Application of multi-step approach for comprehensive identification of microplastic particles in diverse sediment samples

Olga Konechnaya, Christina Schwanen and Jan Schwarzbauer

Olga Konechnaya
Christina Schwanen
Jan Schwarzbauer (corresponding author)
Institute of Geology and Geochemistry of
    Petroleum and Coal,
RWTH Aachen University,
Lochnerstraße 4-20,
52056 Aachen,
Germany
E-mail: jan.schwarzbauer@emr.rwth-aachen.de

ABSTRACT

The tremendous increase of plastic production, its intensive usage in packaging, as transport material, and the insufficient management of plastic garbage have led to a rise in microplastic particles as an anthropogenic contaminant in our environment. To develop appropriate management and remediation strategies for this global pollution problem, reliable and consistent analytical procedures for measuring plastics in the complex matrices need to be designed. The applicability of an easy, robust and fast multi-step approach was tested on three sediment samples from riverine, beach and backwater areas of varying origin, grain size and organic matter content, and is reported here. The optimized method included grain size fractionation, density separation and μ-FTIR analyses. Identification was based on two complementary methods of μ-FTIR measurements, the Image mode for small microplastics (<1 mm) and the ATR method for bigger (1–5 mm) particles. The analyses revealed the identification of several polymers in various grain sizes at different pollution levels. Major findings are the dominance of PET particles and the highest frequency of microplastic particles in the midsize fraction of 100–500 μm. Generally, the method was able to reliably detect microplastic particles in several grain size fractions and down to very low contamination levels of approximately. ten particles per 50 g of sediments with different organic matter content and various grain size characteristics. Moreover, the presented multi-step approach represents a fast, easy and less cost-effective method as an alternative to more expensive and time-consuming methods.

Key words | environmental samples, FT-IR spectroscopy, identification, microplastics, multi-step approach

HIGHLIGHTS

- Easy, robust and fast multi-step approach for microplastic analyses in sediments.
- Reliably microplastic particle detection in several grain size fractions and down to very low contamination levels.
- Applicability tested on on three environmental sediment samples with varying origin, grain size and organic matter content.

INTRODUCTION

The tremendous increase in plastic production, its intensive usage in packaging, as transport material, and the insufficient management of plastic garbage have to a rise of microplastic particles as an anthropogenic contaminant in our environment. Microplastic particles have been investigated more intensively in the last decade and were found almost everywhere in the environment (Fath 2019), including biota (Neves *et al.* 2015; Nelms *et al.* 2019), food (Yang *et al.* 2015;

EFSA Panel on Contaminants in the Food Chain 2016) drinks (Schymanski *et al.* 2018) or even in the polar waters of the Arctic (Barnes *et al.* 2010; Bergmann & Klages 2012). Especially small microplastics (<1 mm) are able to be ingested, to reach organs/tissues and be stored in the intracellular structure (Kershaw & Rochman 2015) (GESAMP 2015). Microplastics can enter the human food chain through ingestion of seafood or other food products and can cause potential human health impacts (Rist *et al.* 2018; Fath 2019). The crucial parameters, which determine the bioavailability of microplastics, are their size, density and abundance (Wright *et al.* 2013). As an example, it has been reported that microplastics between 0.5 and 438 μm can translocate to organs or blood (Brennecke *et al.* 2015; Bergmann *et al.* 2017).

Beside microplastics themselves, additives (bisphenol A, phthalates, triclosan) also exhibit harmful potential. More than 50% of plastics contain hazardous additives or chemical by-products, which, for example, are reported to be carcinogenic (polyethylene terephthalate (PET)) (Li *et al.* 2016) or to lead to reproductive abnormalities (polystyrene (PS), polyvinyl chloride, (PVC)) (Wang *et al.* 2016).

Karbalaei *et al.* (2018) summarized the sources and impacts of microplastics on animals, humans and also provided mitigation options to reduce microplastic pollution around the world. The problems pointed out in this article underline environmental concern regarding microplastics, which are mostly adsorption of persistent organic pollutants (POPs), fragmentation of microplastics, toxicity for animals, damage of maritime equipment and technical synthesis from non-renewable sources. However, to develop appropriate management and remediation strategies for this global pollution problem, reliable and consistent analytical procedures for measuring plastics in complex environmental matrices need to be designed (Hanvey *et al.* 2017).

Several approaches are reported and summarized in reviews (Hidalgo-Ruz *et al.* 2012; Hanvey *et al.* 2017; Miller *et al.* 2017; Shim *et al.* 2017; Li *et al.* 2018; Silva *et al.* 2018; Nguyen *et al.* 2019; Prata *et al.* 2019; Stock *et al.* 2019). These analytical procedures mainly start with separation and concentration steps comprising, for example, grain size fractionation, density separation and filtration (Hidalgo-Ruz *et al.* 2012; Konechnaya *et al.* 2020; Szymańska & Obolewski 2020). These steps are applied partly in combination, as sequential or as single treatment. However, complete separation is often not possible, for example for more highly degraded particles (Biver *et al.* 2018). Finally, the detection for identification or quantification is performed by non-destructive methods such as microscopy or spectroscopy (infrared or Raman spectroscopy) or by

destructive measurements, basically pyrolytic approaches. However, all developed analytical procedures differ in terms of sensitivity, spectrum of detectable polymers, type of sample and analytical quality assessment.

