Detection of Microplastic Contaminant in Marine Environment

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Introduction

• How micro-plastic (MP) end up in ocean?

1. Primary micro-plastic
   ❖ Intentionally manufactured (range of 1nm-5nm)
     ➢ Personal care products: toothpaste, shower gel, scrubs, cosmetics, air blasting

2. Secondary micro-plastic
   ❖ Large plastics: fishing gears, ships, aquaculture, recreational activities
   ❖ Plastic Debris due to:
     ➢ Solar ultra-violet radiation: oxidative degradation
     ➢ Mechanical abrasion: wind, wave, ocean current, animal bite, human activity

Different paths to produce MP

![Diagram of different paths to produce microplastics]

Table 1: Densities and common applications of plastics in the marine environment [41].

Distribution

• Surface
• Benthic Sediment
  ❖ Mostly secondary MP
• Mid Water
  ❖ Ocean currents, resulting in the spread of plastic pollution from source areas
• Sea Ice
  ❖ Microplastic levels in sea ice may be higher than surrounding water bodies because of the concentrating effect of the scavenging phenomenon that accompanies sea ice growth
Selective sampling
- the MP is directly sorted out after identification with the naked eye.
- high amount of large MP and clearly distinguishable from the matrix

Bulk sampling
- uses the entire volume of the sample without any reduction
- mostly applied for sediments.
- have to be separated from other sediment parts like sand or stones

Volume-reduced sampling
- the volume of the sample is minimized
- for example, by filtration or sieving,
- can be applied for water and sediment analyses
Extraction and Isolation

• Need to be separated from both organic and inorganic non-plastic particles
• Density principles
  ➢ using salt solutions of varying densities (sodium chloride, sodium iodide, sodium polytungstate)
• Sediment is mixed with concentrated NaCl solution and vigorously shaken for specific period of time.
• The supernatant is filtered with a filtration unit and a vacuum pump
• The filter (usually nitro-cellulose 0.45 micron-meter) is then dried and sealed in a petri-dish

Reference:
Identification

• The samples need to be purified

• Two different methods have been applied for purification:

  1. chemical degradation

    ✓ treated with different chemicals, mainly 30% hydrogen peroxide (H2O2) solution mixtures of H2O2 and sulfuric acid (H2SO4)

  2. enzymatic degradation of the organic matrix.

    ✓ treated with a mixture of technical enzymes (lipase, amylase, proteinase, chitinase, and cellulase)

Fourier transform infrared (FTIR) spectroscopy

- Source energy through an interferometer and onto the sample.
- The light passes through a beam splitter, which sends the light in two directions at right angles
- One beam goes to a stationary mirror then back to the beam splitter; The other goes to a moving mirror
- Speed of the moving mirror is controlled by using a helium-neon laser beam
- Two beams meet up at the beam splitter and recombine
- the difference in path lengths creates constructive and destructive interference: an interferogram
- The recombined beam passes through the sample
- The sample absorbs all the different wavelengths characteristic of its spectrum
- The detector now reports variation in energy versus time for all wavelengths simultaneously

The longer the path of the moving mirror, the higher the resolution.

Reference: Gable K.; 2013; FTIR Spectroscopy; Oregon State University; Department of Chemistry
A mathematical function called a Fourier transform allows us to convert an intensity-vs.-time spectrum into an intensity-vs.-frequency spectrum. The Fourier transform:

\[ A(n) = \sum X(k) \exp(-2\pi \frac{in}{N}) \]

Reference: Gable K.; 2013; FTIR Spectroscopy; Oregon State University; Department of Chemistry
Microplastic in the surface waters of the Ross Sea (Antarctica): Occurrence, distribution and characterization by FTIR

