



Validation of the identification of microplastics by micro-FTIR and micro-Raman

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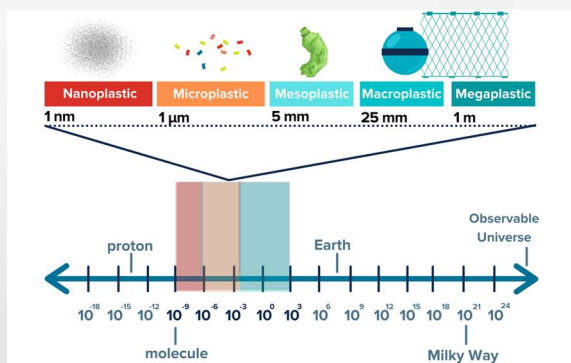


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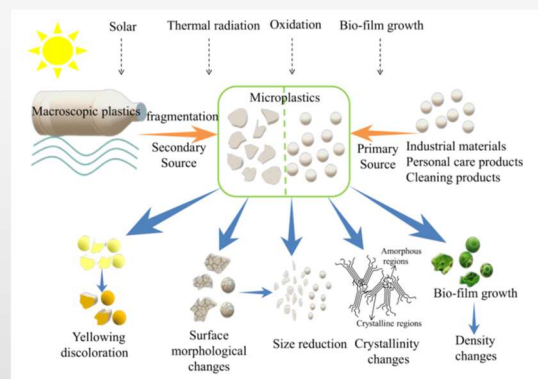
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Introduction

Microplastics (MPs) = “small solid particles composed of synthetic polymers that are non-degradable and less than 5 mm in size” (European Chemicals Agency)



CEN ISO/TR 21960:2020



Marine Pollution Bulletin 142 (2019) 1–14



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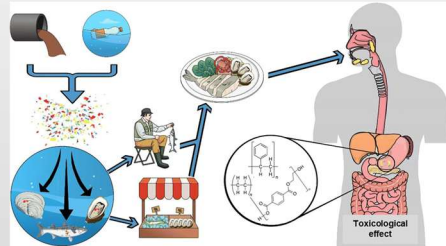
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Introduction



Toussaint et al., *Food Additives & Contaminants: Part A*, 36:5, 639-673 (2019)

- **Microplastics are:**
 - ✓ **Ubiquitous, persistent and cumulative**
 - ✓ Transported by wind, snow and rain, and traveling across entire continents
 - ✓ **Found in soil, air and waters, plants, animals, human diet and drinking water** (even in the Antarctic and on Mount Everest)
- **They can transport, leach and absorb other organic contaminants:**
 - ✓ **Toxic Chemicals** (eg. PCBs, PAHs, PFAS)
 - ✓ Microorganisms



De-La-Torre et al., *Journal of Food Science and Technology*, 2019



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Introduction



Exposure/risk assessment



Source attribution




Environmental monitoring




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Analytical method


Scope: Identification of PE, PP, PET and PS particles, with sizes between 1 µm and 5 mm, in sediment samples




sediment sampling



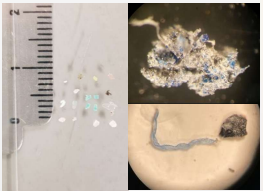
Sieving (5 mm)



H₂O₂ digestion




NaCl density separation



Visualisation & physical analysis

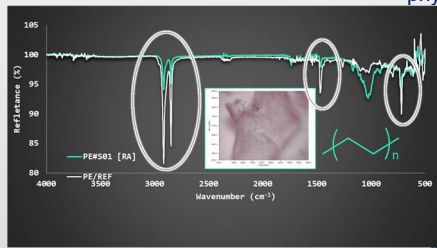
Reference spectra from plastic materials




µATR-FTIR
(4000 cm⁻¹ to 500 cm⁻¹)






←

Identification of particles by µATR-FTIR (manual or automatic)