The main object of this study is to extend and optimize an evaluated and validated multi-step approach based on a sequential grain size and density separation linked to μ-FTIR (Konechnaya *et al.* 2020) by applying the optimized method on various aquatic environmental samples, especially at low contamination levels.

MATERIALS AND METHODS

Samples and sample pretreatment

The examined samples derived from coastal or riverine areas of three different regions. The first sample was a sand sample from a beach in Scotland in June 2018. A second sediment sample derived from an urban river section in Chennai, South India (June 2019). A last riverine sediment was taken near river Tiranë, Albania, in May 2018 upstream of a waste disposal site (Figures of samples and sample locations are exemplified in supplementary material).

Prior to analysis, the sample materials were dried over night at 40 °C. In case of the Albanian sediment, a 100 g sample was taken for analysis. The other two samples were examined in smaller amounts of approximately 50 g.

To prevent potential contamination only non-plastic equipment (glass, metal, ceramic) was used for the whole analytical procedure.

Grain size fractionation and density separation

The sample material was separated into five groups of grain size by liquid separation in a sieve stack (AS 200 from Retsch GmbH). Grain size fractions of 1–5 mm (F1), 0.5–1 mm (F2), 100–500 μm (F3), 50–100 μm (F4) and 20–50 μm (F5) were obtained. Two days after drying at 40 °C in an oven, all fractions were gravimetrically determined and further subjected to density separation using a $ZnCl_2$ solution with a density of 1.7 cm³, which was reused after treatment. To avoid misunderstanding due to laboratory contamination or artifacts, the whole procedure was applied to untreated and unpolluted sediment samples. These blank experiments clearly revealed no microplastics cross contamination. Data on recovery experiments from spiked sediment samples and individual analytical steps

are given in the Supplementary material (Tables S1 and S2) and are discussed in detail by Konechnaya *et al.* (2020).

H₂O₂ treatment and filtration

This additional purification step was applied only for the sample from Albania due to its higher organic matter content.

Hydrogen peroxide (H_2O_2, 30%) in small quantities (20–50 mL) was added to remove labile organic matter from the inorganic sample matrix. The sample was heated up to 60 °C with continuous stirring for 12 h. If needed, as checked by the formation of gas bubbles, more H_2O_2 was added. Then the sample residue was filtrated (filter size 12 μm) and washed with 500 mL deionized water. Finally, the filter containing the sample material was dried over night at 40 °C.

Previously, the treatment with hydrogen peroxide was tested on different sizes of examined small microplastics (up to 1 mm). This test was carried out for a prolonged time of 7 days and all eight polymer types of our study were considered. Beside only a discoloration of polyamide (PA), no further alteration of the materials were observed.

Identification of microplastics by μ-FTIR-spectroscopy

Identification of microplastics was carried out with a Fourier-Transform Infrared spectroscope Spotlight 400. Prior to IR measurements the fractionated particles obtained after filtration were transferred to a pre-prepared KBr pellets (diameter of ca. 1.3 cm) and fixed on the pellet surface with 10–12 tons of pressure for 30 sec. Previously, potassium bromide was pressed to a pellet with 8 tons for 1.5 min.

The mode of IR-detection differed according to the particle size. The attenuated total reflectance – Fourier transform infrared spectroscopy (ATR-FTIR) technique was used to characterize microplastic particles bigger than 1 mm as obtained in fraction F1 (1–5 mm). The prepared KBr pellets were measured by FTIR spectroscopy with a germanium crystal at the ATR objective. IR spectra (image size 100 μm $\times$ 100 μm) were recorded on 10 points over the whole pellet using an MCT (Mercury Cadmium Telluride, cooled down by liquid nitrogen to 77 K) detector in a wavenumber range of 4,000–750 cm^{-1} with the spectral resolution of 16 cm^{-1}. For every spectrum, eight scans were co-added. A background scan without sample material and KBr pellet was carried out prior to running each batch of samples. Full Spectral Image was obtained as result of data collection after ATR-FT-IR measurement. An (ATR) correction was carried out for all measurements.