Abstract

This is the first survey to investigate the occurrence and extent of microplastic (MPs) contamination in sub surface waters collected near-shore and off-shore the coastal area of the Ross Sea (Antarctica). Moreover, a non-invasive method to analyze MPs, consisting in filtration after water sampling and analysis of the dried filter through Fourier Transform Infrared Spectroscopy (FTIR) 2D Imaging, using an FPA detector, was proposed. The non-invasiveness of analytical set-up reduces potential bias and allows subsequent analysis of the filter sample for determination of other classes of contaminants. MPs ranged from 0.0032 to 1.18 particle per m³ of seawater, with a mean value of 0.17 ± 0.34 particle m⁻³, showing concentrations lower than those found in the oceans worldwide. MPs included fragments (mean 71.9 ± 21.6%), fibers (mean 12.7 ± 14.3%), and others (mean 15.4 ± 12.8%). The presence of different types of MPs was confirmed by FTIR spectroscopy, with predominant abundance of polyethylene and polypropylene. The potential environmental impact arising from scientific activities, such as marine activities for scientific purposes, and from the sewage treatment plant, was also evidenced.

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MP 1 – polyethylene (PE) and polypropylene (PP) (57.1%)

MP 2 – polyamide (PA) (2.9%).

MP 3 – polyester (PL) (28.6%)

MP 4 – polytetrafluoroethylene (PTFE) (5.7%)

MP 5 – polymethyl methacrylate (PMMA) (5.7%)

Reference: Cincinelli, A., & et al.; 2017; Microplastics in the surface waters of Ross sea (Antarctica): occurrence, distribution and characterization by FTIR; University of Florence; Chemosphere; 175: 391-400.
Reference: Cincinelli, A., & et al.; 2017; Microplastics in the surface waters of Ross sea (Antarctica): occurrence, distribution and characterization by FTIR; University of Florence; Chemosphere; 175: 391-400.
Conclusion from the study

• Polymers - large range of uses, including packaging, textiles and fishing gears, and for this reason it was not possible to identify specific sources.

• Low density polymers, such as PP and PE, are predominantly found in the sea surface microlayer. They may also be submerged in the first 10 m depth, if they meet water front or are fouled, as in this case, with algae and diatoms that increase their density.

• High density MPs, including PL and PA, are generally mainly found in the benthos, but they can be present in smaller number and temporarily suspended within the water column, when they enter the sea through high-flow rate or owing to turbulent.

Reference: Cincinelli, A., & et al.; 2017; Microplastics in the surface waters of Ross sea (Antarctica): occurrence, distribution and characterization by FTIR; University of Florence; Chemosphere; 175: 391-400.
Microplastic in Aquatic Ecosystems

The contamination of marine and freshwater ecosystems with plastic, and especially with microplastic (MP), is a global ecological problem of increasing scientific concern. This has stimulated a great deal of research on the occurrence of MP, interaction of MP with chemical pollutants, the uptake of MP by aquatic organisms, and the resulting (negative) impact of MP. Herein, we review the major issues of MP in aquatic environments, with the principal aims 1) to characterize the methods applied for MP analysis (including sampling, processing, identification and quantification), indicate the most reliable techniques, and discuss the required further improvements; 2) to estimate the abundance of MP in marine/freshwater ecosystems and clarify the problems that hamper the comparability of such results; and 3) to summarize the existing literature on the uptake of MP by living organisms. Finally, we identify knowledge gaps, suggest possible strategies to assess environmental risks arising from MP, and discuss prospects to minimize MP abundance in aquatic ecosystems.

FTIR imaging was improved by applying focal plane array (FPA)-based detection

Figure 4. FPA-based micro-FTIR chemical imaging of the spectral region $\tilde{\nu} = 1480$–1400 cm$^{-1}$ of a microplastic sample from sediment. A) Overview of the whole sample filter. B) Magnified detail [white square in (A)] of the filter with a polymethyl methacrylate (PMMA) particle [B, red square in (B); red spectrum in (C)] and a polypropylene (PP) particle [B, light blue square in (B); blue spectrum in (C)]. The color bars represent the intensity of the integrated band region. The edge length of a red outlined FPA-detector field is 170 $\mu$m. C) Spectra in black are reference spectra. From Ref. [34a]. Copyright 2015 Löder et. al.

