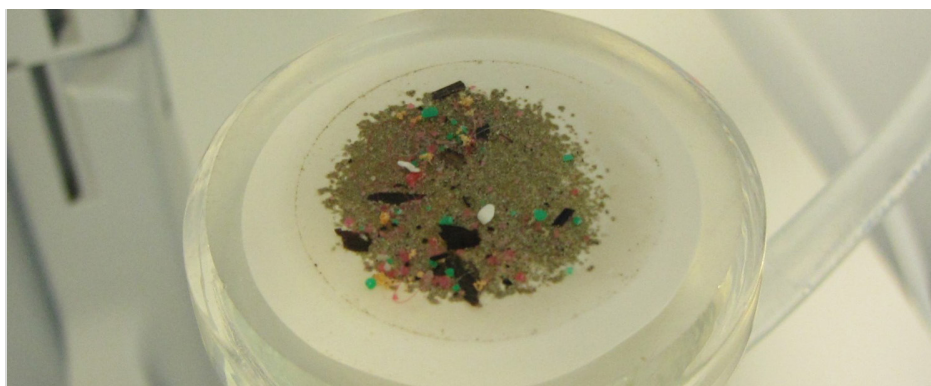


Analysis of Microplastics using FTIR Imaging

Identifying and quantifying microplastics in wastewater, sediment and fauna



Authors

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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) Spectroscopic 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 PTFE-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.

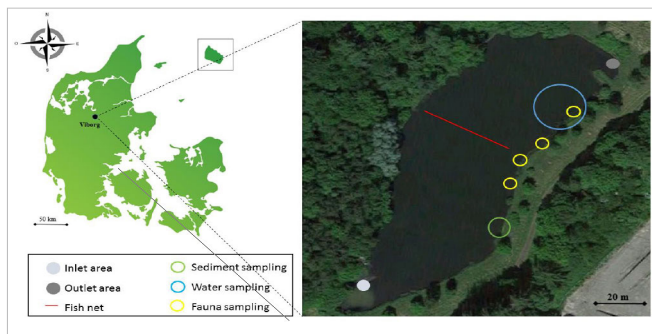


Figure 1. The sampling locations in the wet retention pond in Viborg, Denmark. The water sampling area is shown as a blue circle, and the sediment sampling area by the green circle. The fauna sampling areas are shown by the yellow circles. The red line shows where a fishing net was located. The light 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 Calcium Fluoride (CaF_2) 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.

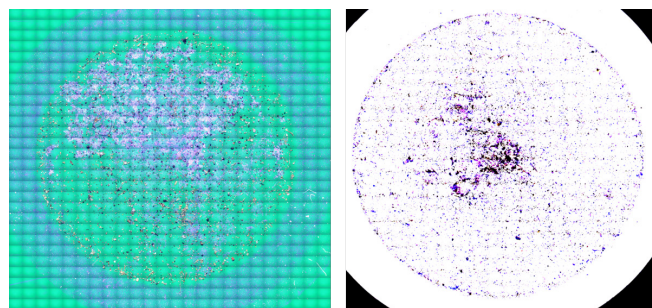


Figure 2. Visible image of a the reflection slide (80-500 μm particle sizes, left) and the CaF_2 transmission window (10-80 μm particle sizes, right). Both images are 10 x 10 mm.

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}
Spectral range	3850-850 cm^{-1}
Total scanning time	3 hours
Total number of spectra	4,194,304

Data Processing

Analysis of the FTIR imaging data was done with the program MPhunter developed at Aalborg University, Denmark, in collaboration with the Alfred Wegener Institute in Germany. MPhunter correlates a number of reference spectra to the spectra obtained by the FTIR Imaging system. It correlates all spectra within the image (4.2 million spectra in this example) using the raw spectra (underivatized) as well as the 1st and 2nd derivatives to each loaded reference spectra, using the entire spectral range, or a selected range of wavenumbers and produces a score between 0 and 1, indicating goodness of fit. The 3 correlations can be weighted individually.

For detecting the microplastics in the sample, an automated algorithm is applied where all reference spectra in the database are compared to all spectra in the map. In this case 113 reference spectra of both plastic polymers and natural materials, having spectra that show similarities to those of the plastics, have been used. The various materials in the spectra-database are assigned to different material groups such as PP, PE, PET, and so on. The algorithm used for detecting microplastic particles applies 2 thresholds of probability score. First the algorithm finds all pixels where the highest probability score (there are in this case 113 probability scores per pixel) belongs to a plastic material and where that score is above the higher threshold. It analyses all the adjacent pixels and adds them to the plastic particle if they have a material belonging to the same material group and which has a probability score above the second threshold.

