Activity: WES N-E-MO-2

Training on marine litter monitoring

Monitoring microlitter on the sea surface with manta trawling: experimental design and survey protocol

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MIO-ECSDE

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MICROLITTER STUDIES IN THE MEDITERRANEAN

The Mediterranean Sea is one of the most studied regions in the world in terms of microplastic contamination


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MICROPLASTICS: FINDING A CONSENSUS ON THE DEFINITION

Thompson et al. (2004) initially coined the term microplastics to describe the accumulation of microscopic pieces of plastic in marine sediments and in the water column of European waters.

In 2009, Arthur et al., proposed an upper size limit to the initial term and microplastics where known as "plastic particles smaller than 5 mm".

This definition was further refined in 2011, when Cole et al. (2011) distinguished microplastics, according to their origin, into primary (produced to be of microscopic dimensions) or secondary (resulting from degradation and fragmentation processes in the environment).

The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP), defines microplastics as 'plastic particles <5 mm in diameter, which include particles in the nano-size range (1 nm).

- There is still no clear consensus on a definition that is extensive enough to encompass all necessary criteria to describe 'microplastics'. This technicality causes several methodological challenges.
- Regarding size, there is still no agreement on the upper and lower size limits to microplastics, even though the most used definition is the one proposed by Arthur et al.

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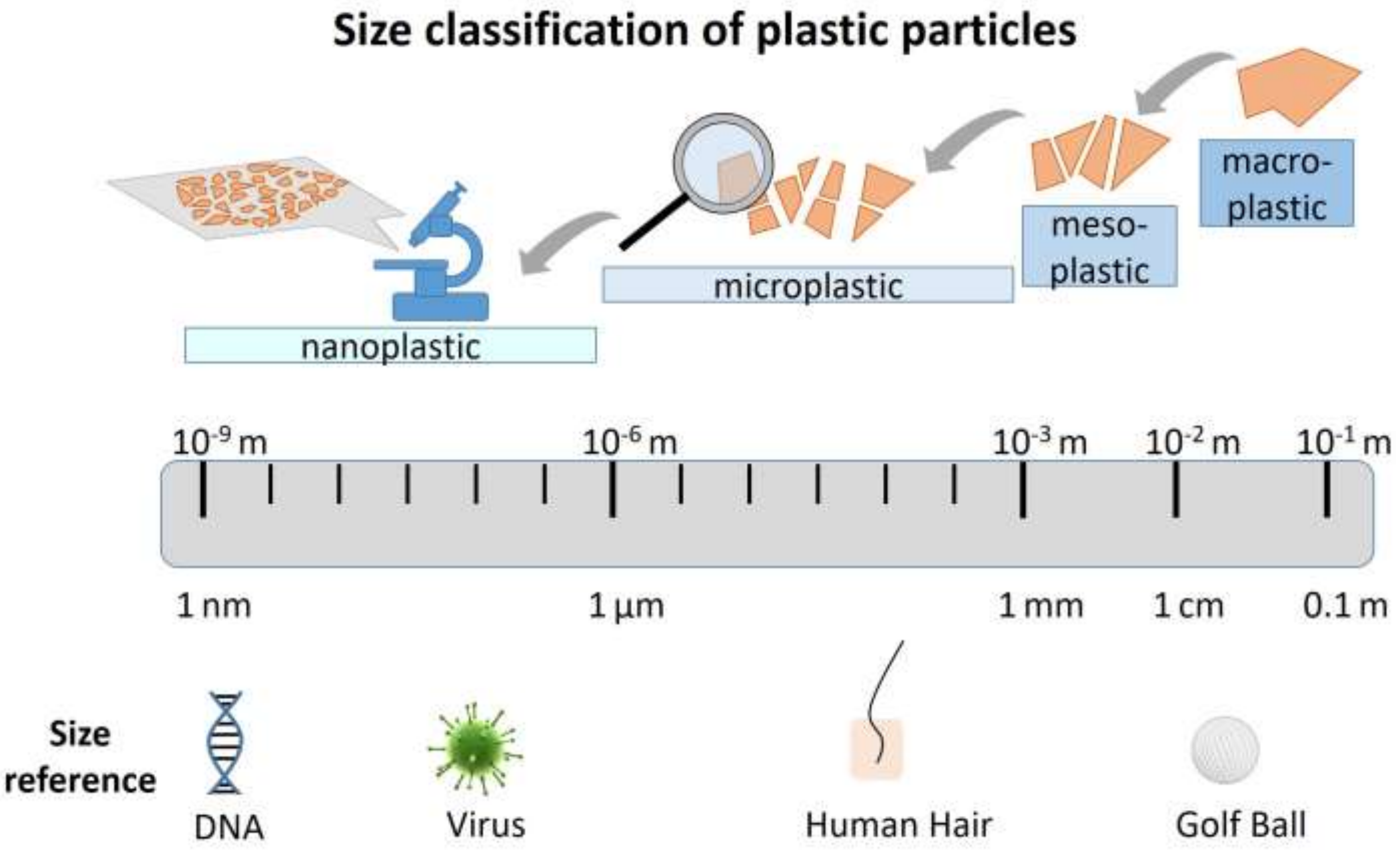
SIZE CLASSIFICATION OF PLASTIC PARTICLES

Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water Source: Frias and Nash, 2019.



CLASSIFYING MICROPLASTICS BY SHAPE & COLOR

Microplastic type	Definition	Potential sources
Fragment	Hard, jagged plastic particle	Bottles; hard, sturdy plastics
Line/fiber	Thin or fibrous, straight plastic	Fishing line/nets; clothing or textiles
Pellet	Hard, rounded plastic particle	Virgin resin pellets; facial cleansers
Film	Thin plane of flimsy plastic	Plastics bags, wrappers, or sheeting
Foam	Lightweight, sponge-like plastic	Foam floats, Styrofoam, cushioning

Microplastic colour is considered important, for studies concerning marine organisms, as some species are thought to potentially ingest microplastics based on a colour preference behaviour

SIZE IS IMPORTANT

- Different sizes of plastic particles or larger plastic objects need different types of equipment to sample them in the ocean and different analytical techniques in the laboratory.
- Size also determines the likely impact on ocean life and human activities such as fisheries.

