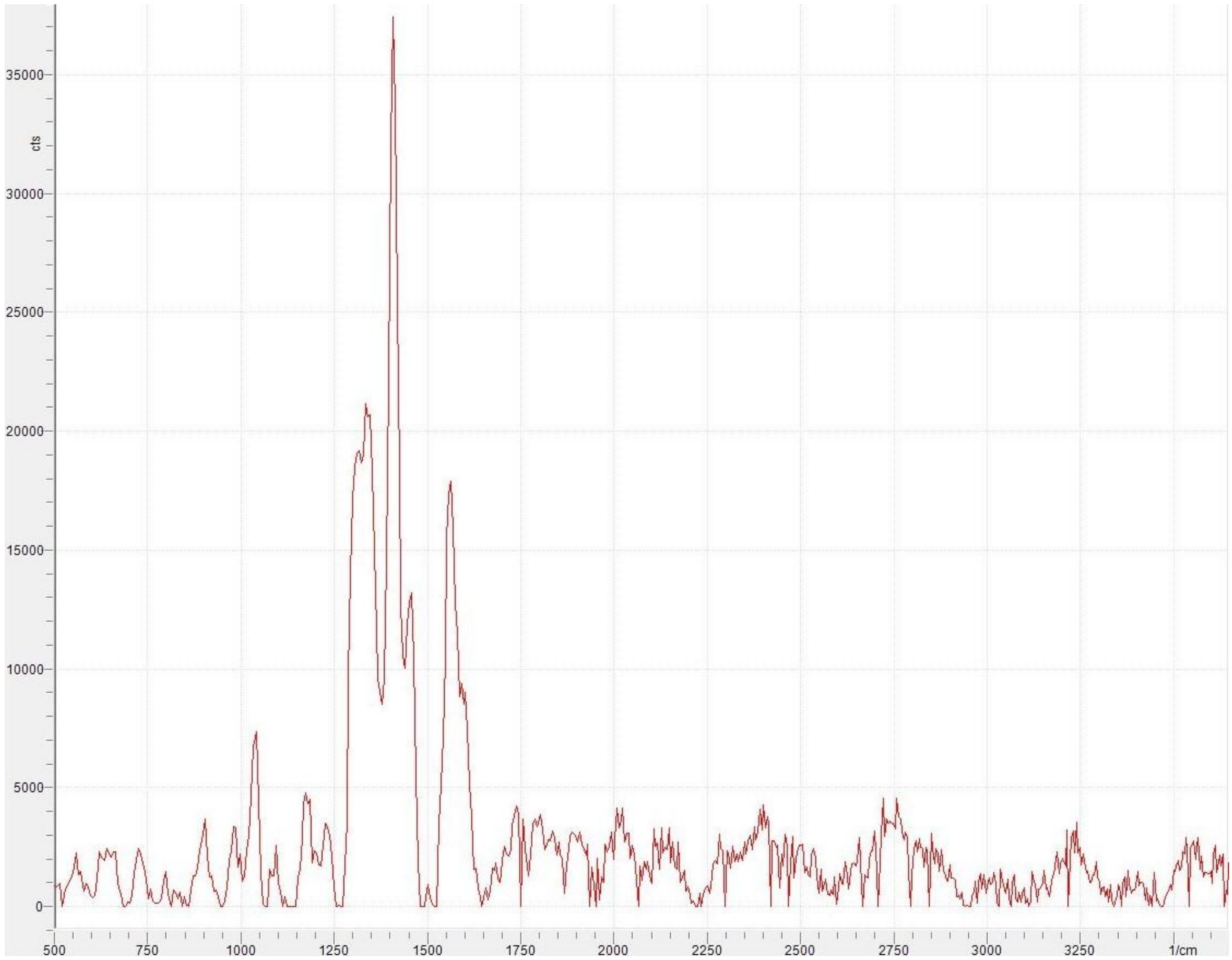

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Extraction

1) **Towards a Consensus Method for the Isolation of Microplastics from Freshwater Sediments,** *Daniel E. Enenche, Christine M. Davidson, John J. Liggat,* ***2024***
2) **Separation and quantification of Microplastics from Beach and Sediment samples using the Bauta microplastic-sediment separator**, *Sabnam Mahat*, ***2017***
3) **Microplastics Monitoring in Marine Environment**, *Agung Dhamar Syakti,* ***2017***
4) **Method for Quantifying and characterization of microplastics in sand beaches,** *Juan Carlos Alvarez-Zeferino, Arely Areanely Cruz-Salas, Alethia Vázquez-Morillas, Sara Ojeda-Benitez,* ***2020***
5) **Microplastics elutriation from sandy sediments: A granulometric approach,** *Mikaël Kedzierski, Véronique LeTilly, Patrick Bourseau, Hervé Bellegou, Guy César, Olivier Sire, Stéphane Bruzaud,* ***2016***

Raman interpretation

1) **Characterization and identification of microplastics using Raman spectroscopy coupled with multivariate analysis**, *Naifu Jin, Yizhi Song, Rui Ma, Junyi Li, Guanghe Li, Dayi Zhang,* ***2022***
2) **A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement**, *Robin Lenz, Kristina Enders, Colin A. Stedmon, David M.A. Mackenzie, Torkel Gissel Nielsen,* ***2015***
3) **Comprehensive Analytical Chemistry, Volume 75, Chapter 5 - Characterization of Microplastics by Raman Spectroscopy**, *Paulo Ribeiro-Claro, Mariela M. Nolasco, Catarina Araújo,* ***2017***
4) **Raman Spectroscopy for the Analysis of Microplastics in Aquatic Systems**, *Veronica Nava, Maria Luce Frezzotti, Barbara Leoni et al.,* ***2021***
