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Detection of microplastics in marine sediments of the German Coast via FT-IR spectroscopy

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Abstract

The presence of microplastic debris in the marine environment has been documented during the past 40 years. With the implementation of the Marine Strategy Framework Directive (MSFD) in 2012 the need for reliable data obtained by standardized methods has been growing in order to realize monitoring programs and subsequent mitigation strategies. In this context a reproducible method for the detection of microplastics in marine sediments was developed in this thesis.

For the analysis of sediments the extraction method is crucial. In this thesis a density separation approach was adapted that involved the recently invented Munich Plastic Sediment Separator (MPSS) built by Hydro-Bios and a highly dense $ZnCl_2$ solution (density about 1.7 g cm^{-3}). The resulting extracted samples from North and Baltic Sea sediments were purified through enzymatic digestion using technical enzymes, i.e., Protease, Cellulase, Chitinase and additionally H_2O_2. This enzymatic digestion protocol proved to be efficient and biologically specific since the biogenic organic matter and not the synthetic polymers were affected. A successful purification was necessary to enable the subsequent analysis. In this context, another essential step is the identification of microplastics in order to quantify them. Since this is especially difficult for small microplastic particles (<500 µm), the highly promising µFT-IR mapping technique was applied. Therefore the pre-treated samples were concentrated on Anodisc filters in a 64 mm^2 area that was completely analyzed using Infrared spectroscopy in transmission mode. Applying this newly developed methodological setup, five North Sea sediment samples could be investigated for their microplastic contamination. The number of microplastic particles per sample detected ranged from 63 to 105. Taking the determined sediment weight of each sample into account the relative abundances of microplastic particles in North Sea sediments ranged from 34 (Sylt, beach) to 74 (German Bight, sublittoral) particles kg^{-1} dry weight sediment. Furthermore, ten different types of plastic polymers could be detected with a high prevalence of polypropylene (PP) in all the samples. Another omnipresent polymer type although not that frequent was polystyrene (PS). Next to low density polymers like polyethylene (PE) also more dense polymers like polyvinyl chloride (PVC) and polyvinyl acetate (PVA) were identified proving the efficiency of the extraction method. The efficacy of the enzymatic digestion was proven by the relatively clean spectra obtained during the µFT-IR measurement. This approach was applied for the first time for marine sediments and presents a first insight into microplastic pollution of German coastal sediments.

1 Introduction

1.1 Topicality and legal framework

The first detection of small plastic debris in the marine environment dates back to the early 1970s (Carpenter et al. 1972; Carpenter & Smith 1972; Colton et al. 1974), and since the discovery of the Great Garbage Patch in the North Pacific by Charles Moore 1997, these small pieces of plastic are of major concern. Macroscopic plastic particles are known to cause serious problems like entanglement of marine mammals, sea turtles, birds and fish (Laist 1987) and ingestion by various species (Derraik 2002; Hammer et al. 2012). However, gradually also the smaller pieces termed 'microplastic', which are not as easily visible to the naked eye also became a considerable topic.

The distribution of macroplastic ranges from the Arctic (Bergmann & Klages 2012) over the subtropical zones with their gyres (Morris 1980; Morét-Ferguson et al. 2010; Davison & Asch 2011; Eriksen et al. 2013) and the tropics (Ivar do Sul & Costa 2007; Jayasiri et al. 2013) to the Antarctic (Barnes et al. 2010). The occurrence of microplastic particles is consequently as widespread as well (Barnes et al. 2009; Browne et al. 2011; Hirai et al. 2011; Ivar do Sul & Costa 2014). Small plastic debris is reported from densely populated areas (Ng & Obbard 2006; Jayasiri et al. 2013) to even remote places and nature reserves (Ivar do Sul et al. 2009; Baztan et al. 2014) worldwide.