For all microplastic particles smaller than 1 mm comprising fractions F2 to F5, an Image mode was used by measuring IR spectra over the entire KBr pellet. All spectra were recorded with the Spotlight 400 FT-IR Imaging System by PerkinElmer, Inc. and analyzed by the corresponding software Spectrum (Version 10.5.3) and Spectrum IMAGE (Version 1.10). The spectral range from 4,000 to 650 cm^{-1} was adopted for the FTIR analysis. A resolution of 8 cm^{1} and 1 scan per pixel were used. The IR measurement of the entire KBr pellet was carried out using View mode 'Compare correlation' and a pixel size of 25 μm to obtain Full Spectral Image (FSM). Positive identifications were determined by a search score higher than 0.7. The obtained IR signals from polymers were classified into eight spectra types according to their composition: (1) polyethylene (PE); (2) polypropylene (PP); (3) polyvinyl chloride (PVC); (4) Polyurethane; (5) polyethylene terephthalate (PET); (6) polystyrene (PS); (7) polyamide; (8) poly (methyl methacrylate) (PMMA).

RESULTS

Three complex environmental samples of different origin were selected for analysis and microplastic identification in five grain sizes (1–5 mm, 0.5–1 mm, 100–500 μm, 50–100 μm and 20–50 μm) using a specifically designed multi-step approach according to Konechnaya *et al.* (2020). Identification was based on two complementary methods of μ-FTIR measurements. For small microplastics <1 mm, identification took place using Image mode and for bigger (1–5 mm) microplastics the measurements were conducted on ATR-μ-FTIR germanium crystal. Simultaneously, up to eight polymer types down to 20 μm could be analyzed and identified by this multi-step approach. Further on, a pretreatment step for organic-rich samples by H_2O_2 addition has been partly applied. All three sediment samples in different grain size distributions containing diverse polymer types were successfully extracted and analyzed as described in the following sections.

Beach sediment from Scotland

The beach sediment sample from Scotland was characterized by a low organic matter content (TOC = 0.24%) and a sandy grain size profile (see Table 1). Accordingly, the grain size fractionation (recovery rate of ca. 99% by weight, see Table 1) revealed the highest content in fraction F3 (100–500 μm) with approximately 75% (see Figure 1). On

Table 1 | Results of gravimetrical determination of Fractions F1 to F5 for Scottish sample after grain size fractionation and density separation

Fraction		Sample [g] after grain size fractionation	Sample [g] after density separation
F1	1–5 mm	5.45	0.007
F2	0.5–1 mm	6.61	0.002
F3	**100–500 μm**	**37.45**	0.024
F4	50–100 μm	0.01	
F5	20–50 μm	0.07	
Total		49.59	

the contrary, fractions F4 and F5 represented less than 0.2%. The low contents of F4 and F5 allowed a direct μ-FTIR measurement without further density separation. However, the coarser grain size fractions were subjected to density separation providing microplastic-containing residues of around one permill (by weight).

In total, only 11 microplastic particles covering five polymer types (PE, PP, PU, PET, PA) were identified within all five grain size fractions (see Table 2). Most of

the microplastic particles (>50%) were found in the smallest fraction F5 (20–50 μm). In fractions F1 to F4, very few particles were found, and only sporadically. No PVC, PS and PMMA polymers were identified in the investigated sandy beach sediment.

Backwater sediment from India

The second sediment sample, derived from a South Indian backwater near Chennai, exhibited very similar properties compared to the Scottish sample, also comprising a low organic matter content (TOC = 0.06%) and a comparable distribution of sediment grain size (Table 3). The recovery rate for the individual grain size fractions also did not differ significantly from the values obtained for the Scottish beach sample. Thus, density separation was performed also only for the coarser fractions, F1 to F3, which represent the main proportion of the sediment material (>96% in the fractions F2 and F3).

In total, 18 microplastic particles were identified in all five fractions F1 to F5 (Table 4). Approximately two thirds
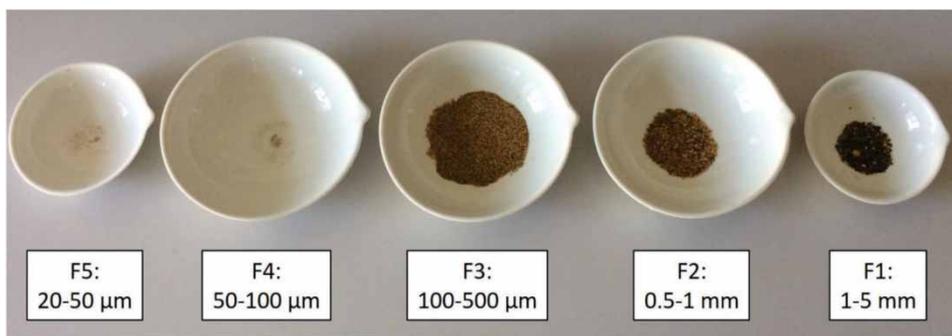
Figure 1 | Scottish sediment after grain size fractionation and drying.