5) **The effect of weathering environments on microplastic chemical identification with Raman and IR spectroscopy: Part I. polyethylene and polypropylene**, *Samantha Phan, Jacqueline L. Padilla-Gamiño, Christine K. Luscombe,* ***2022***
6) **Identification of Microplastics Using a Custom Built Micro-Raman Spectrometer**, *Unnimaya, Mithun N, Jijo Lukose, Manju P Nair, Anu Gopinath, and Santhosh Chidangil,* ***2022***
7) **Deep learning assisted ATR-FTIR and Raman spectroscopy fusion technology for microplastic identification**, *Haoze Li, Shihan Xu, Jiahao Teng, Xiangheng Jiang, Han Zhang, Yazhou Qin, Ying sheng He, Li Fan,* ***2025***

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CONSIDERATIONS

In the fingerprint region of Raman spectra (ca. 500 to 1800 cm–1) are shown the following peaks, relatable with:

- 1042: C-C
- 1300: CH3, CH CH2 twisting
- 1334: bending mode of the CH and the twisting mode of CH2 group
- 1345: –CH2- bending
- 1407:
- 1452: CH3 and CH2 bending
- 1560:
- 1590-1600: phenyl ring vibration

HDPE is characterized by three peaks: the C-C bond symmetric stretching vibrations at 1066 and 1130 cm^{-1}, and the CH_2 torsional vibration at 1298 cm^{-1}. Peak at 1345cm^{-1} is specific for the Carbon, generated by the probable combustion of organic matter by the laser.

Protocol	Sub-sample	Shape	Composition	Polymer type
NB4/2		fibre	***	
NB5/1	A	particle	**	HDPE
	B	particle	**	HDPE
	C	particle	**	HDPE
	D	particle	**	HDPE

*Microplastics identified as a priority polymer (PE, PP, PET, PS, PVC, PA, PU, PMMA, PTFE, PC)
**Microplastics identified as a synthetic polymer or chemically modified natural polymer that is not on the list of priority polymers
*** other (e.g. minerals, natural polymers, other) or unidentified