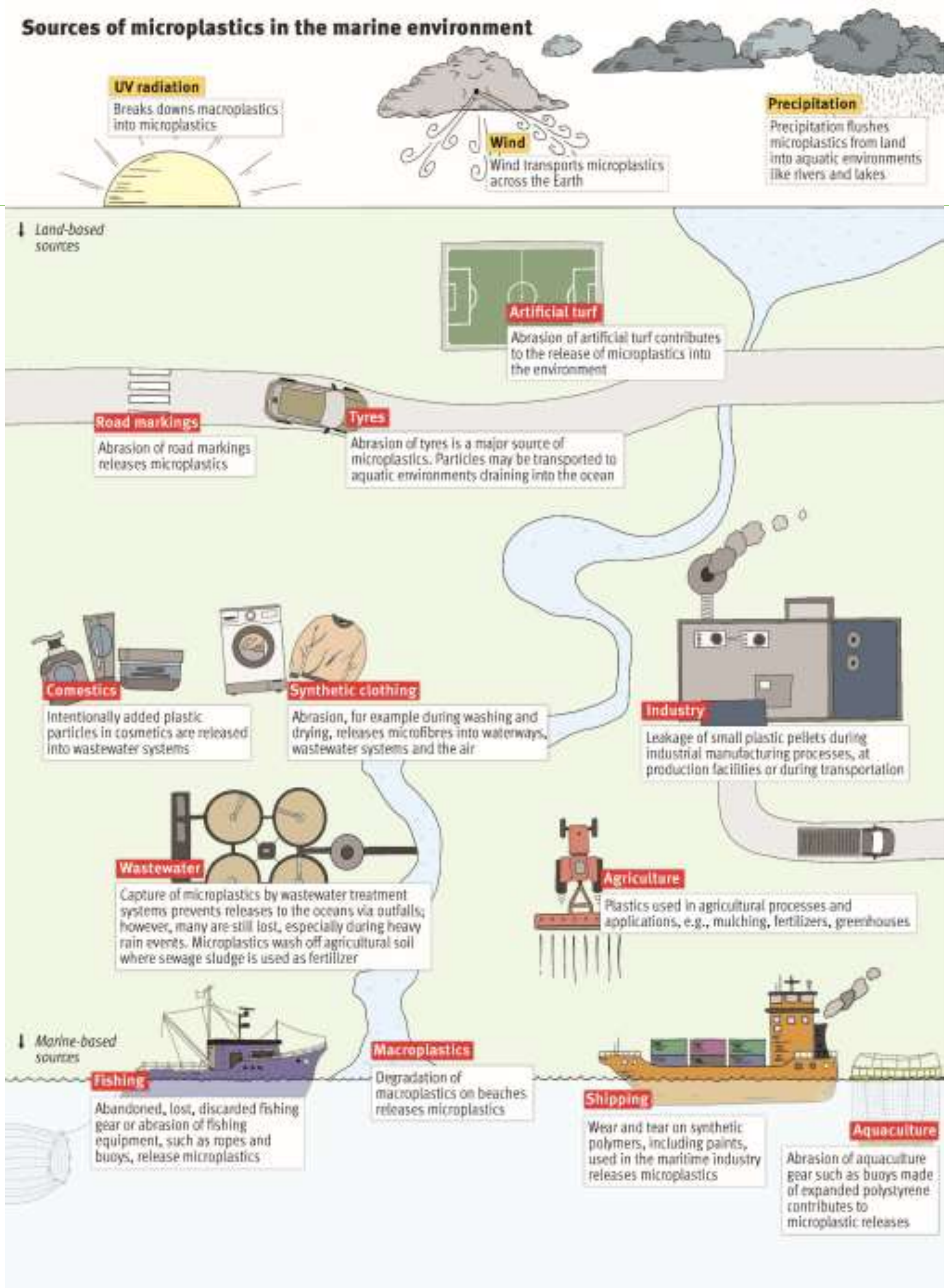
TYPES OF MICROPLASTICS

Primary microplastics

manufactured for the purpose of being added to (or used in the production of) other products

Secondary microplastics

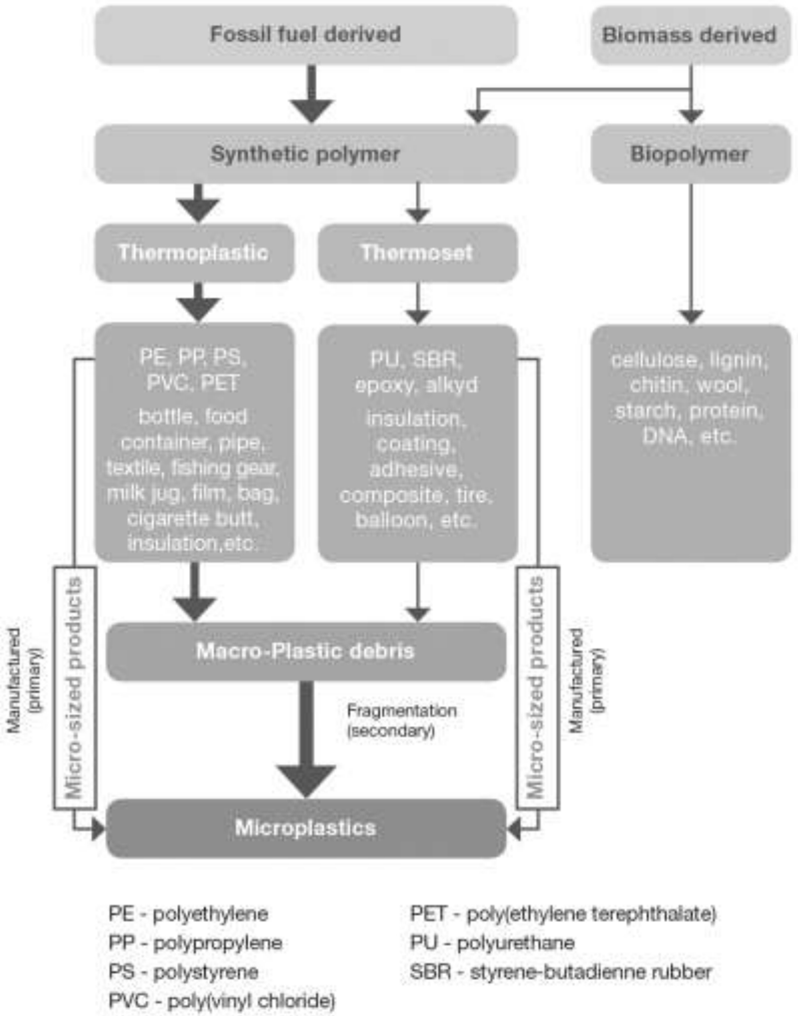
created by the fragmentation and degradation of macroplastics



MICROPLASTICS TERMS & DEFINITIONS IN THE RPMMM

- Primary microplastics: tiny particles designed for direct commercial use (such as cosmetics, detergents and paints components), or for indirect use (such as pre-production pellets).
- Secondary microplastics: the fraction of microplastics in the marine environment which results from the breakdown of larger plastic items into numerous tiny fragments due to mechanical forces and/or photochemical processes, as well as from other degradation sources such as water bottles, fibres in wastewater from washing clothes and particles of rubber lost from tyres due to normal wear.