Table 2 | Polymer particles found in five grain size fractions of the Scottish sample using ATR (F1) and image mode (F2 to F5)

Polymer	Fraction 1 1-5 mm	Fraction 2 0.5–1 mm	Fraction 3 100–500 μm	Fraction 4 50–100 μm	Fraction 5 20–50 μm	Total
PE	–	–	–	1	1	2
PP	1	–	–	–	1	2
PVC	–	–	–	–	–	–
PU	–	–	–	–	1	1
PET	–	1	1	–	3	5
PS	–	–	–	–	–	–
PA	–	1	–	–	–	1
PMMA	–	–	–	–	–	–
Total	1	2	1	1	6	**11**

Table 3 | Results of gravimetrical determination of fractions F1 to F5 for Indian sample after grain size fractionation and density separation

Fraction		Sample [g] after grain size fractionation	Sample [g] after density separation
F1	1–5 mm	1.64	0.001
F2	0.5–1 mm	10.26	0.012
F3	**100–500 μm**	**37.16**	0.004
F4	50–100 μm	0.22	
F5	20–50 μm	0.01	
Total		49.28	

of the microplastics particles were found in fractions F3 and F4, containing PP, PVC, PU and PET particles. Of note, the abundance of microplastics particles correlated well with the relative proportions of individual grain size fractions.

River sediments from Albania

The third sediment sample, derived from the Tiranë river (near a waste disposal site in Albania), differed significantly in organic matter content and grain size composition. It exhibited a higher organic matter content (TOC = 1.4%) and was dominated by the fine-grain size fraction. Of note, for the grain size fractionation a total recovery rate of only 69% was detected. That points to the assumption that more than a third part of the sample was represented by the grain size fraction less than 20 μm. However, the highest sediment content was observed in the third fraction, F3, with around 48% and the grain size of 100–500 μm and in fraction F4 with 33% and grain size of 50–100 μm. But also fraction F5 contained more than 15% of sediment sample. Thus, the grain size distribution showed a completely different composition as compared to the Scottish and Indian samples with most of the sediment amount (>96%) in grain size fractions smaller than 500 μm.

Due to this more uniform grain size distribution, the density separation procedure was applied to all five fractions. Further on, because of its high organic matter content, an H_2O_2 treatment was applied to fractions F2 to F4 as an additional purification step (Table 5). For these three fractions, the sample quantity was successfully reduced from ca. 0.6 g to 0.09 g. Consequently, 85% of material was successfully removed by H_2O_2 application, representing the labile organic fraction.

Table 4 | Polymer particles found in five grain size fractions of the Indian sample using ATR (F1) and Image mode (F2 to F5)

Polymer	Fraction 1 1-5 mm	Fraction 2 0.5–1 mm	Fraction 3 100–500 μm	Fraction 4 50–100 μm	Fraction 5 20–50 μm	Total
PE	–	1	–	–	–	1
PP	–	–	1	–	–	1
PVC	1	–	1	–	–	2
PU	–	1	2	1	1	5
PET	–	1	4	3	–	8
PS	–	–	–	–	–	–
PA	–	–	–	–	1	1
PMMA	–	–	–	–	–	–
Total	1	3	8	4	2	**18**

Table 5 | Results of gravimetrical determination of fractions F1 to F5 for Albanian sample after grain size fractionation, density separation and H_2O_2 treatment

Fraction		Sample [g] after grain size fractionation	Sample [g] after density separation	Sample [g] after H_2O_2 treatment
F1	1–5 mm	1.27	0.63	
F2	0.5–1 mm	0.79	0.20	0.06
F3	**100–500 μm**	32.74	0.33	0.02
F4	**50–100 μm**	22.98	0.07	0.01
F5	20–50 μm	10.86	0.02	
Total		68.64	1.25	

Table 6 | Polymer particles found in five grain size fractions of the Albanian sample using ATR (F1) and Image mode (F2 to F5)

Polymer	Fraction 1 1-5 mm	Fraction 2 0.5–1 mm	Fraction 3 100–500 μm	Fraction 4 50–100 μm	Fraction 5 20–50 μm	Total
PE	12	3	18	7	10	50
PP	4	2	6	7	9	28
PVC	1	–	–	2	–	3
PU	–	1	7	14	11	33
PET	–	6	10	60	39	115
PS	–	1	4	4	2	11
PA	–	–	6	4	4	14
PMMA	–	–	2	2	1	5
Total	17	13	53	100	76	259

In total, 259 microplastic particles were identified in all five fractions. Hence, also with respect to the level of plastic contamination, the third sample differed. Most of the microplastic particles were found in the fractions F3 (53 microplastics) to F5 (76 microplastics) with the maximum of 100 particles in fraction F4 (Table 6). Dominant plastic types were PE, PET, PU and PP. PE and PP were the only polymers that were found in all five fractions, with a total number of 50 and 28, respectively. The highest abundance was observed for PET with 115 particles in total. In all fractions below 1 mm, the polymers PP and PU were identified with a frequency of around 30 particles. On the contrary, PS, PA, PMMA and PVC were identified only sporadically as well as in lower quantity. Finally, the grain size fraction F4 was the only fraction in which all polymer types have been identified together.