Owing to this global problem of pollution of the world`s oceans, legislative efforts have been made, like the Annex V of the "International Convention for the Prevention of Pollution from Ships", in short MARPOL, which was established in 1973/78 and implemented in 1988. "Annex V pertains to the Prevention of Pollution by Garbage from Ships and prohibits discarding of plastics into the sea worldwide." (IMO 2014).

Also many other more regional conventions and projects attend to the subject of plastic pollution in the marine environment. One of these, concerning the North Sea, is the "Convention for the Protection of the Marine Environment of the north-east Atlantic", better known under the abbreviation OSPAR, which sets quality objectives, promotes the monitoring of the current environmental status and manages potential actions and measures (OSPAR 1992; Dekiff et al. 2014). An equivalent for the Baltic Sea is the Helsinki Convention with the Helsinki Commission (HELCOM) as governing body (UNEP 2009). However, the beach monitoring recommended by both conventions focuses mainly on macroplastic so far.

The problem of microplastic litter in the marine environment is gaining more importance in current political discussions. Thus “microplastic” is specifically mentioned in the “European Marine Strategy Framework Directive” (MSFD). The MSFD aims to reach a good environmental status of the marine environment until 2020 (Zarfl et al. 2011). In addition to topics like “Invasive Species” and “Biodiversity” also “Anthropogenic Pollution” is of concern. “Marine Litter” – committed as descriptor 10 from the 11 descriptors – is defined as “any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment.” (Galgani et al. 2010).

In this context the EU has decided to reduce the use of lightweight plastic bags by banning or raising higher taxes on them (EUCommision 2013). Such a ban of plastic bags already exists in most cities in California, USA (Plasticbaglaws 2014). In New York the Attorney General Schneiderman even pleads to ban cosmetics that contain microplastics (Office 2014).

“These legislative efforts reflect societal awareness of open ocean and coastal pollution.” (Baztan et al. 2014).

1.2 Plastic - some general information

“Plastics are synthetic organic polymers, which are derived from the polymerization of monomers extracted from oil or gas.” (Cole et al. 2011)

Depending on the monomers used each polymer has its specific chemical structure, weight, size and properties (PlasticEurope 2014). Roughly 1.6 million liters of crude oil are necessary to produce 100 million plastic bags (Royte 2007). About 100 billion of plastic bags were placed on the EU market in 2010 (EUCommision 2013).

In 2012 the global production rose to 288 million tons of plastics of which the European part of the production represented 57 million tons (PlasticsEurope 2013). The demands of Polypropylene (PP), Polyethylene (PE) and Polyvinylchloride (PVC) are particularly high in Europe (PlasticsEurope 2013).

The plastic polymer types vary considerably in density, which ranges from 0.85 g cm^{-3} (PP) to 1.41 g cm^{-3} (PET) or 1.56 g cm^{-3} (PVC) (Morét-Ferguson et al. 2010; Claessens et al. 2013). While these values refer mostly to the raw material, there are other factors that influence the specific density of the particles. For instance fouling or degradation can alter the density as well as additives added during the production process (Morét-Ferguson et al. 2010). These additives ensure the final plastic product various properties like color, shape,

durability and serve e.g. as plasticizers, antioxidants or flame retardants (Hammer et al. 2012; Fries et al. 2013).

Next to density the shape and size of plastic particles are crucial points of characterization of microplastic particles. The size definition referring to the term ‘microplastic’ varies between studies and an upper limit in the size range of microplastics of 1-20 mm can be found in the literature (Hidalgo-Ruz et al. 2012). Despite this inconsistency there is a recommendation worked out on a workshop held by the NOAA 2008 to define particles < 5 mm as microplastic (Arthur et al. 2009). Whereas in some publications there is a further subdivision into small microplastic particles (S-MPP) < 1mm and large microplastic particles (L-MPP) 1-5 mm (Imhof et al. 2012; Vianello et al. 2013), the division into 500 µm - 5 mm and another fraction <500 µm is more common due to the mesh size of sieves frequently used (Hidalgo-Ruz et al. 2012).