MICROPLASTICS CLASSIFICATION OVERVIEW



MICROLITTER MONITORING APPROACHES

Beach sediments

Sea surface

Seafloor sediments

Biota

MICROLITTER STUDIES IN MEDITERRANEAN BEACH SEDIMENTS

Location	Size	Sampling			Extraction					Identification	Reference
		Beach zone	n	Depth (cm)	Drying duration/ Temp	Extraction process	Stirring time/ speed	Settling time	Repeat extractions		
Slovenian coast	250µm-5mm	Entire beach	3	5	24 h/60 ºC	1.2 kg/L NaCl	2 min manually	30 min	2	Visual (microscope)	Laglbauer et al., 2014
Slovenian coast	250 µm-5mm	Sublittoral zone	3	ND	24 h/60 ºC	1.2 kg/L NaCl	2 min manually	30 min	2	Visual (microscope)	Laglbauer et al., 2014
North-western Adriatic coast, Italy	≤5mm	High tide mark	6	5	48 h/50 ºC	Optical	-	-	-	FTIR-ATR	Munari et al., 2017
Mediterranean coastline, Morocco	1.25- 4.75 mm	Entire beach	3	5	1 h/65 ºC	Optical	-	-	-	-	Alshawafi et al., 2017
Northern coast of Crete isl.	≥2mm	Entire beach	12-18	10	-	Optical	-	-	-	-	Karkanorachaki et al., 2018
Mediterranean Sea (Esp, Fr, It, Gr, Tr, Is)	0.3- 5mm	High tide line	5	5	48 h/60 ºC	1.2 kg/L NaCl	2 min/ 900 rpm	8 h	3	Visual (microscope) and Raman spectroscopy	Lots et al., 2017
Northern Tunisian coast	≥1mm	ND	3	2-3	air	1.2 kg/L NaCl	5 min manually	ND	ND	FTIR-ATR	Abidli et al., 2018
Kea isl., Aegean Sea	1-2 mm	Upper beach	3-4	3	ND	1.2 kg/L NaCl	ND	ND	ND	FTIR-ATR	Kaberi et al., 2013
Kea isl., Aegean Sea	2-4 mm	Upper beach	3-4	3	ND	Optical	-	-	-	Visual and FTIR-ATR	Kaberi et al., 2013
Samos isl., Greece	≥1.2 µm	Beach and sublittoral zone	27	0-5, 5-10, 10-15 cm	ND	1.2 kg/L NaCl	ND	ND	ND	ND	De Ruijter et al., 2018

Source: Vlachogianni, et al., 2018. State-of-the-art methods to monitor marine litter and its impacts on biodiversity. Interreg Med Plastic Busters MPAs project.

MICROLITTER STUDIES IN THE MEDITERRANEAN SEA SURFACE

Photo: Thomais Vlachogianni

Location	Sampling						Identification	References
	Compartment	Net	Mesh size	Mouth	Vessel speed	Time		
North Western Mediterranean Sea Tuscan coast	Sea surface/Water column	Surface samples: Manta-net Vertical hauls: WP2 plankton net	Manta trawl net: 330 μm WP2 net: 200 μm	Manta trawl: 0.5 × 0.25 m WP2: 0.57 m diameter	Manta trawl: 2 – 3 knots WP2: 0 knots	Manta trawl: 20 min WP2: up to 100 m	FTIR	Baini et al., 2018
Western Mediterranean Sea PelagosSanctuary	Sea surface	Manta-net	330 μm	0.5 × 0.25 m	3 – 4 knots	30 min	FTIR	Fossi et al., 2017
Western Mediterranean SeaGulf of Lion	Sea surface	Manta-net	780 μm	0.5 x 0.15 m	2.5 knots	20 min	Visual (microscope)	Schmidt et al., 2017
Aegean-Levantine Sea, Turkish	Sea surface	Manta-net	333 μm	0.4 x 0.2 m	-	-	FTIR	Güven et al., 2017
Aegean-Levantine Sea	Sea surface	Manta-net	333 μm	0.2 × 0.6 m	2 knots	15 min	Visual (microscope)	van der Hal et al., 2017
Western Mediterranean Sea (Ligurian Sea)	Sea surface	Neuston net	200 μm	0.6 x 0.2 m	2.5 knots	60 min	FTIR	Pedrotti et al., 2016
Western Mediterranean Sea and Adriatic Sea	Sea surface	Neuston net	200 μm	1 × 0.5 m	1.5 – 2 knots	5 min	FTIR-ATR	Suaria et al., 2016
Whole Mediterranean	Sea surface	Manta-net	333 μm	0.6 x 0.25 m	3.13 knots	15 - 30 min	Visual (microscope)	Ruiz-Orejón et al., 2016
Adriatic Sea	Sea surface	Neuston net	300 μm	0.6 × 0.15 m	3 knots	20 min	Chemical analysis	Gajšt et al., 2016
Western Mediterranean Sea	Sea surface	Neuston net	200 μm	0.6 x 0.25 m	1.5 knots	20 min	Visual (microscope)	Fossi et al., 2016
Aegean-Levantine Sea Turkey	Sea surface	Manta-net	333 μm	0.6 × 0.25 m	2 knots	20 min	Visual (microscope)	Gündoğdu and Çevik, 2017
Western Mediterranean Sea AsinaraNational Park PelagosSanctuary	Sea surface	WP2	200 μm	57 cm diameter	0.772 m/s	20 min	Visual (microscope)	Panti et al., 2015
Whole Mediterranean	Sea surface	Neuston net	200 μm	1.0 × 0.5 m	2 – 3 knots	15 min	Visual (microscope)	Cózar et al., 2015
Western Mediterranean Sea	Sea surface	Manta-net	330 μm	0.6 × 0.15 m	1.4 m/s	45 - 90 min	Visual (microscope)	Faure et al., 2015
Western Mediterranean Sea (Corsica)	Sea surface	WP2 0.2 mm	200 μm	0.6 x 0.25 m	2.5 km/h	20 min	Visual (microscope)	Collignon et al., 2014
Western Mediterranean Sea (Sardinian coast)	Sea surface	Manta-net	500 μm	-	2 knots	20 min	Visual (microscope)	de Lucia et al., 2014
Adriatic and Ionian Seas	Sea surface	Manta-net	330 μm.	0.6 × 0.24 m	< 3 knots	30 min	Visual (stereomicroscope); ATR-FTIR spectroscopy	Zeri et al., 2018

Source: Vlachogianni, et al., 2018. State-of-the-art methods to monitor marine litter and its impacts on biodiversity. Interreg Med Plastic Busters MPAs project.