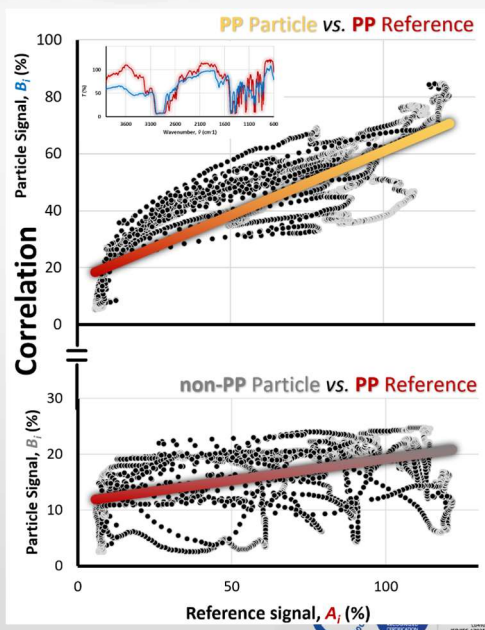
PE – polyethylene; PP – polypropylene; PET – polyethylene terephthalate; PS – polystyrene.




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


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Analytical method

- Automatic µATR-FTIR identification: Involves assessing the match (correlation) between reference and particle spectra.
- Spectral comparison parameters:
 - » signal requirements
 - » wavenumber range
 - » signal processing
 - » match algorithm
 - » target value for the match



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Validation

- Collection of micro-FTIR spectra & Evaluation of contamination and
- Match determination by spectral comparison parameters
- Distribution of the match was simulated
- From the density distribution of:
 - Positive case $P5 \gg P$
 - Negative cases $P95 \gg N$
- Validation parameters were calculated FP and $LR(+)$
- Decision on the validity of the method
 - $P5 \gg P > P95 \gg N$
 - $FP < 5\%$ $LR(+)> 19$

Note: $P5 \gg P$ is associated to a TP of 95 %

Examination uncertainty

$LR(+)=\frac{TP}{FP}$

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Match methods

Spectra exclusion	Type of spectral intensity	Type of processing	Match algorithm	Signal weighting	Wavenumber weighting
Yes	I_i vs $\tilde{\nu}_i$	Original	r		
No	I_i^{-1} vs $\tilde{\nu}_i$	First derivative	ρ	Yes	No
	I_i^c vs $\tilde{\nu}_i$		C_{PE}	No	$\tilde{\nu}_i$
			r_w		$1/\tilde{\nu}_i$
			ρ_w		
			C_{PE_w}		

----- An example where after no exclusion of some wavenumber range, the Spearman correlation coefficient, weighted for signal and inverse wavenumber, based on the first derivative of transmittance spectra is collected (options combination 2|1|2|5|1|3)

- 240 match methods were studied

r : Pearson's CC
 ρ : Spearman's CC
 C_{PE} : Alternative CC
 r_w : Weighted Pearson's CC
 ρ_w : Weighted Spearman's CC
 C_{PE_w} : Weighted Alternative CC
 I : Original signal
 I^{-1} : Inverse signal
 I^c : Complementary signal
 $\tilde{\nu}$: Original wavenumber
 $1/\tilde{\nu}$: Inverse wavenumber

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Results

- Experimental part:
 - » Analysis of 34 sediments from Ria de Aveiro, Ria Formosa and Mira River (Portugal)
- 319 microplastics were identified.
- PET and PS were identified in one step
- PE and PP were identified in two steps

Top three valid match methods for PE identification

Match Method	P5»P	FP(%)	LR(+)	n(+)	n(-)
1 1 1 4 1 1	0.9765	0.696	137	72	140
1 1 1 1	0.9802	0.729	130	71	140
1 3 1 1	0.9802	0.729	130	71	140

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Results

Collecting micro-Raman spectra from PET (Polyethylene terephthalate) and non-PET particles

Sample name	Correlation	sample S/N ratio	Sample auto identification	sample highest absolute intensity peak	sample Raman shift of said peak	Note
S001-PET 06 (F)	0.99238897	13	PET	33632	1618	the Raman shift of the highest absolute peak corresponds to PET characteristic peaks

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Best-performing match algorithms, considering all collected spectra and spectra with S/N larger than 10. The $P5 \gg P$ is the match threshold, FP the false positive rate, LR the likelihood ratio ($LR = TP/FP$; TP is the true positive rate), and n_P and n_N the number of considered PET and non-PET cases