DISCUSSION

This study aimed at testing the applicability of a specifically designed multi-step approach for simple identification of microplastic particles in complex environmental samples. The approach has been optimized for detecting up to eight common polymers (PE, PP, PVC, PU, PET, PS, PA, PMMA) in different grain size fractions of particulate matter samples (Konechnaya *et al.* 2020). Here, the developed multi-step method has been applied on environmental sediment samples of different origin and locations, with varying grain size, diverse organic matter content and different levels of contamination. In the course of the study, some further optimization steps in sample preparation have been included to determine microplastic in particular at very low amounts or concentration levels. Mainly an H_2O_2 pretreatment for organic-rich samples as well as the extension of

the grain size fractionation down to 20 μm due to the importance of the very fine fractions in risk potential assessments (Wang & Wang 2018).

Optimization of parameter settings

The main focus was laid on the μ-FTIR-based detection and identification of microplastic particles. This includes some critical steps and analytical restrictions, which have been considered in more detail, comprising the sediment sample from Albania and the complementary usage of FTIR measurement modes.

In environmental studies on soils and sediments, microplastic particles are usually filtered after enrichment procedures such as density separation. FTIR analyses are dominantly performed on the filter material itself. In the literature, the usage of polycarbonate membrane filter (compare (Vianello *et al.* 2013; Wang & Wang 2018), cellulose filter (Mani *et al.* 2019) or aluminum oxide filter (Löder & Gerdts 2015; Bergmann *et al.* 2017) are reported.

An investigation into 10 different filters for reflectance μ-FTIR mapping/imaging revealed that aluminum oxide filters and polycarbonate filters exhibit the best performance and are suitable for chemical mapping (Löder & Gerdts 2015). However, a focal plane array (FPA) detector has been used to detect microplastic particles down to a size of 20 μm but the effect of filtration material for other detectors has not been considered.

An alternative and novel approach, which was used in our study, is the transfer of microplastic particles on the surfaces of potassium bromide (KBr) pellets. It is an important step for fixation and comprehensive measurement, since the highly stable fixation on the plane area of the surface of the pellet reveals a representative concentration of particles of one sample. Further on, this fixation of the whole particles

on a small and defined surface area allows high-quality FTIR measurements by enhancing an Image mode FTIR measurement. Both aspects improve distinctly the sensitivity and reproducibility in particular for analyses of low-contaminated samples.

A further important aspect in μ-FTIR analyses is the comprehensive but reliable identification of the whole set of microplastic particles per sample. Of note, microplastic particles can consist of copolymers exhibiting at least two different monomer units, leading consequently to a more complex IR spectrum. Furthermore, a superimposition by adsorbed microorganisms or biofilms can interfere with the FTIR measurement (Harrison *et al.* 2011). Here, the

mode of detection plays an important role in particular with respect to the different particle sizes. For large size fraction ($>500\,\mu m$), usually ATR-FTIR is used to investigate and analyze microplastics. In comparison, for small microplastics ($<500\,\mu m$) μ-FTIR in Image mode or on focal plane array (FPA) detector is used (Bergmann *et al.* 2017; Lorenz *et al.* 2019; Mani *et al.* 2019). Exemplifying the different approaches, comparable microscopic and spectroscopic images are given in Figures 2 and 3 of the backwater sediment from India. The microscopic picture (Figure 2(a)), the area selected for IR measurements by ATR (Figure 2(b)) and the spectra image build up by punctual ATR analyses (Figure 2(c)) of a KBr pellet representing the fraction F3 of
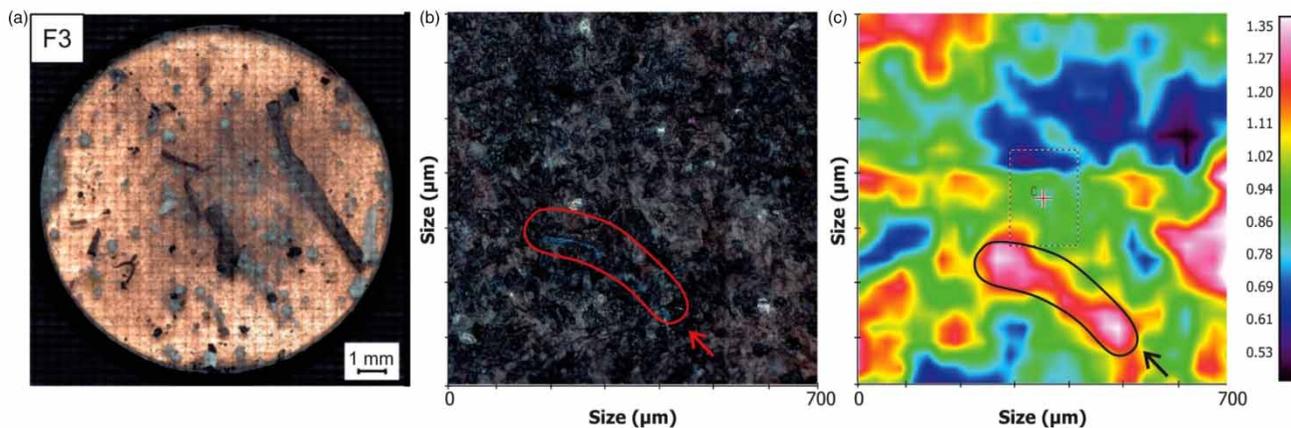