INDICATIVE FLOATING MICROPLASTICS DENSITIES

Location	Habitat	Date	Density	References
North Western Mediterranean Sea Tuscan coast	Sea surface/Water column	2013-2014	Surface: 69,161.3 ± 83,243.9 items/km^2 Vertical: 0.16 ± 0.47 Items/m^3	Baini et al., 2018
Western Mediterranean Sea Pelagos Sanctuary	Sea surface	2014	82,000 ± 79,000 items/km^2	Fossi et al., 2017
Western Mediterranean Sea (Ligurian Sea)	Sea surface	2013	125,930 ± 132,485 Items/km^2 ± SD	Pedrotti et al., 2016
Western Mediterranean Sea and Adriatic Sea	Sea surface	2013	400,000 ± 740,000 items/km^2 1.00 ± 1.84 Items/m^3	Suaria et al., 2016
Adriatic Sea- Slovenian coastal waters	Sea surface	2014	472,000 ± 201,000 Items/km^2 ± SD	Gajšt et al., 2016
Gulf of Trieste	Sea surface	2014- 2015	444,182 ± 563,190 items/km^2	Zeri et al., 2018
Western Mediterranean Sea	Sea surface	2012	0.31 ± 1.17 Items/m3 ± SD	Fossi et al., 2016
Western Mediterranean Sea, Asinara National Park, Pelagos Sanctuary	Sea surface	2012–2013	0.17 ± 0.32 Items/m^3 ± SD	Panti et al., 2015
Whole Mediterranean	Sea surface	2013	243,853 Items/km^2	Cózar et al., 2015
Western Mediterranean Sea (Sardinian coast)	Sea surface	2013	0.15 ± 0.11 Items/m^3	de Lucia et al., 2014
Ligurian and Sardinian Sea	Sea surface	2011	0.62 ± 2.00 Items/m^3 ± SD	Fossi et al. 2012
Western Mediterranean Sea	Sea surface	2010	116,000 Items/km^2	Collignon et al., 2012
Archipelago of Zadar	Sea surface	2015	127,135 ± 294,847 particles/km^2	Palatinus et al., 2019

MICROLITTER STUDIES IN MEDITERRANEAN SEAFLOOR SEDIMENTS

Location	Sampling	Depth	Laboratory analysis	References
32"22.90 N 31"43.130 E	25 cm^2 Core sampling, 1-5mm	1176-4848	Density (NaCl) separation, visual counts, 4 categories (fibres, pellets, films, spherical)	Van cauwernberghe et al., 2013
NW basin, canyons & slope	Canyons/slopes/abyssal plain, ROV/core sampling, 0.32-5mm	300-3500	Density (NaCl) separation, visual counts, fibers & particles separation, FTIR analysis	Woodall et al., 2014
Eolian Islands	Undisturbed sediment (5 cm depth) collected by scientific scuba divers, using wide mouth glass jars	30	Surface sediment, sieving, visual observation, MSFD categories (5)	Fastelli et al., 2016
Malta	0.1-m2 van Veen grab at eight sampling stations	4-22	Density (NaCl) separation, visual counts, 3 categories (fibrous, rounded and irregular)	Romeo et al., 2015
Croatia	sediment collected by scientific scuba divers, using wide mouth glass jars in 10 sites. Three replicates for each site	3-15	Density (NaCl) separation of sieved fractions, MSFD categories (5)	Blăsković et al., 2017
Balearic Islands	Superficial core sampling (0-3.5 cm) with scuba diving, 1- 5 mm	8-10 m	Density (NaCl) separation of sieved fractions, MSFD categories (5)	Alomar et al., 2016

Source: Vlachogianni, et al., 2018. State-of-the-art methods to monitor marine litter and its impacts on biodiversity. Interreg Med Plastic Busters MPAs project.

COMPOSITION OF FLOATING MICROPLASTICS

The detected plastic types are diverse but some are predominant on the sea surface because of their widespread use and their buoyancy:

- poly(ethylene) (PE) frequent in food packaging (e.g. in films and bottle caps)
- poly(propylene) (PP), used as packaging material and plastic parts in various industries
- poly(amides) (PA) and poly(styrene) (PS)

