Analysis of Microplastics using FTIR Imaging

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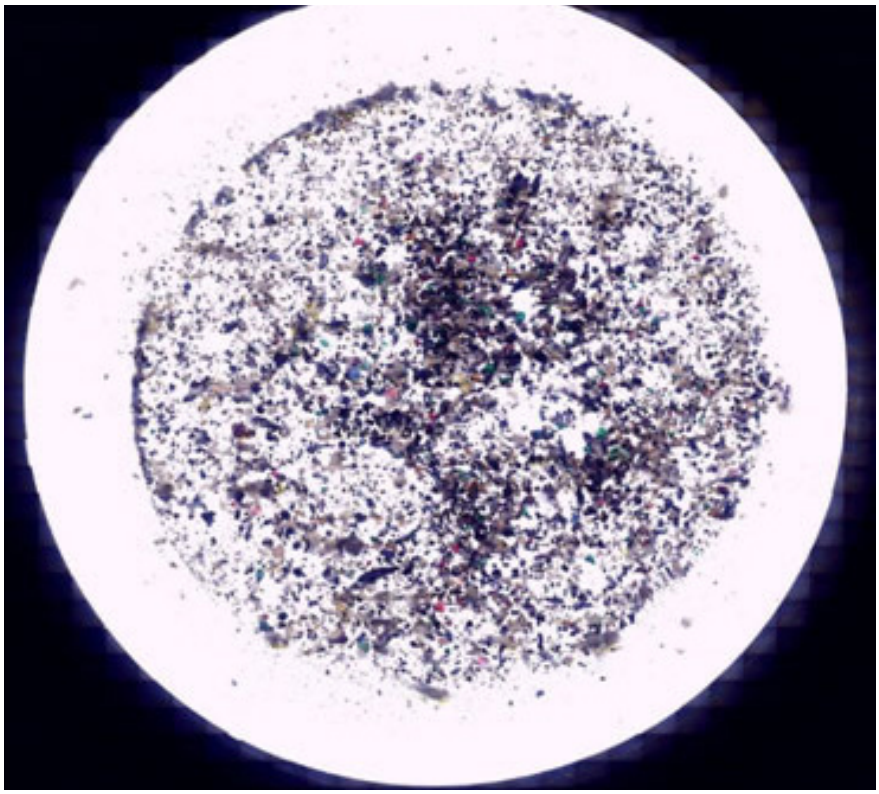

Introduction

In recent years, plastic pollution has received an increasing amount of interest from researchers, politicians, and the public. Microplastics (<5 mm) are a particular concern as they are suspected to accumulate in the environment and aquatic life [1].

Microplastics originate from various sources and can remain in the environment for hundreds of years before they finally decompose. However, the accumulation level and the effects on the environment and aquatic life are poorly understood. This is partly due to a lack of standard analysis protocols and current analytical techniques that are prohibitively time consuming and thus impractical.

Previously published studies relied on visual identification of plastics in samples to quantify them [2]. In this study, reliable methods for microplastic extraction from environmental samples were developed. Fourier Transformed Infrared (FTIR) Spectroscopy imaging was used to identify and quantify the types of microplastics [3,4].

Experimental

Samples

Samples were collected over a period from a wet retention pond in Viborg, Denmark, and included sediment, water, three-spined stickleback fish, and leeches. The aquatic animals were not analyzed in depth, but used solely to validate the detection of microplastics in fauna matrices.

The pond receives stormwater runoff and retains pollutants from roads which may lead to a high microplastic concentration.

A total of 50 L of water was collected from the pond. Each sampling batch of 10 L of water was collected in 2x5 L media storage bottles with Teflon-coated screw caps. Sampling locations are shown in Figure 1.

Sediment samples were collected 1-2 m from the edge of the pond with a glass corer (see Figure 1 for sampling locations), 5 cm in diameter. The top layer of each sediment sample was transferred to a glass jar.

Fish samples were caught with a net, placed across the pond, as shown in Figure 1. Other fauna samples were collected with a landing net, before being placed in glass bottles with pure ethanol. They were then placed on ice and stored at -20 °C in the laboratory.

