Microplastics in the Adriatic Sea: Occurrence, characterization, distribution and environmental management

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Plastic litter: Environmental and Human Health concern?

Litter has been recognized as a serious problem to the environment:

Several intergovernmental Organizations (IMO, FAO, UNESCO-IOC, UNIDO, WMO, IAEA, UN, UNEP, UNDP) have undertaken a number of scoping activities to provide an initial assessment of fate, sources and effects of microplastics in the marine environment (GESAMP - Working Group 40)

At European level, plastic litter has become one of eleven descriptors defining a Good Environmental Status (GES) of the aquatic environment in the European Marine Strategy Framework Directive (MSFD, 2008/56/EC)

EU member states are thus obliged to monitor litter (including micro litter), in coastal areas, and if necessary take measures to reduce the input at least to levels where “Properties and quantities of marine litter do not cause harm to the coastal and marine environment” (EC, 2010).
Plastic litter and microplasics: sources, loadings and definitions

The increasing applications of polymers in everyday products inevitably resulted in the widespread presence of plastic waste in the natural environment.

Plastics in the last decade represent 50-60% of municipal solid waste, 30-40% of industrial waste and between 15-25% of all hospital waste in developed countries. They tend to be generated in much higher quantities in wealthier regions of the world.

“Primary microplastics”: synthetic polymer microbeads (0.1μm<MBs<5mm) including solid, hollow, amorphous, solubilized particles, etc. involved in daily cleansing or grooming health care products.

Although most of the plastic materials are persistent and do not biodegrade easily, under the influence of solar UV radiations, high temperature, wave and air friction, do degrade and fragment into small particles, termed “secondary microplastics”.

<table>
<thead>
<tr>
<th>Size</th>
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<tbody>
<tr>
<td>Macroplastic</td>
<td>&gt; 5 cm</td>
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<tr>
<td>Mesoplastic</td>
<td>5cm–5mm</td>
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<tr>
<td>Microplastic (MP)</td>
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<td>Large MP (LMP)</td>
<td>5mm–1 mm</td>
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<tr>
<td>Small MP (SMP)</td>
<td>1mm–0.1 μm</td>
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<tr>
<td>Nanoplastic</td>
<td>&lt; 0.1 μm</td>
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</table>
Secondary microplastics: formation and interaction with the biosphere

Marine environment

- Plastics litter eventually undergo physical and biological fragmentation and chemical degradation leading to the formation of small plastic pieces.

[Diagram showing various processes related to secondary microplastics]

Wright et al., 2013
Standardized and trustworthy analytical methods are needed

- Without proper analytical methods we cannot:
  - Assess the amount of microplastic in the environment
  - Distinguish which are the most important sources
  - Quantify impacts of microplastic

- We (the scientific community) do not (yet) have the final answer to how microplastic should be analyzed
- Over the later years, certain methods have shown promising results, while others have been deemed unsatisfactory
Challenges

Analyzing for microplastic in the environment, there are many ways of doing:

- **Microplastic identification**
  - How to safely distinguish artificial polymers from naturally occurring substances?

- **How to report results**
  - Particle sizes: What is the “size” of a particle? Particle mass: How best to quantify the mass of a particle?

- **Document the validity of the analysis** – this is often forgotten …

- **Document uncertainties** – this is often forgotten …
Methodological criteria for detection and characterization: the way for the environmental management

Multi-tiered approach combining:

✓ Rapidity
✓ Accuracy
✓ Sensitivity
✓ Execution costs

Tools for Environmental Management

Tier 1 - “Fast screening”

• Collection of techniques able process large amounts of samples with adequate resolution

Tier 2 - “Confirmation”

• Collection of techniques to process limited amounts of samples with high resolution and detailed info
Phase 1: Extraction & purification techniques comparison

Automatic / semi automatic system

Automatic extraction systems (MDDS)
Curtesy Marte Haave, Unresearch.

Physical separation
Removing inorganic matter by density separation

Manual set up

VS

Standardized laboratory operative procedure

6th IMDC, San Diego, CA 12-16 March 2018
Phase 2: comparison of sample preparation techniques

....... find the needle in the haystack!

- Sample oxidation? …
- Alkali degradation? …
- Enzymatic driven degradation? …

![Diagram showing different sample preparation techniques and their comparison based on time and efficiency](image-url)
Extraction/purification methods drawbacks ....

Lesson learned.....

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Analyses</th>
<th>CA</th>
<th>ePS</th>
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Dehaut et al., 2016 Env Poll 215, 223-233

.... Nanoplastics?
Phase 3: Characterization

Overview of the best available technology:

The repetitive fingerprint-like molecular composition of plastic polymers allows for a clear assignment of a sample to a certain polymer origin.

**Pyrolysis-GCMS**: pyrolysis-gas chromatography (GC) in combination with mass spectrometry (MS) can be used to assess the chemical composition of potential microplastic particles by analyzing their thermal degradation products.

**Raman spectroscopy**: during the analysis with Raman spectroscopy the sample is irradiated with a monochromatic laser source. The interaction of the laser light with the molecules and atoms of the sample (vibrational, rotational, and other low-frequency interactions) results in differences in the frequency of the backscattered light when compared to the irradiating laser frequency.

**FTIR** spectroscopy: similar to Raman spectroscopy, infrared (IR) or Fourier-transform infrared (FTIR) spectroscopy offers the possibility of accurate identification of plastic polymer particles according to their characteristic IR spectra.