Raman Spectroscopy:
- the sample is irradiated with a monochromatic light source
- normally a laser Light sources used are normally in the visible range
- RS can easily be coupled to a standard optical microscope in so-called Raman microspectroscopy (RM).

FTIR and RM are non-destructive techniques that characterize the sample by exciting vibrations in specific functional groups, but they are time consuming.


Figure S. Two Raman microspectroscopy studies showing the size distribution of MP including small particles (< 50 µm). A) Relative microplastic particle size distribution in the European and subtropical Atlantic. Dark grey bars represent particle length. Light grey bars show the size as a geometric mean of length and width. The images show the smallest (left) and largest (right) MP particles found and confirmed by RM. B) Size distribution of MP (blue and black) and pigmented (red) particles from Lake Garda beach sediment. Pigments in colored MP as well as pigmented microparticles can be identified due to the strong RM signal of pigments. From Ref. [24] and Ref. [28], both copyright 2015 Elsevier.
Impacts

• MP - physical harm ranging from internal abrasions, intestinal blockage, and internal and external wounds, to starvation, debilitation, and finally death.
• The impact of MP on humans is not yet fully understood.
• Many chemicals that are used in plastic production and are known to be toxic.
  ▪ for example, bisphenol A (BPA), polybrominated diphenyl ethers (PBDE), and tetrabromobisphenol (TBBPA) have already been detected in human tissues and biological fluids.
  ▪ It has also been reported that additives, for example, di(2-ethylhexyl)phthalate (DEHP), can leach from medical supplies made of PVC and accumulate in the blood of haemodialysis patients.

Removal method: ATR-FTIR

- The sample is placed on an ATR crystal
- The surface is irradiated with an evanescent wave.
- This enables an FTIR analysis of larger MP

What are the advantages of ATR?

**Minimal sample preparation**—place the sample on the crystal and collect data

**Fast and easy cleanup**—simply remove the sample and clean the surface of the crystal

**Analysis of samples in their natural states**—no need to heat, press into pellets, or grind in order to collect spectra

**Excellent for thick or strongly absorbing samples**—ideal for difficult samples like black rubber

Reference: FTIR Sample Techniques: Attenuated Total Reflection; 2017; ThermoFisher Scientific; Canada
Several fungi have been shown to be able to use plastics as the sole source of nutrients.

**Graphical Abstract**

![Graphical Abstract](image)

**Abstract**

Plastic yearly production has surpassed the 300 million tons mark and recycling has all but failed in constituting a viable solution for the disposal of plastic waste. As these materials continue to accumulate in the environment, namely, in rivers and oceans, in the form of macro-, meso-, micro- and nanoplastics, it becomes of the utmost urgency to find new ways to curtail this environmental threat. Multiple efforts have been made to identify and isolate microorganisms capable of utilizing synthetic polymers and recent results point towards the viability of a solution for this problem based on the biodegradation of plastics resorting to selected microbial strains. Herein, the response of the fungus *Zalerion maritimum* to different times of exposition to polyethylene (PE) pellets, in a minimum growth medium, was evaluated, based on the quantified mass differences in both the fungus and the microplastic pellets used. Additionally, molecular changes were assessed through attenuated total reflectance Fourier transform Infrared Spectroscopy (FTIR-ATR) and Nuclear Magnetic Resonance (NMR). Results showed that, under the tested conditions, *Z. maritimum* is capable of utilizing PE, resulting in the decrease, in both mass and size, of the pellets. These results indicate that this naturally occurring fungus may actively contribute to the biodegradation of microplastics, requiring minimum nutrients.

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observed biomass variation and the percentage of removed plastics in the tested periods.

A positive correlation was noted, as an increase in weight percentage for *Z. maritimum* corresponded to an increase in the mass loss percentage of the plastic particles.