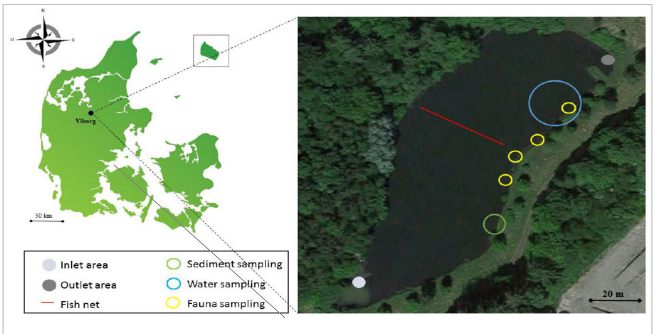

Figure 1. The sampling locations in the wet retention pond in Viborg, Denmark. The water sampling area is shown as a blue circle, and the sediment sampling area by the green circle. The fauna sampling areas are shown by the yellow circles. The red line shows where a fishing net was located. The light and dark gray dots show the location of the inlet and outlet area respectively.

Sample preparation

All glassware was rinsed three times before use and all equipment, samples etc. were kept covered to prevent contamination by airborne microplastics.

One major challenge in the microplastic analysis of environmental samples is the removal of organic/biological matter. Due to the hydrophobic nature of many plastics, organic matter will aggregate onto its surfaces and must be removed before the microplastic can be characterized spectroscopically. Oxidation by H_2O_2 was selected as the main pretreatment as this treatment would preserve the plastic while removing organic material.

The plastics in the water samples were concentrated by sieving and flushing with ethanol before evaporation of the ethanol.

Sediment samples were sieved and freeze dried before oxidation by H_2O_2 to remove organic matter. Gravimetric separation was then used to separate the inorganic and organic fractions.

To prepare the fauna samples, 60 mL of 5 M KOH was added per 1 g of dry weight freeze-dried sample. The solution was then stirred for 48 h at 45 °C. Ultrapure water was added before sieving of the sample.

The final concentrated plastic particle samples from each of the three sample types were suspended in ethanol. Samples with particle sizes > 80 µm were deposited onto an infrared reflective glass slide (MirrIR, Kevley Technologies) for reflection mode FTIR imaging analysis. Particles < 80 µm were deposited onto a Zinc Selenide (ZnSe) infrared transparent window, which was then dried for subsequent analysis in transmission mode. This left the microplastic particles adhered to the slide, ready for analysis via FTIR imaging.

To validate the method, some replicate samples were spiked with between 30-36 red 100 µm polystyrene beads.

Instrumentation

To identify and quantify microplastics in the samples, a Fourier Transform Infrared (FTIR) imaging system was used. The system comprised an Agilent Cary 620 FTIR microscope coupled to an Agilent Cary 670 FTIR spectrometer. The microscope is equipped with an 128 x 128 pixel Focal Plane Array (FPA) detector and is capable of simultaneously acquiring 16,384 spatially resolved spectra over an area of 700x700 microns per tile using 15x magnification. The instrument can operate in reflection and transmission mode. The settings are shown in Table 1.

Table 1. FTIR imaging settings used for the analysis.

Settings for Reflection and Transmission mode	
Focal Plane Array Size	128 x128
Objective	15x
IR Pixel size	5.5 µm
Number of scans per tile	30
Number of mosaic tiles	16 x 16
Total Measurement area	9.8x9.8 mm
Spectral Resolution	8 cm^{-1}

Results and Discussion

The FTIR images of the samples were analyzed to identify and quantify the plastics present. This analysis requires removal of most materials other than the subject investigated. The sample preparation methods were optimized for each different sample type e.g., water, sediment, fish to achieve this.

A rough analysis was initially conducted, where materials without a C-H stretch IR absorbance between 3000-2800 cm^{-1} were rejected. This ruled out most of the particles present. Spectral comparison of the most common plastics (listed in Table 2) was used to analyze the remaining particles [5, 6]. When a plastic was detected, the material of the particle was noted down together with the dimensions.

Table 2. Common plastics, likely to be found in wastewater.

Polyethylene (PE)
Polypropylene (PP)
Polyvinyl chloride (PVC)
Zinc stearate
Polystyrene (PS)
Styrene Butadiene Rubber
Polyurethane (PUR)
Polyamide/Nylon (PAM)

The spectral analysis was done by individually assigning the peaks in the IR spectra. This reduced the risk of both false positive and false negative plastic particles [4, 7]. To reduce the time required only one tile-column, approximately in the center of the sample slide/window, was analyzed (see Figure 2).