True Positive rate 95 %

False Positive rate 5×10^{-7} %

$P5 \gg P = 0,6244$

Performance

Spectra with $S/N > 10$ ($n_P = 80$ and $n_N = 33$)			
Match	$P5 \gg P$	$FP / \%$	LR
1 1 r	0.6244	4.90×10^{-7}	1.94×10^8
3 1 C_{PEw} 1 1	0.9664	8.09×10^{-5}	1.17×10^6
3 1 C_{PEw} 1 2	0.9734	0.000537	1.77×10^5
3 1 C_{PE}	0.9325	0.282	336
1 1 ρ	0.3471	0.507	188
1 1 C_{PE}	0.5703	0.620	153
1 2 r	0.3438	0.627	151
3 2 C_{PE}	0.3089	1.33	71.2
1 2 C_{PE}	0.3051	1.45	65.6
1 1 r_w 1 2	0.5072	1.68	56.5
1 1 r_w 1 1	0.4692	2.93	32.4

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Experiment "Circumnavigation of STS Sagres" (2020)

- **Period:** 05/01/2020 – 09/05/2020
- **Route:** Lisboa – Canary Islands – Cape Town – Rio de Janeiro (Brazil) – Buenos Aires (Argentina) – Cape Town (South Africa) – Lisboa
- **Water samplings collected stations:** 12

• 1 (10)

• 2 (10)

• 3 (10)

• 4 (13)

• 5 (8)

• 6 (5)

• 7 (5)

• 8 (1)

• 9 (3)

• 10 (2)

• 11 (1)

MP (quantity of stations with # MP observed)

* - Circumnavigation interrupted due to Covid-19

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Experiment "Circumnavigation of STS Sagres" (2020)

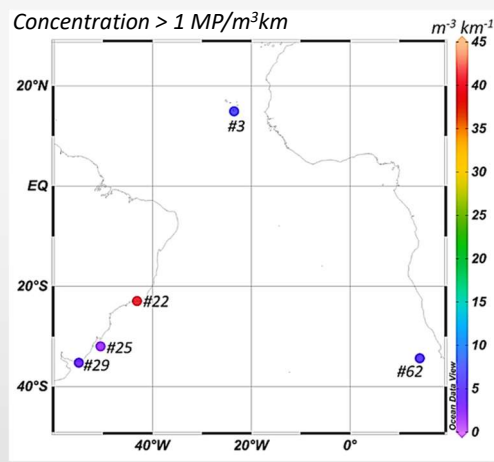
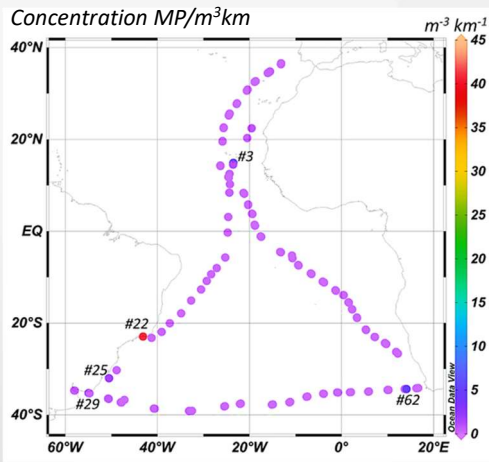


Table 1: Estimated MP contamination with uncertainty, for 99% confidence level, in studied linear Atlantic Ocean paths

Sampling	Concentration (m-3 km-1) §	Date	Time interval (hh:mm:ss)	Start coordinates	End coordinates	Linear length (km)	Water volume (m3)	Note
#3	5.9 ± 5.2	19/01/2020	01:01:00	14° 54.55' N; 023° 29.97' W	14° 54.55' N; 023° 29.97' W	0.00	1.36	Close to Santiago
#22	41 ± 27	09/02/2020	01:09:00	22° 54.57' S; 043° 09.70' W	22° 54.58' S; 043° 09.18' W	0.9	0.41	Rio de Janeiro
#25	1.6 ± 1.6	19/02/2020	00:17:00	31° 57.24' S; 050° 30.46' W	31° 58.62' S; 050° 31.58' W	3.1	0.81	
#29	3.4 ± 3.4	04/03/2020	00:25:00	35° 11.80' S; 054° 59.15' W	35° 13.15' S; 054° 55.69' W	5.8	0.19	
#62	4.9 ± 2.4	23/03/2020	01:01:00	34° 20.54' S; 013° 58.17' E	34° 20.85' S; 014° 06.04' E	12.1	0.46	Close to South Africa

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Experiments "Sagres and Mar Aberto" (2021 and 2022)