Figure 2 │ Microscopic image of the area (700 μm × 700 μm) of a KBr pellet (F3: 100–500 μm) of the Indian sediment (a), the selected area for ATR-FTIR analyses (b) and the resulting FSM (Full spectral image) (c) as built up by the individual ATR-FT-IR measurement. A marked PET particle is indicated by the intensive absorbance in red color in (c) and the visible blue PET particle in (b). The full colour version of this and other figures is available in the online version of this paper, at http://dx.doi.org/10.2166/wst.2020.600.
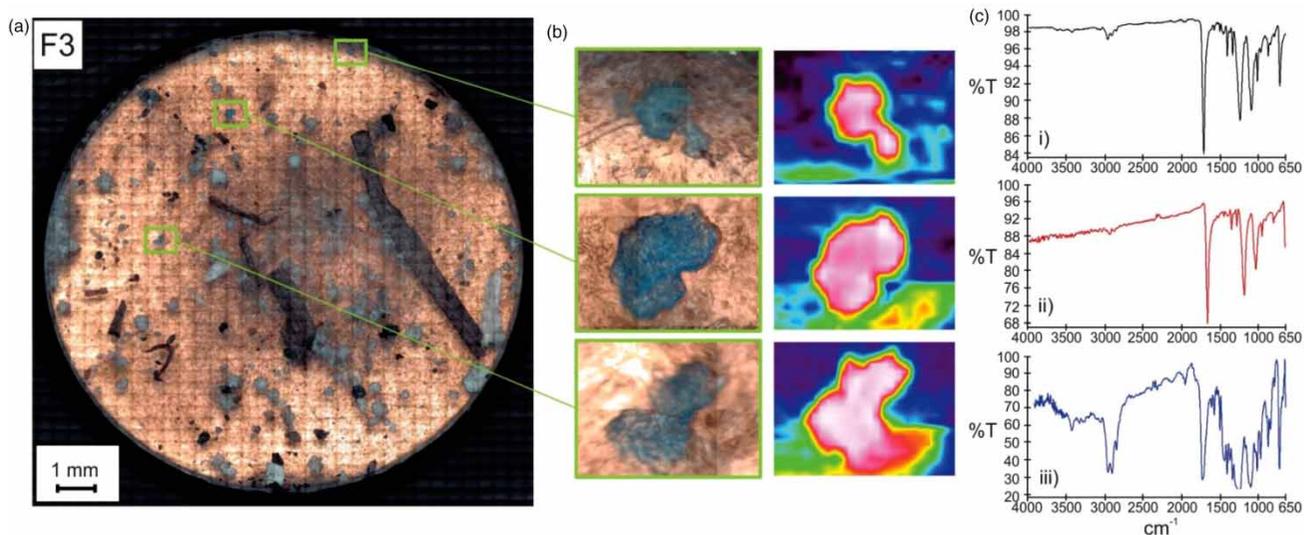

Figure 3 │ KBr pellet of the grain size fraction F3 of the Indian sediment sample (a) (see also Figure 2) with identified PET particles on it (i – iii) using Imaging mode (b). In addition, the corresponding IR spectra are illustrated in (c).

the Indian sediment sample are shown in Figure 2. Here, the detection of one PET particle is illustrated.

Figure 3 represents the visible image of the entire KBr pellet of the same grain size fraction (Figure 3(a)), but measured by FTIR Image mode. Selected areas with three identified PET microplastic particles accompanied by the corresponding IR spectra are illustrated.

In order to obtain reliable good-quality spectra by ATR-μ-FT-IR, some crucial preconditions are required. Firstly, a close contact between the sample and the crystal surface is essential. Secondly, all particles have to be analyzed sequentially, which extends the analysis time and needs an experienced applicant. Since only aliquots of areas can be investigated intensively, the area of measurement on KBr pellets or filters needs to be representative for the whole sample. Of note, the selected area of measurement also determines the spatial resolution of the IR detection, as illustrated in Figure 2. Here, an area of $700 \times 700\,\mu$m (near the maximum selectable area) has been chosen but the spatial resolution of the PET particle is worse, which extends compared to the corresponding visible image. For better spatial resolution, areas of $50 \times 50\,\mu$m to $100 \times 100\,\mu$m are recommended, but these areas represent only a very small aliquot of the total KBr pellet surface. Thirdly, particles with smaller grain sizes are often overlooked, impacting the reliability of quantitative results.