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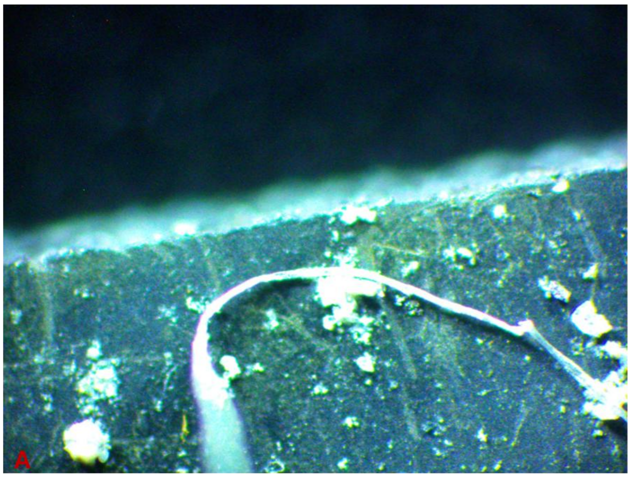

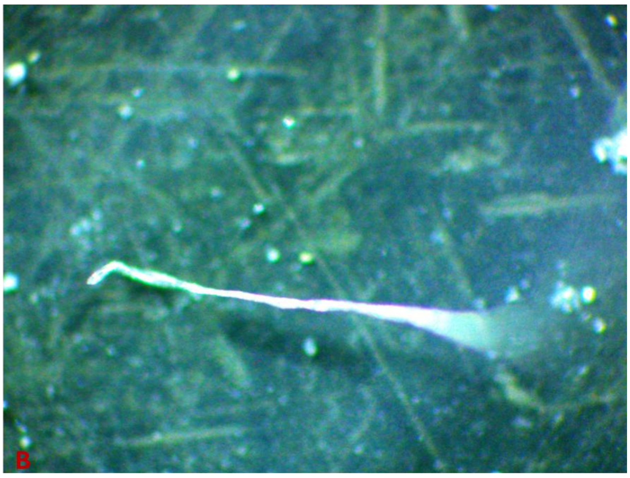

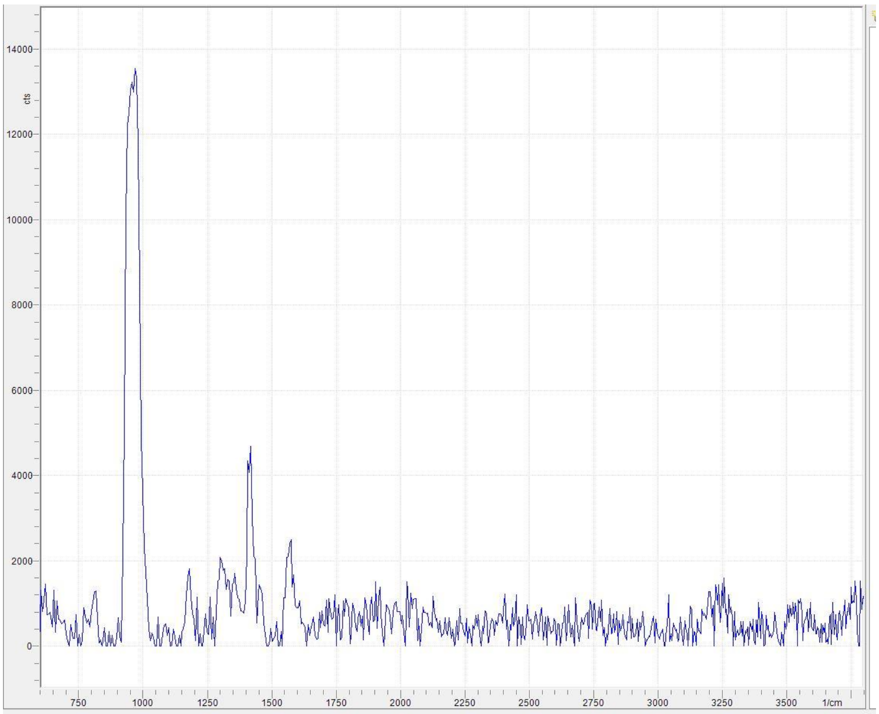

Raman spectrum fibre B

1182: C-O stretching vibrations
1406,1420: CH_2-methilene bending
1573: aromatic C=C stretching or amide-related vibrations in polyamides or nylons

These peaks suggest that the fibre is polyester, and polyethylene terephthalate (PET) is a prime candidate.