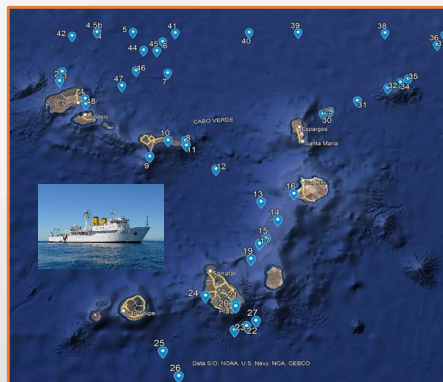
Sagres + 1st Mar Aberto

2nd Mar Aberto

3rd Mar Aberto



(31/12/2020 – 11/02/2021)
2 + 44 stations

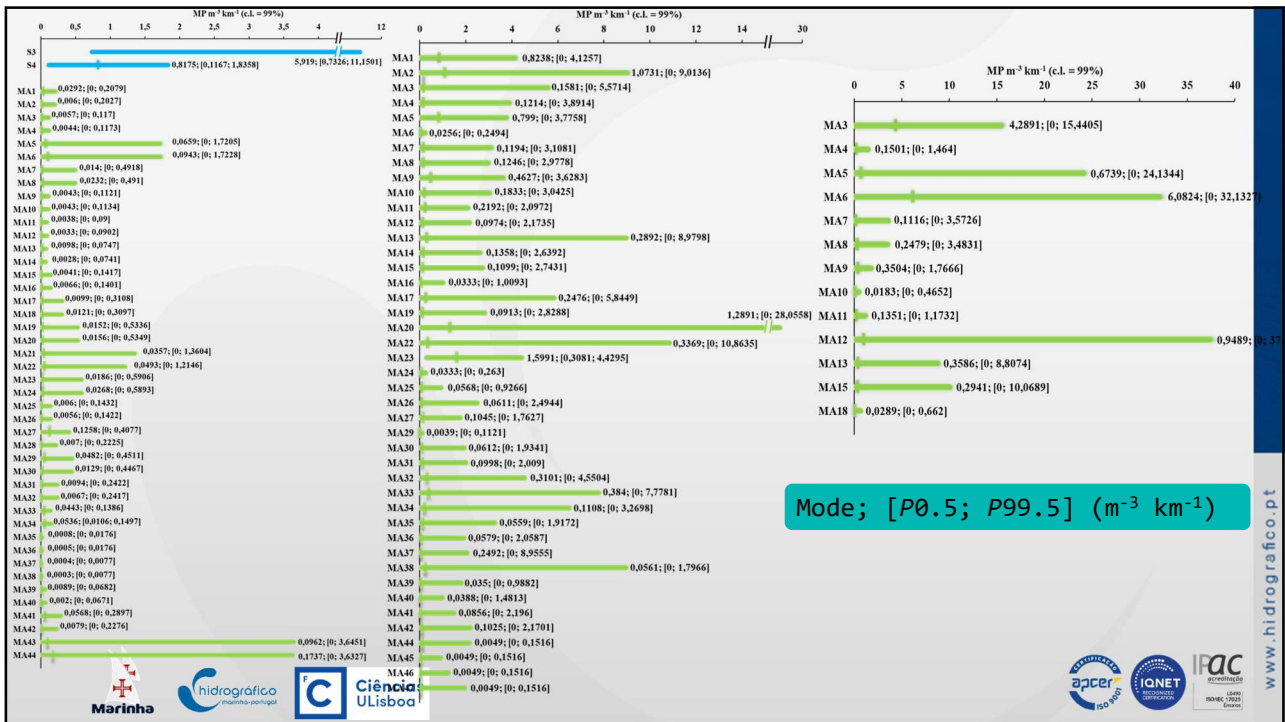


(30/10/2021 – 15/11/2021)
48 stations



(20/11/2022 – 25/12/2022)
16 stations

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Final remarks

- ✓ High-quality data is critical for understanding pollution levels, supporting environmental monitoring programs, and ultimately protecting both ecosystems and human health.
- ✓ Validation of the identification of microplastics is essential.
- ✓ Every step—from sampling to laboratory analysis—introduces uncertainties.
- ✓ Quantification of the uncertainties is a requisite of good practice.
- ✓ Recognizing and controlling these sources of variability is essential.

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