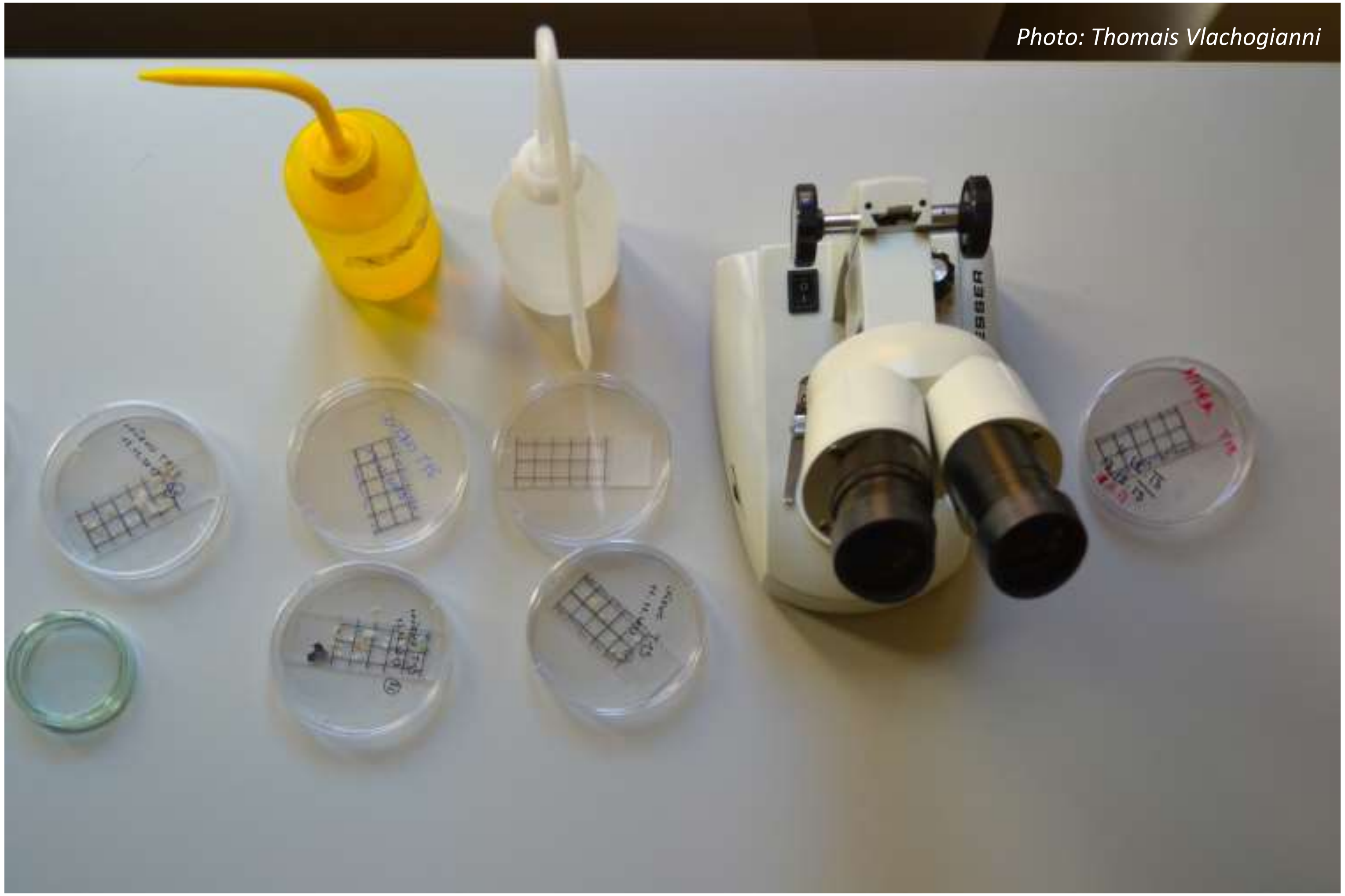


Photo: Thomais Vlachogianni

MONITORING MARINE MICROLITTER ON THE SEA SURFACE WITH MANTA TRAWLING

MARINE MACROLITTER MONITORING | KEY ELEMENTS

- **Monitoring Strategy**
  - Site selection
  - Frequency & timing
  - Sampling unit
- **Survey Protocol**
  - Litter 'sampling'
  - Sample processing
  - Microplastics identification & classification

SITE SELECTION CRITERIA

Low density areas (e.g. open sea)

High density areas (e.g. close to ports)

Other selected areas e.g. in estuaries, in the vicinity of cities, in local areas of touristic, recreational or commercial traffic





FREQUENCY & TIMING OF THE SURVEYS

Spring:
April

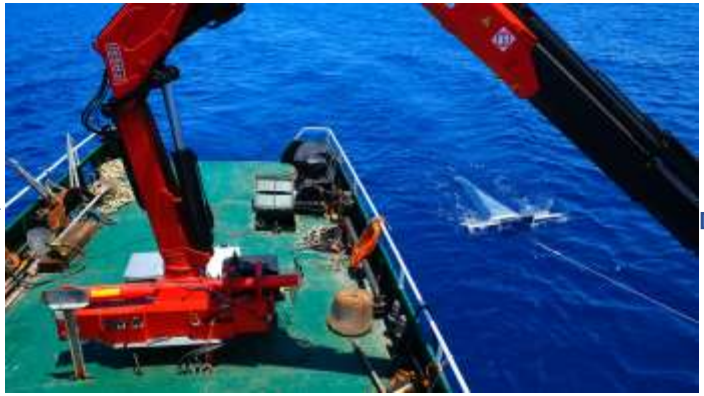
Autumn:
October

THE SAMPLING UNIT

Manta trawl equipped with a flowmeter
Mouth opening: 60 x 15 cm
Mesh size: 330 μm
Sampling duration: 30 minutes
Vessel speed: 1.5 – 2.5 knots

- ✓ The sampling should be conducted using small to medium-sized vessels in low wind conditions (0-2 Beauforts).
- ✓ All tows should be conducted from the ship's side and beyond the ships' wake.
- ✓ Both the starting and ending positions should be recorded with GPS, along with the track.

THE SAMPLING PROCEDURE – STEP-BY-STEP



- ✓ The **sample collected in the cod-end** should be **rinsed** with seawater on a **300 µm metallic sieve** and transferred to glass jars filled with seawater.
- ✓ Any **natural debris items**, such as leaves, twigs, seaweed, etc., should be **rinsed separately** above the sieve and removed from the sample.
- ✓ The samples should be **stored in 70% ethanol solution** for further analysis.

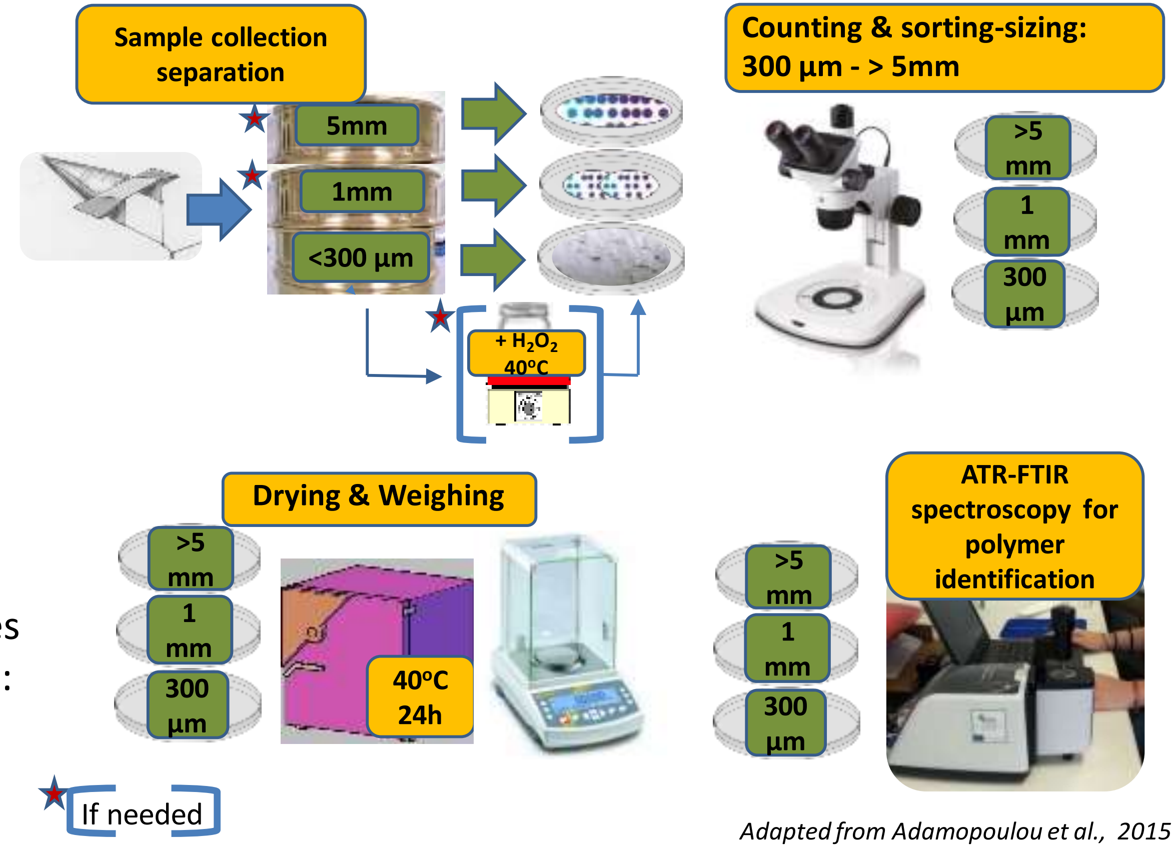
SAMPLE PROCESSING

Microlitter is classified in:

- ✓ **Large Microlitter LML (1mm-5mm)**
- ✓ **Small Microlitter SML (300µm – 1mm)**

- ✓ In case of samples **poor in natural particles and organic material**, transfer the sample into a petri dish and observe under a stereomicroscope. Measure the particles' longest dimension using an image analysis software, count and classify into the sizes classes. For the determination of weight, transfer the characterized MPs into three pre-weighted petri dishes according to size classes, dry at 40°C and weigh.

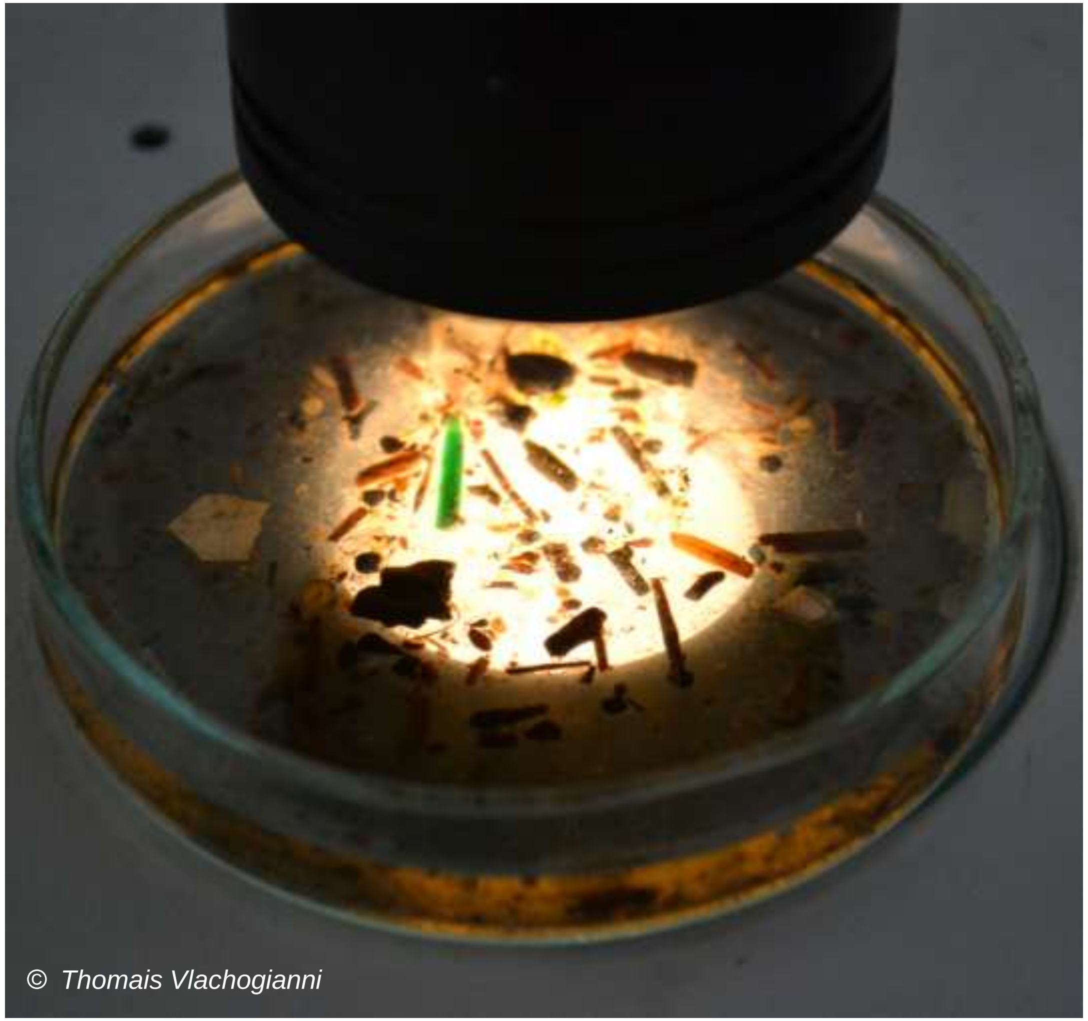
- ✓ In case of **high natural organic matter content** in the samples (LML or SML) a step of peroxide digestion precedes filtration: Add 15% hydrogen peroxide (H2O2) with 1:1 (sample:H2O2) volume ratio and boil on a hot plate (approx.40ºC) until the digestion is complete (no natural organic material should be visible). Collect the digested material with deionised water and continue with filtration, drying and mass determination.