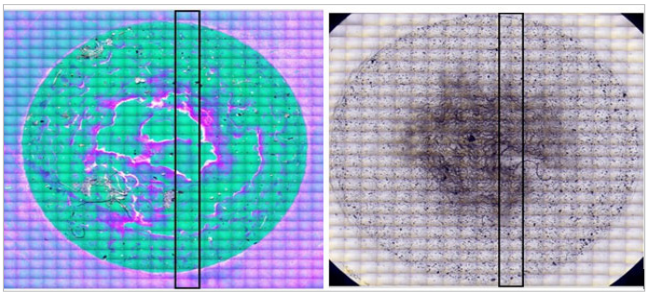

Figure 2. The FTIR image of a slide (left) and the zinc selenide transmission window (right) with a deposited water sample. The black boxes indicate the part of the 10 x 10 mm measurement area that was analyzed.

All spectra assigned as plastic were noted as well as the plastic type. The tiles were analyzed one by one, by zooming in on the particles, assigning the peaks and comparing the full visual image with the IR image. The initial screening showed that most of the particulate matter remaining on the tile was of other origin than plastic i.e. mineral particles (illustrated in Figure 3).

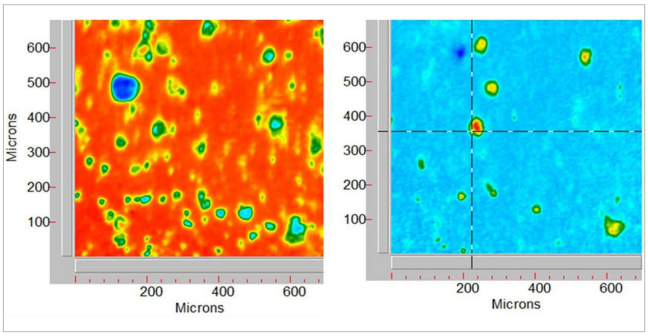

Figure 3. A tile image from the 20-80 µm water sample. To the left is the infrared image before applying the initial screening, based on a absolute peak height ~ 2200 cm^{-1} to highlight scatter and thus all particles. On the right, is the infrared image after the initial screening, based on integrated peak area of 3000-2800 cm^{-1} to highlight plastic particles. The initial screening showed that most of the particulate matter remaining on the tile was of other origin than plastic.

The screening showed that some colorful spherical shaped particles got excluded as well. This issue was also addressed in another study [4], which reported unusable IR spectra when the material was irregular shaped.

Quantifying the plastic content

The plastic spectra were assigned into one of two categories: 'high' and 'likely', based on the confidence in the spectral identification. 'High' represents a spectrum completely identical to the reference spectrum and 'likely' represents a spectrum where most peaks were present. Figures 4 and 5 show examples of the spectra obtained. However, all particles identified as being plastic were included in the results, shown in Figure 6.

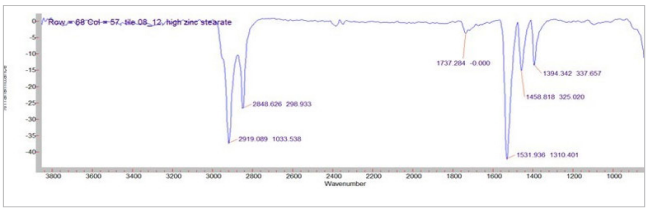

Figure 4. The IR spectrum of zinc stearate, designated 'high', located in a fish sample.

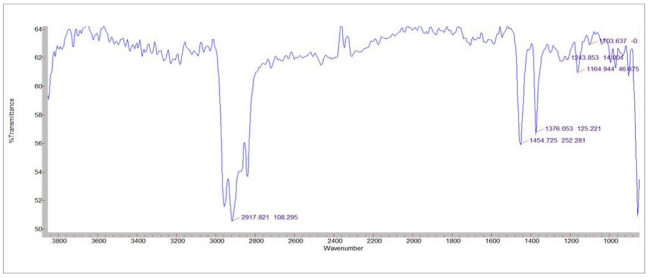

Figure 5. The IR spectrum of polypropylene, designated 'likely', located in a sediment sample.