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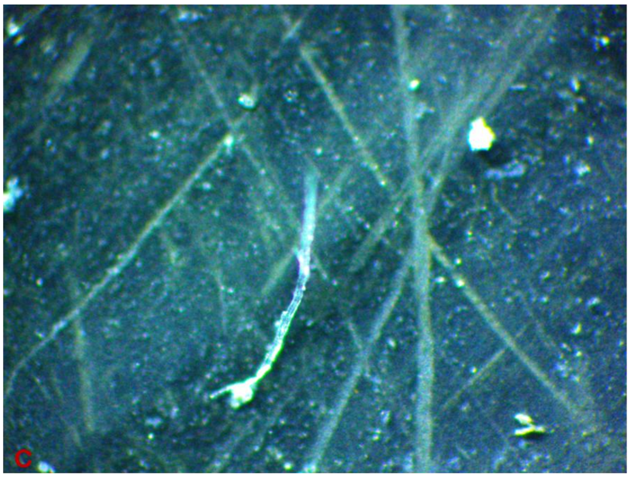

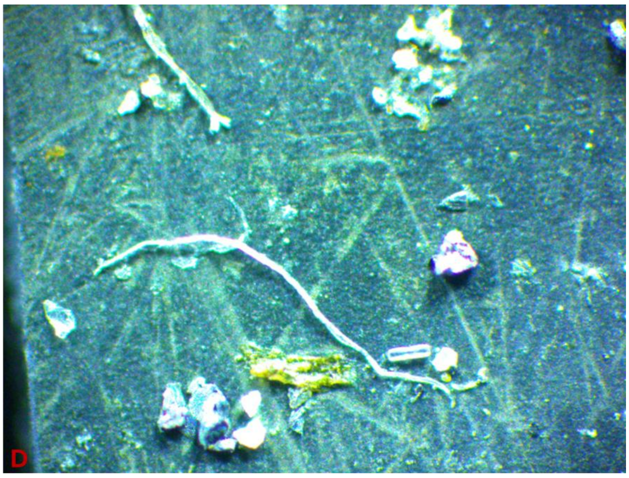

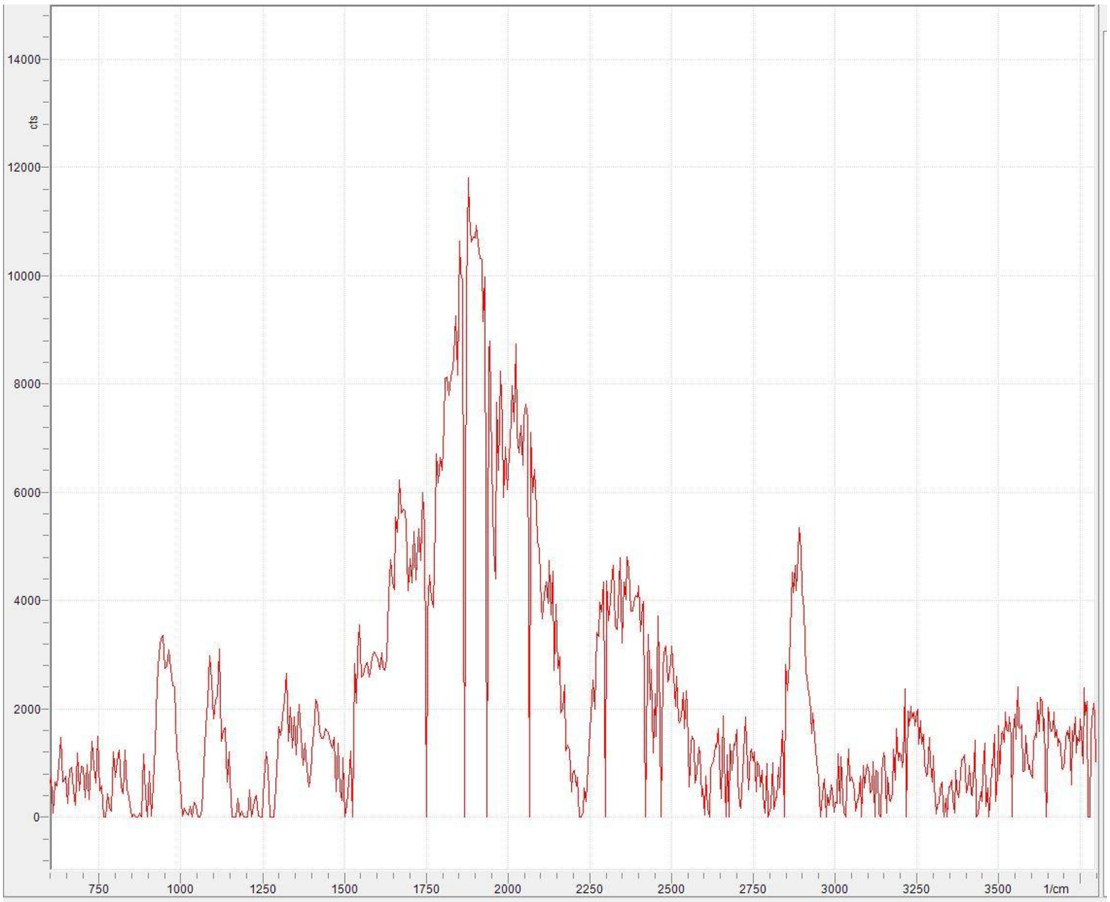

Raman spectrum fibre D

943-961: C-C stretching or CH2 rocking vibrations
1086: vibrational C–C symmetric stretching