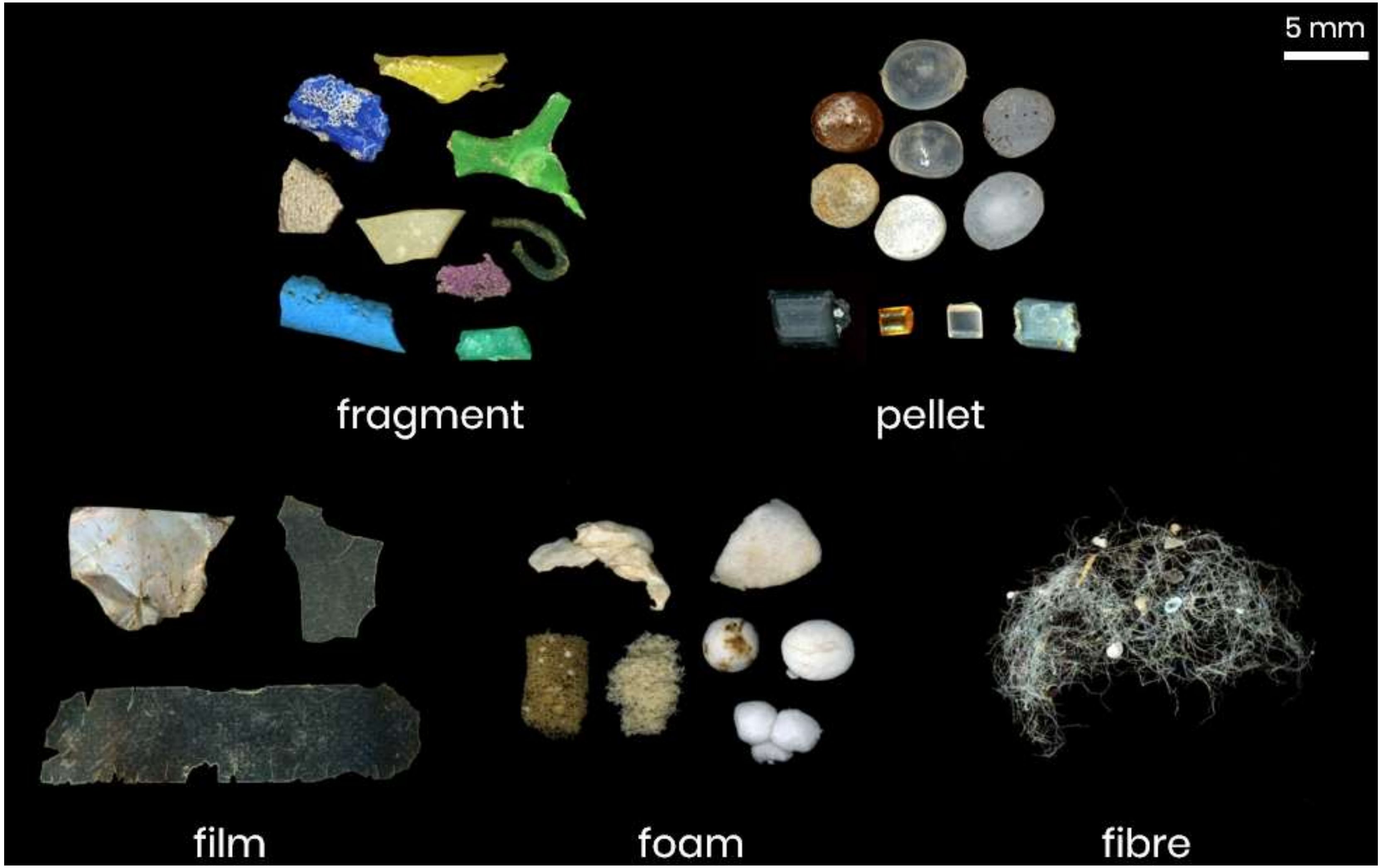
Adapted from Adamopoulou et al., 2015

SAMPLE ANALYSIS

- ✓ Microplastics sorted, counted and characterized by **type** on the basis of the following categories: pellet, fragment (granule, flake), fibre, film, filaments, microbeads, foam (expanded polystyrene-PS), in line with the MSFD TGML guidelines.
- ✓ The most common **colours** of microplastics identified are the following: black, blue, white, transparent, red, green, multicolour, other.
- ✓ For the identification of the **polymer type** it is recommended to use an ATR-FTIR spectrometer or Raman spectroscopy or Pyrolysis-Gas chromatography-mass spectroscopy (Py-GCMS). FT-IR spectroscopy is mostly used in microplastic studies and in particular ATR-FTIR is considered fast, low cost and adequate for analyzing particles >300 μm, in size like the ones collected with manta nets.



MICROPLASTICS SORTED BY TYPE



MICROPLASTICS – REPORTING UNITS

Microlitter counts (N) are reported as follows:

- N per km^2 or N per m^2, based on the start - end transect coordinates and the dimensions of the manta net mouth.
- N per Km^3 or N per m^3, based on flow meter indication and relevant formula.

Microlitter mass is reported as follows:

- g per km^2 or g per m^2
- g per Km^3 or g per m^3

EQUIPMENT NEEDED

Sampling equipment

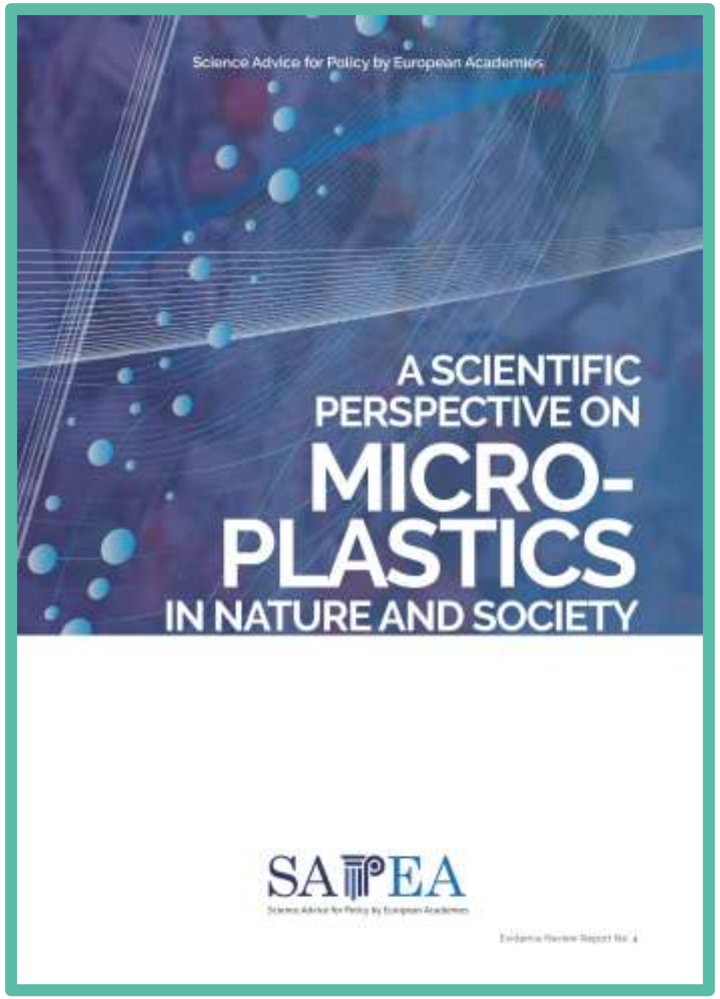
- Manta net with wings and cod end (mesh size: 330 µm)
- Oceanographic flowmeter
- Submersible water pump with a hose (for rinsing the net) or other equipment for net rinsing
- GPS
- Glass jars with caps or plastic bottles (500 ml) (one or more per each sample; when on the sea is a lot of sea grass, than you need 2 - 3 plastic bottles per sample)
- Sample container – cool box
- Screw driver
- Sieve (max 0.3 mm mesh size; preferable with smaller mesh size)
- Large bowl or washbasin (to prevent spillage of sample when emptying cod-end; 5 l <)
- Tap/fresh water source (tap/hose/squirt bottle)
- Squirt bottles2 x (one for water; one for alcohol)
- Tweezers (longer)
- Metal spoon
- Funnel (Ø 20 cm)
- Latex gloves without powder
- 70 % ethanol