On the contrary, the entire surface of a KBr pellet can be analyzed simultaneously in μ-FT-IR Image mode. This method provides more reliable results in particular for smaller grain sizes. However, this mode results either in a lower quality of spectra in adequate analysis time (as illustrated in Figure 3) or very time and data storage consuming measurements at higher optical resolution (e.g. $4\,\text{cm}^{-1}$ or lower). Consequently, Image mode analyses need an optimized parameter setting for balanced measurements.

Generally, the detection of microplastic particles requires the complementary application of different modes of μ-FTIR measurement depending on the grain sizes. This points to the usefulness of grain size separation prior to μ-FTIR detection. A flexible application has already been suggested by Wagner & Lambert (2018) by applying ATR-FTIR measurements for microplastics $>500\,\mu$m and to use FTIR microscopy in transmission mode for smaller microplastic particles.

Comparison of three investigated sediment samples

The three samples investigated differ in terms of material, regional origin and contamination level. However, the results demonstrate clearly an appropriate application of the developed simple multi-step approach including the differentiated μ-FTIR-based identification.

Further on, some general observations can be pointed out by the overall results. Most of the microplastic particles (approximately 80%) were found in the finer grain size fractions with $<500\,\mu$m (Tables 2, 4 and 6). In detail, 73% of microplastic particles in the Scottish beach sediment were concentrated in the grain size fraction smaller than $500\,\mu$m, the Indian sediment sample contained 78% of microplastic particles smaller than $500\,\mu$m and in the Albanian sample even 88% of the detected microplastics were found in the grain size fraction $<500\,\mu$m. However, the individual grain size fractions with maximum amounts differ (Indian, F3: 8 of 18 microplastics; Albanian, F4: 100 of 259 microplastics; Scotland, F5: 6 of 11 microplastics). Similar results have been reported by Bergmann *et al.* (2017) with 99% of all microplastics found in the grain size fraction $<150\,\mu$m in Arctic deep sea sediments and by Mani *et al.* (2019), who detected in sediments of the Rhine River a proportion of 96% of microplastic particles within grain sizes $<75\,\mu$m. Further on, Haave *et al.* (2019) identified 95% of microplastics in the $<100\,\mu$m fraction in sediments of various fjords in Norway, and Lorenz *et al.* (2019) found 98% of microplastics in the fraction $< 100\,\mu$m in North Sea sediments.

Interestingly, the most frequent polymer type found in all three environmental samples of our study was PET, with an overall particle number of nearly 50% for all samples studied (see Figure 4). PE, PP, PA and PU were also detected in all samples but in minor amounts. On the contrary, PA and PVC were found only sporadically and PMMA as well as PS were detected only in the Albanian sediment. This is partly in contrast to other studies, in which the most frequent plastic fragments in environmental samples were PE, PP and PA (e.g. Hidalgo-Ruz *et al.* 2012). Polyethylene was detected as the most abundant microplastic particle (38%) in Arctic deep-sea sediments followed by polyamide (22%), and polypropylene (16%) (Bergmann *et al.* 2017). Microplastic particles identified in the Northwestern Pacific Ocean were dominated by PE (58%) as well as PP (36%) (Pan *et al.* 2019). However, in the sediments of the Rhine River, mostly polyacrylate/polyurethane copolymers were detected (>70%). In Norwegian sediments, the microplastic particles in the larger grain size fractions were dominated by PA (larger microplastics), whereas in the smaller fractions polyacrylate/polyurethane copolymers were more abundant (Haave *et al.* 2019).

As a second aspect, a clear correlation between grain size distribution and microplastic particle distribution is evident for the Indian and Albanian samples. In both samples,
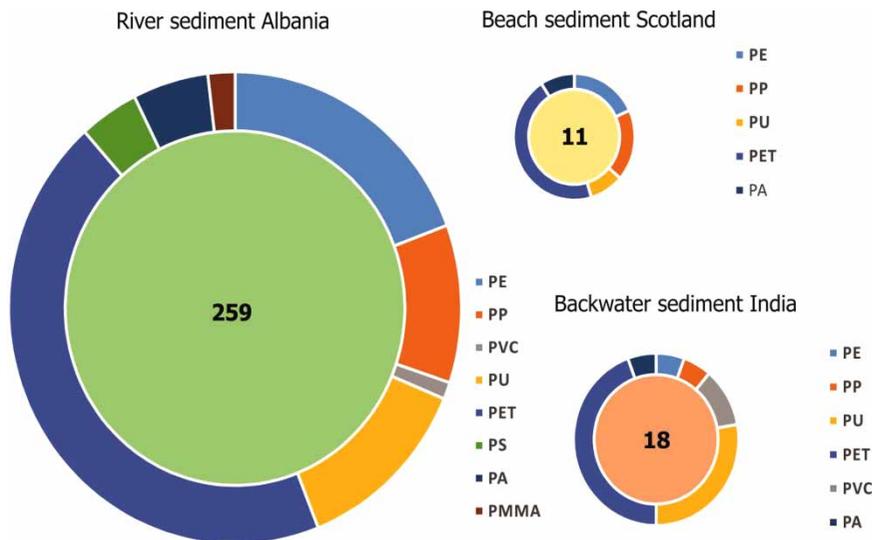
Figure 4 │ Distribution by polymer type of all detected particles of all three sediment samples.