1116: C–O–C stretching
1745: C=O from the ester
2890: C–H stretching

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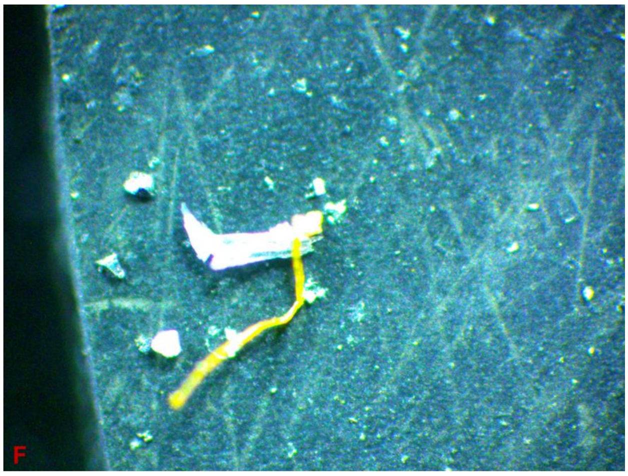
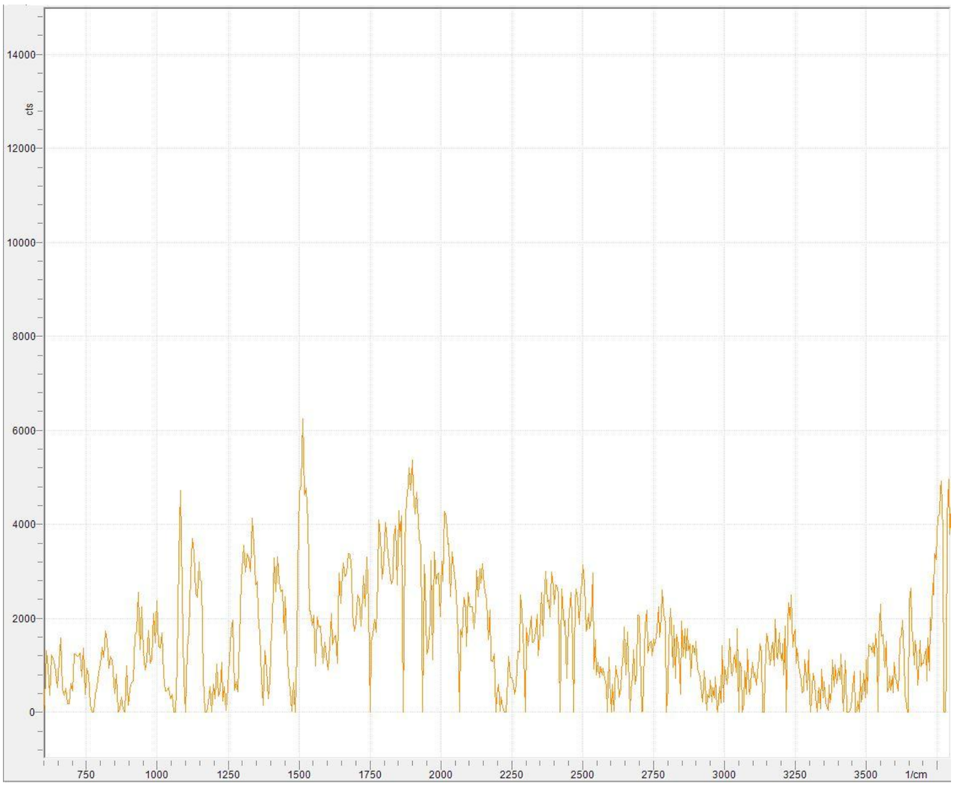
The combination of these peaks suggests the fibre could be polyethylene terephthalate (PET).