In the present correlation, the raw spectrum was given the weight 0 (meaning it was not taken into account) while the 1st and 2nd derivatives each were given the weight 1, meaning the final score was an average of the 1st and 2nd derivative scores. The reason for not including the raw spectrum is that sloping baselines (due to sample shape size related optical scattering) tend to give misleading results, an issue which is not encountered when using the derivatives. The graphical output can be either a color correlated image, where each pixel is color coded against the nearest spectral match and/or a second image can be generated to show a heat map for a specific user selected reference material.

The identified plastic particles are then analyzed for the longest distance between pixels of the particle, yielding the major dimension of the particle. The minor dimension is found by assuming the particle shape is an ellipse and knowing the area of the particle in the scan. The third dimension, the thickness, is assumed as being 0.67 times the minor dimension. The volume is calculated assuming the particle is an ellipsoid. The mass is calculated from the volume and the density of the identified plastic material. These particle parameters are then displayed in tabular format for easy export. See Table 3 for an example.

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 full correlation map against all 113 reference spectra for the transmission measurement (10-80 μm particles sizes, is presented in Figure 3.

At first glance it is clear that the majority of particles are of natural origin such a cellulose and protein. In particular, the fibrous particles can be seen and are of cellulosic origin.

Figure 4 shows a comparison of a pixel identified as polypropylene compared to the reference spectrum for polypropylene, using both raw spectra (underivatized) and a first derivative. This demonstrates quite clearly the benefit of applying a derivative as scatter related spectral offsets or slopes (due to the particulate nature of the sample) are very effectively minimised, providing for a better correlation to the reference spectrum.

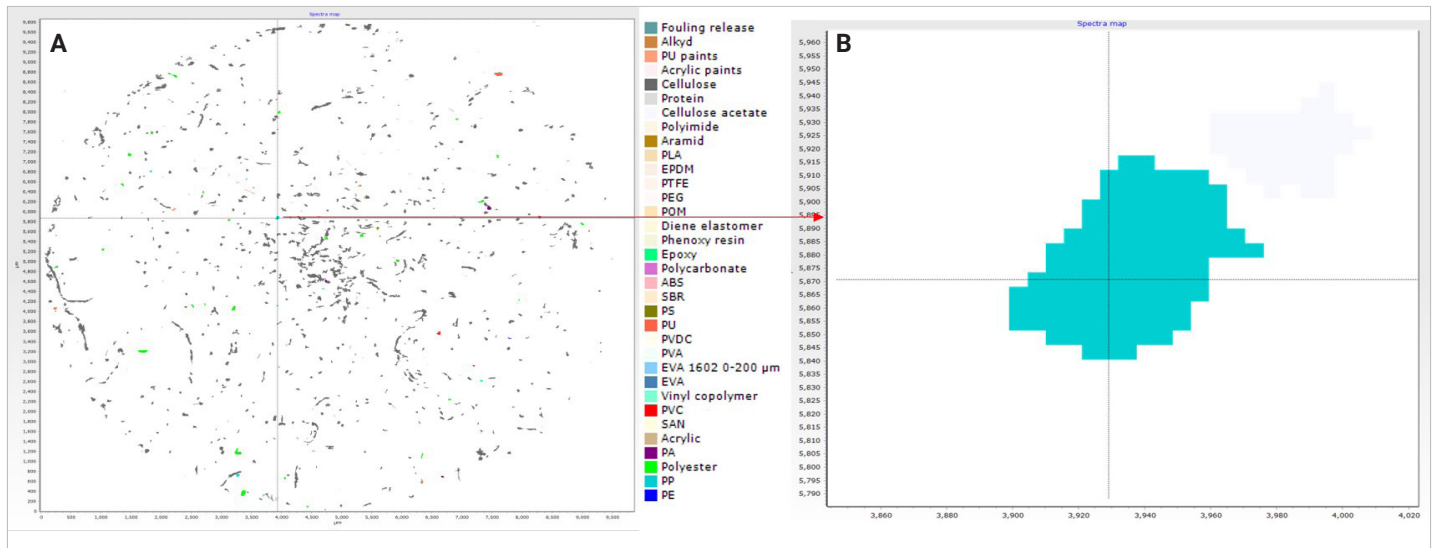


Figure 3. A. Full 10x10mm automatic correlation image. Each Particle is color coded based on the identified plastic (or natural material) type. B. Zoomed in region of ~200x200 microns, to show the level of detail for this Polypropylene particle. Note, each pixel is 5.5 microns.