The FTIR-ATR spectra of *Z. maritimum* before, during and after exposure to PE microplastics.

The spectrum for *Z. maritimum* was similar to those reported for other fungi.

**Reference:** Paco, A.& et al.; 2017; Biodegradation of polyethylene microplastics by the marine fungus Zalerion maritimum; Elsevier; Science of the Total Environment 586 (2017) 10–15
Rapid and Efficient Method for the Detection of Microplastic in the Gastrointestinal Tract of Fishes

**ABSTRACT:** The rising evidence of microplastic pollution impacts on aquatic organisms in both marine and freshwater ecosystems highlights a pressing need for adequate and comparable detection methods. Available tissue digestion protocols are time-consuming (>10 h) and/or require several procedural steps, during which materials can be lost and contaminants introduced. This novel approach comprises an accelerated digestion step using sodium hydroxide and nitric acid in combination to digest all organic material within 1 h plus an additional separation step using sodium iodide which can be used to reduce mineral residues in samples where necessary. This method yielded a microplastic recovery rate of ≥95%, and all tested polymer types were recovered with only minor changes in weight, size, and color with the exception of polyamide. The method was also shown to be effective on field samples from two benthic freshwater fish species, revealing a microplastic burden comparable to that indicated in the literature. As a consequence, the present method saves time, minimizes the loss of material and the risk of contamination, and facilitates the identification of plastic particles and fibers, thus providing an efficient method to detect and quantify microplastics in the gastrointestinal tract of fishes.

**Reference:** Roch, S., & Brinker, A.; 2017; *Rapid and Efficient Method for the Detection of Microplastic in the Gastrointestinal Tract of Fishes*; Universitu of Konstanz; American Chemical Society; 51: 4522-4530
Table 1. Amount of Chemicals Used in the Described Digestion Method for Different Sample Weights

<table>
<thead>
<tr>
<th>wt of sample (g)</th>
<th>NaOH volume (mL)</th>
<th>HNO₃ volume (mL)</th>
<th>water volume (mL)</th>
<th>final volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>5</td>
<td>17.5</td>
<td>2.5</td>
<td>25</td>
</tr>
<tr>
<td>1–3</td>
<td>10</td>
<td>36</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>3–5</td>
<td>25</td>
<td>72</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>5–10</td>
<td>50</td>
<td>144</td>
<td>6</td>
<td>200</td>
</tr>
<tr>
<td>10–15</td>
<td>75</td>
<td>221</td>
<td>4</td>
<td>300</td>
</tr>
</tbody>
</table>

*Sodium hydroxide, 1 mol L⁻¹. bNitric acid, 65%. cFinal concentration of HNO₃ is 10 mol L⁻¹.

Figure 1. Efficiency of the digestion method. (A) Gastrointestinal tract (GIT) of a whitefish (Coregonus lavaretus) before digestion. (B) Filter after the completion of digestion steps. (C) Recovered polystyrene (PS) particles after digestion fluorescing under UV light. (D) Mean (+SD) recovery rates of PS particles after the first and second digestion steps (black bars) and after density separation (white bars) for each size class (n = 6).

Figure 3. Fourier transform infrared spectroscopy (FTIR) spectra of common polymer types before (gray lines) and after (black lines) digestion. The spectra were averaged from five particles of each type, baseline-corrected, smoothed, and normalized.

Reference: Roch, S., & Brinker, A.; 2017; Rapid and Efficient Method for the Detection of Microplastic in the Gastrointestinal Tract of Fishes; Universiti of Konstanz; American Chemical Society; 51: 4522-4530
Conclusion

- The advantages of FTIR lie in the proper identification of plastic types without strong interference from fluorescence, and the already automated analysis of filters.
- However, only the identification of particles down to 20 mm has been realized up to now, which neglects the environmentally important smaller particles.
- The analysis of non-transparent/dark particles is difficult and the samples have to be dried thoroughly since water shows strong IR bands that interfere with the analysis.
- More trials should run to properly find a digestion method in aquatic biota and in human eventually.
- However, the microplastic is only 3% according to the Boyan Slat: Plan to Rid the Sea of Plastic and other scientists also can help to remove macroplastic from the ocean.