Raman spectrum fibre F

1087: vibrational C–C symmetric stretching
1129: C-C symmetric stretching
1331: bending mode of the CH and the twisting mode of CH_2 group
1513, 1515: aromatic C=C stretching
1884, 1905

Raman spectrum suggests polymer is potentially polystyrene (PS).

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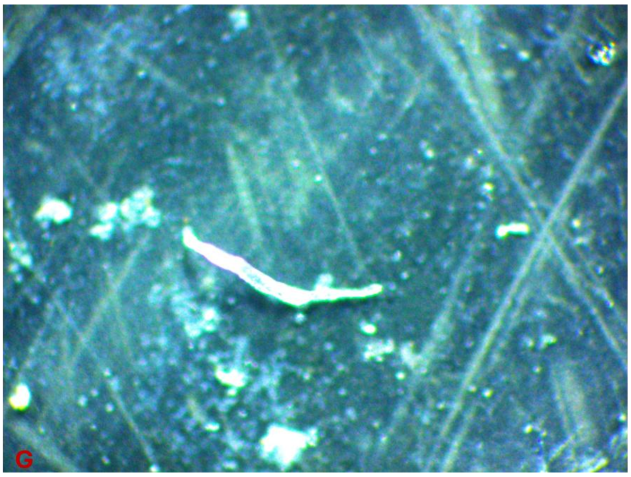