the highest amounts of microplastic particles were identified in those grain size fractions that represented the highest mass abundance (Indian sample F3, Albanian samples F3 and F4). This correlation was not visible for the lowest contaminated sample, the Scottish beach sample. That might be either the result of the very low number of detected microplastic particles or the effect of different sample types (beach vs. river samples). Of note, sediment and soil samples are known to be heterogeneous and analyzing a single or a low number of samples may not be representative for the site (Haave *et al.* 2019). Hence, based on the limited number of samples in this study as well as the problems of representative sampling, these assumptions remain for further research.

Methodological comparison with other studies and limitations of multi-step approach

Several studies reported on identifying microplastics in sediments by μ-FTIR (Hidalgo-Ruz *et al.* 2012; Bergmann *et al.* 2017; Hanvey *et al.* 2017; Peng *et al.* 2017; Wang & Wang 2018; Haave *et al.* 2019; Lorenz *et al.* 2019; Mani *et al.* 2019; Pan *et al.* 2019). However, all studies differ in various parameters among each other and also in comparison to this study. Since these variations need to be considered in particular for comparison of results, as well as the applicability of the analytical procedures, they will be exemplified in the following text by selected reports.

Peng *et al.* (2017) investigated sediments from China, extracting samples by density separation according to

Thompson *et al.* (2004) and he considered six types of polymers (PE, PP, PET, PA, PS, polyester). Here, microplastic particles were placed on a KBr surface similar to our study. However, NaCl solutions have been used for density separation, which can cause problems with particles of higher density than $1.2 \, \mathrm{g} \, \mathrm{L}^{-1}$ such as PET or PVC.

Bergmann *et al.* (2017) published the first study providing data on the contamination of Arctic deep-sea sediments, indicating an enormous amount of microplastics found at 2,340–5,570 m depth, down to 11 μm. For their study, they used density separation by Micro Plastic Sediment Separator (MPSS) and Fenton's reagent for precleaning of organic-rich sediment samples. They detected a very wide spectrum of 18 polymer types, with the most frequent occurrence of PE (38%), PA (22%) and PP (16%). Almost 80% of the investigated microplastics were <25 μm. However, Bergmann used an FPA detector for their μ-FTIR analyses, which takes an enormous analysis time of up to around 13 h for each individual measurement. Further on, Bergmann analyzed large microplastic particles >500 μm by ATR-FTIR and smaller 500 μm by FPA-FTIR, comparable to our approach. Hereby, their study revealed high amounts of small microplastics up to 11 μm. Of note, these very small particles often reveal problems for FTIR analyses due to the IR beam thickness. Nevertheless, Duis & Coors (2016) were able to sufficiently detect microplastic particles down to 1–2 μm of grain size.

Further recent studies reported on microplastic pollution in sediments of the Rhine River and in Norwegian sediments (Haave *et al.* 2019; Mani *et al.* 2019). These studies

also covered a wider range of polymer types in a grain size range of 11–500 μm by FPA μ-FTIR microscopy and imaging analysis. Both studies reported the dominant appearance of micro plastic particles in the smaller grain size fractions (approximately <100 μm).

In summary, most of the μ-FTIR based studies on the detection of micro plastic particles in sediments exhibit restrictions either by time and cost-consuming analyses (as discussed above) or by restricted ranges of selected polymer types, coarser grain size fractionation, limited density separation ranges or lower sensitivity.

CONCLUSIONS

In this study, the applicability of an easy, robust and fast multi-step approach was tested on environmental samples. Three sediment samples from riverine, beach and backwater areas with varying origin, grain size and organic matter content were selected. In the course of the investigation, a recently developed multi-step approach (Konechnaya *et al.* 2020) was extended by grain size fractionation down to 20 μm and an additional H_2O_2-based purification step for more organic-rich samples. μ-FTIR analysis was applied in a complementary approach using Image mode measurements for smaller grain size fractions (<1 mm) and ATR-FTIR analyses for coarser grain sizes. Further specific conditions of this multi-step approach were the usage of KBr pellets for sample preparation and the preselection of the eight most relevant polymer types.

The analyses of microplastic particles in the sediment samples revealed the identification of several polymers in various grain sizes at different pollution levels. Major findings are the dominance of PET particles and the highest frequency of microplastic particles in the midsize fraction of 100–500 μm.

Generally, the method was able to reliably detect microplastic particles in several grain size fractions and down to very low contamination levels of approximately 10 particles per 50 g of sediments with different organic matter content and various grain size characteristics. Moreover, the presented multi-step approach represents a fast, easy and less cost-effective method as an alternative to more expensive and time-consuming methods.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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