**SEM, SEM-EDS, ESEM-EDS spectroscopy**: Scanning electron microscopy/ energy dispersive X-ray spectroscopy are techniques used for characterizing surface morphology and determining the elemental composition of polymers.
Advantages and disadvantages

Size ranges and analytical methods

Microplastic range: 1 – 5000 μm

- Increasing uncertainty
  - Micro-ATR-FTIR (single point analysis of particles on a filter)

- Increasing uncertainty
  - ATR-FTIR (particles hand-picked, analyzed on bench)

- Possibly down to a few μm (not proven)
  - Imaging μFT-IR using filters, windows, or slides

- Imaging μRaman – possible methods, not well proven

- Macro Raman (particles hand-picked, analyzed on bench)

- NIR (pre-sorting) + Hy-Spec
  - Imaging NIR (not well proven)

- TDU-Pyr-GC/MS; TED-GC-MS

100 nm   500 nm

1 μm  10 μm  100 μm  1000 μm  10000 μm

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Flowchart of the selected analytical methods

Sediments

**Extraction:**
- Automatic extraction systems (MDDS)

**Purification:**
- Permeation → Enzymatic degradation → oxidation

**Detection:**
- Tier 1 (Screening) + Tier 2 (Confirmation)

Biota

**Extraction:**
- Sample loading (0.5 - 1.0 gr)
- Enzymatic degradation (cellulase/protease/chitinase) - 24h
- Oxidation 24h
- Final washing

**Purification:**
- Permeation → Enzymatic degradation → oxidation

**Detection:**
- Tier 1 (Screening) + Tier 2 (Confirmation)

Seawater

**Extraction:**
- Filtration on stainless steel sieves (10-50 -100 – 200 µm)

**Detection:**
- Enzymatic degradation → oxidation

**Time**

Permeation → Enzymatic degradation → oxidation
Occurrence, characterization and distribution of microplastics in the marine environment: the Adriatic Sea case study

Sediments

Tier 1:
- Autofluorescence analysis of the extracted particles
- SEM analysis extracted particles

Tier 2:
- Raman spectra
- ... corresponding GCMS pyrogram

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Biota: microplastic litter in the gastrointestinal tract of wild fish

average no. of particles/individual ...

<table>
<thead>
<tr>
<th>Abundance Mean of polymers</th>
<th>PKX</th>
<th>1.45±0.04</th>
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</thead>
<tbody>
<tr>
<td>PET</td>
<td>1.74±0.05</td>
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</tr>
<tr>
<td>PA</td>
<td>1.76±0.04</td>
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<tr>
<td>PP</td>
<td>1.85±0.05</td>
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<tr>
<td>PE</td>
<td>1.85±0.04</td>
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<tr>
<td>PK</td>
<td>1.52±0.08</td>
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<tr>
<td>PET</td>
<td>1.61±0.09</td>
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<tr>
<td>PA</td>
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<tr>
<td>PE</td>
<td>1.71±0.08</td>
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<tr>
<td>PP</td>
<td>1.72±0.08</td>
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</table>
Occurrence, characterization and distribution of microplastics in the marine environment: the Adriatic Sea case study

Biota: microplastic litter in tissue of native blue mussels

Particles size distribution:
- < 100 µm D5
- 80µm < D4 < 100µm
- 40µm < D3 < 80µm
- 20µm < D2 < 40µm
- 10µm < D1 < 20µm

Fragments
- Relative abundance %
- PVC
- PLY
- PET
- PP
- PS
- PE

Fibers
- Relative abundance %
- PVC
- PLY
- PET
- PP
- PS
- PE

PE > PP > PET > ….
Nylon > PE….

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Basis for risk management: microplastics … environmental and human concern?

Possible scenario?
Help with the understanding of possible biological effects

Background

- Ingestion of microplastics has been demonstrated in various marine organisms with different feeding strategies.
- Microplastics may act as “vectors” of chemical pollutants such as additives or being adsorbed from seawater.
- … facilitate the adsorption of these chemicals on microplastics at concentrations orders of magnitude higher than those detected in seawater.
- Under physiological conditions it has been suggested that pollutants adsorbed on plastics might be released to organisms (Browne et al., 2013; Oliveira et al., 2013).
Do they represent a potential source of chemical exposure within marine food webs?

- Potential “trojan horse” effect induced by the occurrence of plastic particles in different environmental scenarios?

- Rank biological responses induced by pollutants adsorbed and eventually released under physiological conditions

…. An example …..

Study: “Comparative effects of ingested PVC micro particles with and without adsorbed benzo(a)pyrene vs spiked sediments on the cellular and sub cellular processes of the benthic organism Hediste diversicolor”

Organisms were exposed to:

- **Natural sediments** (no b[a]p; no plastic particles, C),
- **Virgin plastic** particles in sediment: 500 (LC-MPS) and 5000 particles/Kg (HC-MPS),
- **b[a]p spiked plastic** particles in sediment: 500 (S-B[a]P_LC) and 5000 particles/Kg (S-B[a]P_HC),
- **Spiked sediments** (1 mg/L b[A]p) without plastic particles (b[a]p-Sed),

Gomiero *et al.*, 2018
Results

According to the responses of the selected endpoint tests (Mortality, lysosomal membrane stability, ROS production, mitochondrial activity, phagocytosis, lipofusin content, genotoxicity) …

….. and the body burden accumulation

✓ Microplastic (PVC) particles can efficiently adsorb the organic contaminants like benzo(a)pyrene from the marine environment seawater and can assist in transferring this compound to the representative benthic species (*H. diversicolor*),

✓ Enhance the bioaccumulation rate of providing evidence of the potential transfer and bioaccumulation of environmental pollutants in a species representative of the benthic environment.
Thank you