**Fig. 1** Summary of currently used physical and chemical characterization methods for microplastic analysis.

<table>
<thead>
<tr>
<th>Identification method</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| Microscopy            | - Simple, fast, and easy | - No chemical confirmation  
- High possibility of false positive  
- High possibility of missing small and transparent plastic particles  
- No polymer composition data |
| Microscopy (+FTIR/Raman) | - Plastic confirmation of subset samples  
- Polymer composition of major or typical plastic types | - Possibility of false positive  
- Possibility of missing small and transparent plastic particles  
- Representativeness of polymer types with spectroscopic analysis of subset samples |
| FTIR spectroscopy     | - No possibility of false positive data by chemical confirmation of all the plastic-like particles  
- Reduction of false negative data  
- Non-destructive analysis  
- Detection of down to 10 μm plastics in size  
- Automatic mapping (FPA-reflectance) | - Expensive instrument  
- Laborious work and time consuming for whole particle identification  
- Contact analysis (ATR) |
| Raman spectroscopy    | - No possibility of false positive data by chemical confirmation of all the plastic-like particles  
- Reduction of false negative data  
- Detection of down to 1 μm plastics in size  
- Non-destructive analysis  
- Non-contact analysis | - Expensive instrument  
- Laborious work and time consuming for whole particle identification  
- Interference by pigments |
| Thermal analysis      | - Simultaneous analysis of polymer type and additive chemicals (pyro-GC/MS) | - Destructive analysis  
- A few polymer identification (DSC)  
- Complex data (pyro-GC/MS) |

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**Reference:** Shim, W.J., Hong, S.H., & Eo, S.E.; 2017; *Identification methods in microplastic analysis a review*; Anal. Methods, 9, 1384.
Figure 6. Overview of the MP sizes analyzed by means of the different identification methods [pyrolysis gas chromatography coupled to mass spectrometry (Py-GC/MS), attenuated total-reflectance (ATR)-FTIR, Raman spectroscopy (RS), FPA-based micro-FTIR, visual identification, and Raman microspectroscopy (RM)]. The smallest reported particle from the environmental samples is indicated with a cross. Please note RM and micro-FTIR are also applicable for particles larger than 500 μm, but ATR-FTIR is more reasonable for larger sizes. Visual identification can applied to all sizes, but should only be used for particles larger than 500 μm, if at all. Data taken from Refs. [3a, 10a, 12 b, 27, 29, 34a, 37, 51b].

Raman Microspectroscopy Imaging of PVC in *Daphnia Magna*

Figure 8. A) Fluorescence microscopy of *Centropages typicus* containing 7.3 μm PS beads (dorsal view). B) CARS microscopy (spectral range: 2775–3103 cm⁻¹) of 3.4 μm microplastic (yellow dots) accumulated in the alimentary canal (ac) of the copepod *Temora longicornis*. Beads (blue dots) further adhered to the exterior of the copepod’s urosome (u), furca (f), and posterior swimming legs (sl). C) CARS of 3.4 μm microplastic (red dots) adhered to the external surface of the posterior swimming legs of *T. longicornis*. Scale bars: 50 μm. Modified from Ref. [12a] Copyright 2013 American Chemical Society.

Scanning Electron Microscopy (SEM) Images

Figure 7. SEM images showing examples of the rich microbial community on MP: A) algae (pennate diatom) with possible prosthocercal filaments produced by Hyphomonas-like bacteria; B) filamentous cyanobacteria; C) stalked predatory suctorarian ciliate in foreground covered with endosymbiotic bacteria (inset), along with diatoms, bacteria, and filamentous microorganisms; D) microbial cells pitting the surface. Scale bars: 10 μm. From Ref. [15b] Copyright 2013 American Chemical Society.