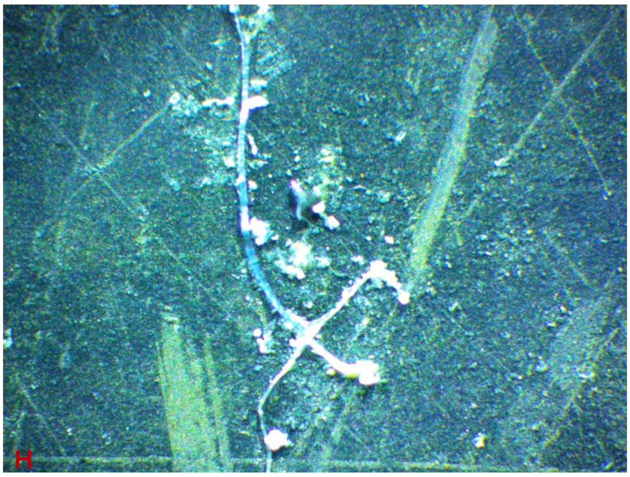

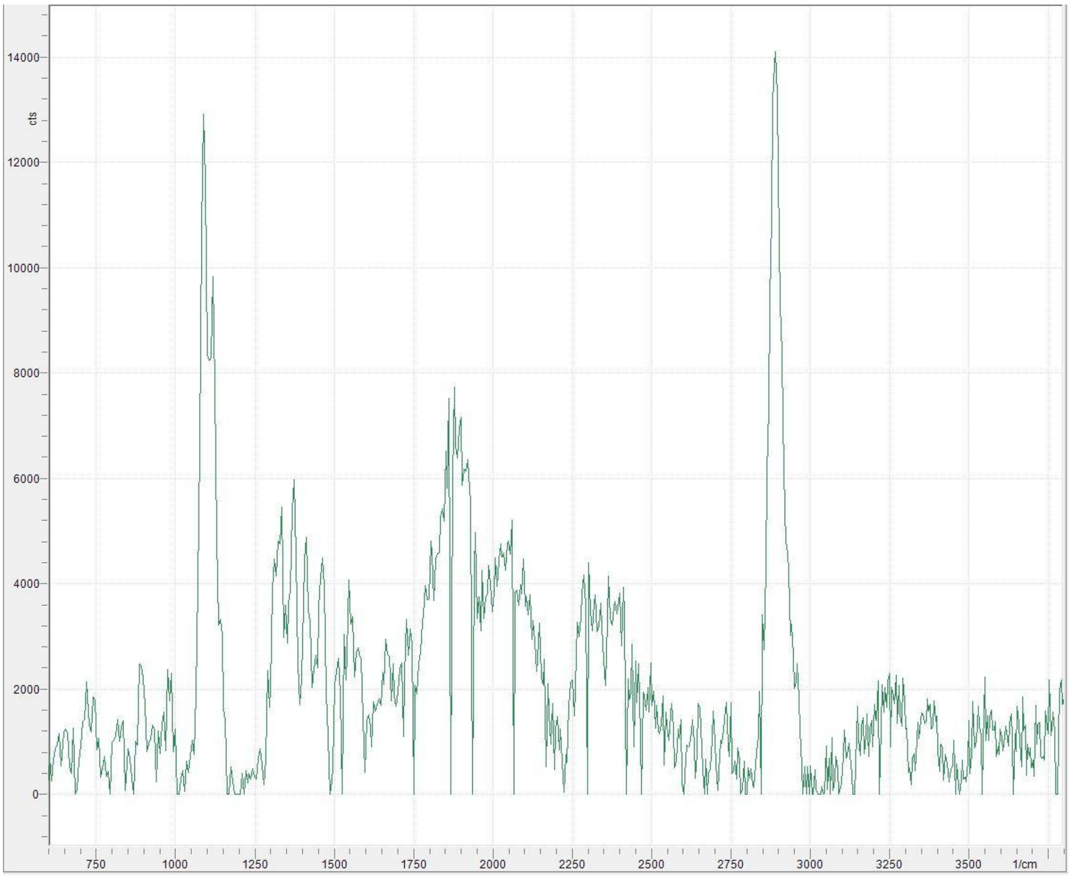

Raman spectrum fibre G

1086, 1116: C-C stretching vibrations
1332: bending mode of the CH and the twisting mode of CH_2 group
1373: C-H vibration in methylene or methyl groups
1412: C-H vibration
1462: asymmetric bending of CH_3 and CH_2 bending
2889: C–H stretching

The peaks appear to be characteristic of aliphatic and aromatic synthetic polymers, such as polyethylene terephthalate (PET), polyethylene or polypropylene. The presence of peaks at

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approximately 1332 cm^{-1} and 2889 cm^{-1} suggests the presence of polymer chains containing methylene or methyl groups, typical of synthetic polymers. In addition, the peak at 1086 cm^{-1} suggests the possible presence of esterified groups, such as those in PET.

Water Analysis Protocol

Water samples are filtered using a vacuum filtration system with a silicon filter (pore size ≤ 0.45 µm). The filters are then observed with the RAMOS 120 optical microscope (Ostec, Milan, Italy). This simplified protocol enables efficient identification and characterisation of microplastics in water.

CONSIDERATIONS

In approximately 800 mL of water, 8 fibres were identified, 4 of which, through Raman analysis, gave us the certainty of their anthropic and polymeric nature.

Protocol	Sub-sample	Shape	Composition	Polymer type
NB_3	A	fibre	***	
	B	fibre	**	PET
NB_4	C	fibre	***	
	D	fibre	*	PET
	E	fibre	***	
NB_6	F	fibre	**	PS
	G	fibre	*	PET
NB_9	H	fibre	***	

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**Microplastics identified as a synthetic polymer or chemically modified natural polymer that is not on the list of priority polymers
*** other (e.g. minerals, natural polymers, other) or unidentified

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