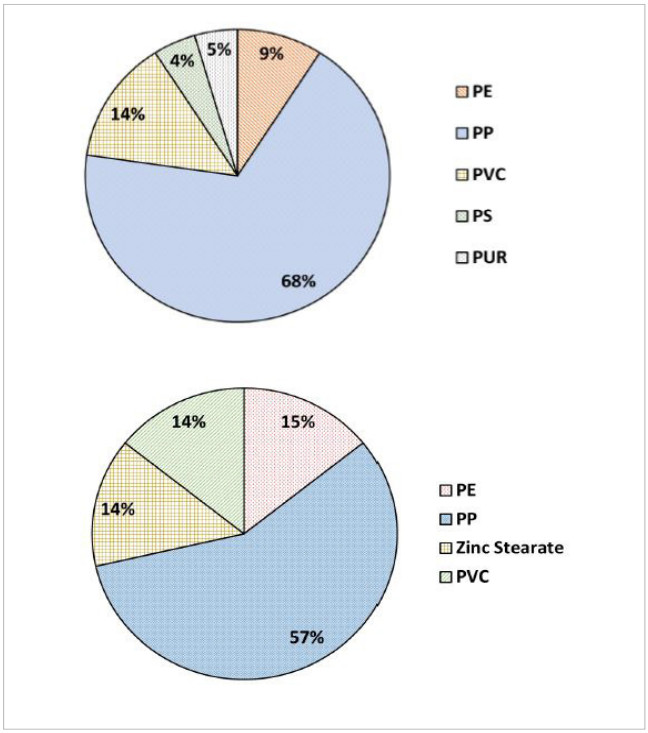

Figure 6. The composition of plastic particles found in sediments (upper) and water (lower). Refer to Table 2 for a description of the acronyms.

The most abundant plastics in the pond were polypropylene and PVC. The sediment samples were found to contain more types of plastics present, compared to the water samples.

In the fish sample one zinc stearate particle was detected. The analyzed volume corresponds to 7.3% of one stickleback fish which indicates that the method was able to detect microplastics, but no concentration was calculated.

The number of particles present in an aquatic environment could potentially play a significant role in the impacts of microplastic on the aquatic fauna [7]. In this study, the quantification of plastic was done by determining the number of plastic particles present in the analyzed sample volume. When quantifying plastic particles, the sample preparation method should be considered as there is the potential it may increase the quantity of particles by breaking larger particles into smaller particles, e.g., via processes such as sonication, mechanical stirring and scraping.

Based on the FTIR analysis, the plastic concentration in the sediment was determined to be 5.2 x 10^5 particles/kg dry sediment, equivalent to 26 mg/kg dry sediment. The plastic concentration in the water samples was determined to be 1.1 x 10^2 particles/L, equivalent to 4.5 µg/L. No plastic particles were found in the leech-sample, however this result may not mean that plastics were not present, they were just undetectable via this method possibly due to being smaller than 20 microns (the lower size fraction limit in this study) and different sample preparation techniques may be required for animal tissue.

Method validation

The study protocols were validated by spiking samples with a known quantity of polystyrene particles. The particles were quantified after the sample preparation and FTIR quantification method was applied. A high recovery rate was observed for most samples, as shown in Figure 7, with recoveries ranging from 97% in a water sample through to 64% in a sediment sample.

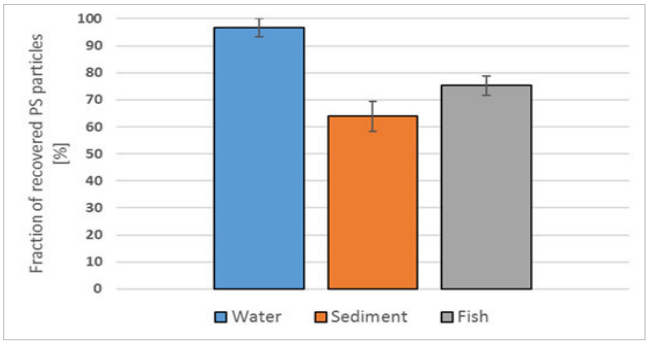

Figure 7. The fraction of recovered polystyrene (PS) beads from spiked samples. The recoveries were: 97% in the water sample, 64% in a sediment sample, and an average of 75% in two fish samples. The error bars on the blue and orange column were calculated as the possibility of a miscount due to the amount of matter on the filter containing the recovered particles. The error bar calculated for the fish sample was calculated as the standard division.

The low recovery achieved for the sediment sample indicates that studies of soil and sediment have a risk of underestimating the plastic concentration. The sediment samples in this study had the highest concentration of plastic, with the prepared samples visibly containing colorful plastic particles. The presence of particles similar in shape and color to the red polystyrene particles may have complicated the count of the recovered polystyrene.

Conclusions.

The study's methods were able to successfully recover, identify, and quantify microplastics in organic-rich samples such as sediment, water, and fish.

Based on the results of the study it can be concluded that microplastic is present in the wet retention pond from which the samples were taken.

FTIR imaging proved to be an accurate way to detect and quantify microplastics. Combined with H_2O_2 oxidation, FTIR imaging is a strong candidate to be a standard method in microplastic analysis, allowing further study and understanding of microplastics in the environment.

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