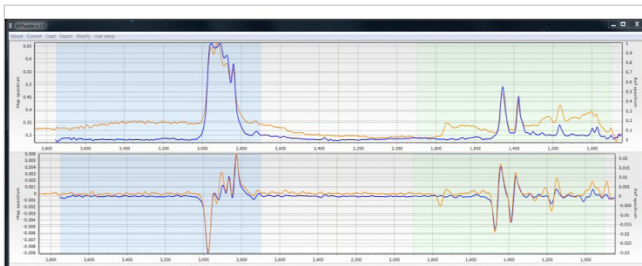


Figure 4. Screen capture from Mphunter, showing blue the reference polypropylene spectrum overlaid with a pixel identified as polypropylene. Upper pane shows raw spectra (underivatized). Lower pane shows the same spectra after a first derivative.

With this process occurring for all spectra, in this example 4.2 million spectra, it becomes a very efficient and accurate method to quantify and chemically identify the particles present. The percentage by mass and by particle count is presented in Table 2.

A more detailed analysis can be conducted using the MPhunter particle information output, which lists each identified particle, its coordinates, polymer group (chemical ID), area, major and minor dimensions, volume and mass. See Table 3.

Table 3. MPhunter derived detailed particle information. This analysis had 871 particles identified. Only for the first 4 are shown here for clarity.

MP ID	Coordinates (pixels)	Coordinates (µm)	Polymer group	Area on map (µm ²)	Major dimension (µm)	Minor dimension (µm)	Volume (µm ³)	Mass [ng]
MP_1	1416;630	7788;3465	pe	968	74.3	16.6	6423	6.102
MP_2	111;914	611;5027	pp	182	17.8	13	943	0.896
MP_3	333;1238	1832;6809	pp	938	44.8	26.7	10002	9.502
MP_4	464;1500	2552;8250	pp	61	13.3	5.8	140	0.133

Table 2. List of particle ID by % mass and by % particle count.

Particle ID	% by mass	% by particle count
PE	0.01%	0.11%
PP	0.30%	1.03%
Polyester	3.11%	3.22%
Polyamide (PA)	0.37%	0.69%
PVC	0.15%	0.23%
Polyurethane	1.21%	1.49%
Polystyrene	0.05%	0.11%
Epoxy	0.02%	0.23%
POM	0.01%	0.11%
Cellulose Acetate	0.15%	0.23%
Protein	1.90%	10.57%
Cellulose	92.98%	82.18%
PU paints	0.10%	0.23%
Alkyd	0.16%	0.46%

Quantifying the plastic content

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 5.2×10^5 particles/kg dry sediment, equivalent to 26 mg/kg dry sediment. The plastic concentration in the water samples was determined to be 1.1×10^2 particles/L, equivalent to 4.5 $\mu\text{g/L}$. 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 5, with recoveries ranging from 97% in a water sample through to 64% in a sediment sample.

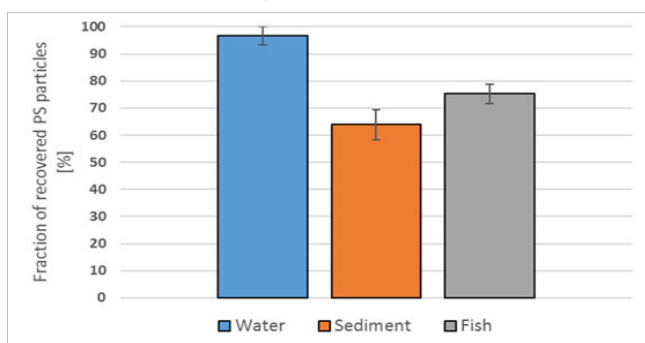


Figure 5. The fraction of recovered polystyrene (PS) beads from spiked samples. The recoveries were: 97% in the water sample, 64% in a sediment sample, and an average of 75% in two fish samples. The error bars on the blue and orange column were calculated as the possibility of a miscount due to the amount 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, combined with the MPhunter software, proved to be an rapid and accurate way to automatically identify and quantify microplastics and other materials. 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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More Information

This application note was extracted from a study titled “Microplastic in Water, Sediment, Invertebrates, and Fish of a Stormwater Retention Pond”, presented at the Society of Environmental Toxicology and Chemistry (SETAC) Conference in May 2017 by Kristina B Olesen, Diana A Stephansen, Nikki van Alst, Marta Simon, Alvise Vianello, Fan Liu, Jes Vollertsen, Department of Civil Engineering, Aalborg University, Denmark.

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