Sample separation equipment

- Stereomicroscope (min. 80x zoom; recommended also: transmission light with dark field, polarisation contrast and ring light)
- Object glasses (marked – number of a sample, date of analysis)
- Micro tweezer and tweezer
- Glass petri dishes
- Lab coat
- 70 % ethanol
- 3 Sieves with mesh sizes: 5mm; 1mm; 0.3 mm or smaller
- Squirt bottle 2x (one for distilled water; one for alcohol)
- Latex gloves without powder
- Filtered water or distilled water
- Analytical laboratory scale
- Multiwell plate provided by NIC for storing the microlitter particles

MICROPLASTICS – WHAT DO WE KNOW?

- A lot is already known about microplastics, and more knowledge is being acquired, but some of the evidence remains uncertain and it is by its nature, complex (for instance, differences in size, shape, chemical additives, concentrations, measurements, fates, unknowns, human factors, actions).
- There is a fair knowledge of microplastics concentrations for freshwaters and the ocean surface, but little is known about concentrations and implications of microparticles below the ocean surface.
- Little is known with respect to the human health risks of microparticles; we have no evidence of widespread risk to human health from nanoparticles at present.
- Most microplastics go in and out of most organisms, and as with many chemicals, ‘the poison is in the dose’. Most effect studies are performed using concentrations that are much higher than those currently reported in the environment, or using very small microplastics for which limited exposure data exists, or using spherical ones which are not representative of real-world types of particles, or using relatively short exposure times. Currently, it is not known to what extent these conditions apply to the natural environment. This limits the reliability of the risk assessments.



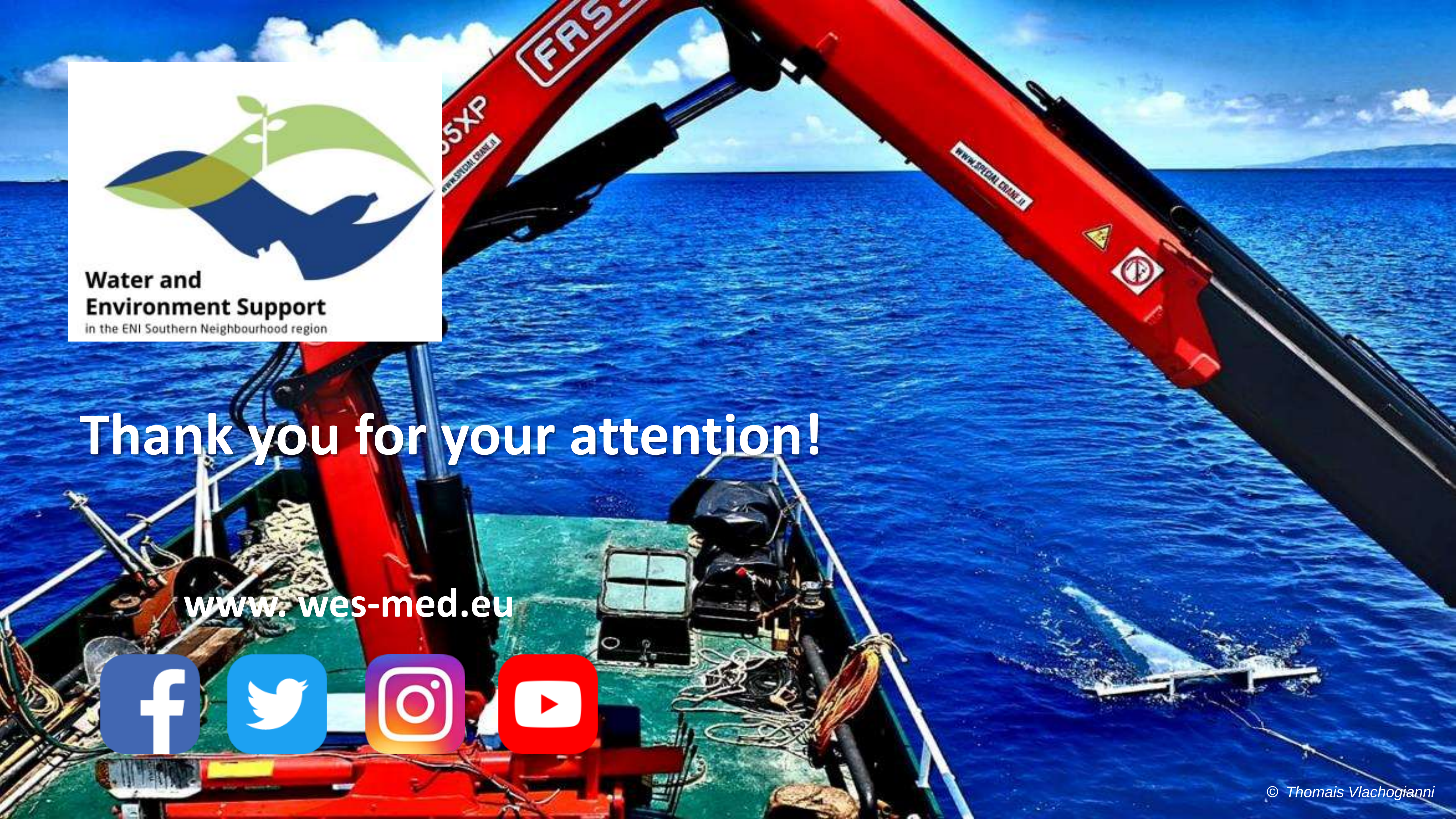
KNOWLEDGE GAPS AND RESEARCH NEEDS

- Despite a growing number of papers, knowledge is still lacking to fully understand the distribution and concentration and chemical composition of microplastics in the Mediterranean Sea
- The different studies are uneasy to compare because of the heterogeneity of the Mediterranean Sea environment (hydrodynamic features, seasonality) and the variety of methodologies used (e.g. sampling, microplastic extraction, analysis, size classes considered, concentration units)
- Lack of quality assurance/quality control (QA/QC) instruments. No organisations to-date offer proficiency training or testing in microplastics samples processing.

Photo: Thomais Vlachogianni

DATA GAPS

ANALYTICAL METHODS RESEARCH NEEDS



Water and Environment Support
in the ENI Southern Neighbourhood region

Thank you for your attention!

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