Mainly two groups of microplastic are distinguished. The first group, the so called “primary microplastic” (Cole et al. 2011), contains small plastic particles that were produced either as raw material for the production of other plastic products, termed virgin/resin pellets or as exfoliants in peeling and for other purposes in other cosmetic products e.g. toothpaste or makeup (Zitko & Hanlon 1991; Fendall & Sewell 2009) or as abrasive for industries e.g. in air blasting (Gregory 1996; Andrady 2011). The other group includes all kinds of fragments of meso- and macroplastic, mostly originating from the degradation of post-consumer products. Degradation takes place mainly due to photo-oxidative processes by UV radiation or mechanical abrasion by wave action as well as thermo-oxidative and biological processes (Hadad et al. 2005; Andrady 2011; Cole et al. 2011; Imhof et al. 2012) and results as a consequence in a decreasing size but an increasing number of smaller plastic fragments. Although all these degradation processes exist they are orders of magnitude slower in some parts of the marine environment than in others. Whilst the degradation by UV radiation plays an important role in beached and floating plastic particles it is less effective for particles lying at the sublittoral or being buried by sediment (Andrady 2011). Also the relatively cool temperatures in sea water decelerate chemical degradation. As chemical reactions are dependent on temperature, a decrease in temperature would slow down degradation processes according to the Q_{10} temperature coefficient. So in general, special circumstances in the oceans like less heat and UV radiation, higher pressure or haline conditions hinder the dissolution in the marine environment (Barnes et al. 2009; Andrady 2011).

Based on their desirable properties like durability, corrosion-resistance as well as lightweight and certainly their relatively inexpensive production, plastics are used for a lot of industrial and commercial applications. However, these are the same reasons why plastics are so

abundant and cause so many problems when entering the marine environment (Zurcher 2009; Cole et al. 2011).

1.2.1 Sources and transfer routes

The origin of microplastics introduced into the marine environment can be ascribed to two superordinated sources – land-based or sea-based.

Plastic waste from land reaches the marine environment mostly via rivers originating from landfills, close to shore industries and concerning mainly microplastic, sewage treatment plants (Fendall & Sewell 2009; Browne et al. 2011). But also waste left behind by beachgoers represents a notable problem.

While these land-based sources contribute a great amount of debris in urban regions sea-based sources are more important in remote areas. Plastic litter reaching the marine environment directly on sea is mostly derived from cargo ships, ferries, cruiseliners or fishing boats (Hammer et al. 2012). Waste goes overboard accidentally, e.g. by loss of containers, or deliberately, despite the MARPOL Convention which prohibits such illegal dumping.

Moreover all these plastics entering the marine environment are prone to break down into microplastics due to the degradation processes mentioned above.

Once plastic debris enters the marine environment it will, according to its specific density, either settle to the sea floor or float and be transported by currents and winds to the gyres or washed ashore (Ivar do Sul & Costa 2014).

Especially the buoyant plastic particles represent a solid substrate in the partially vast ocean. Floating plastic debris will be covered by macromolecules and microorganisms very rapidly as any form of solid substrate in the marine environment because of its scarcity (Ye & Andrady 1991; Lobelle & Cunliffe 2011). The developing biofilm will be taken over by a known succession of organisms that will grow on the particles, i.e., first algae like diatoms and filamentous seaweeds, followed by invertebrates like hydroids, barnacles, encrusting bryozoans as well as tunicates, molluscs and tubeworms. The composition of this fouling community can change depending on the water conditions and season (Ye & Andrady 1991; Zurcher 2009; Andrady 2011).

Due to this biofouling the plastic particles gain weight and will eventually sink to the sea floor or stay in different layers of the water column along the pycnocline. These particles can be defouled again through detachment and grazing and can therefore rise again to the surface

where the succession could restart anew (Zurcher 2009). However, this has not been investigated in detail so far.

1.2.2 Impacts

Given the widespread distribution of microplastics in the marine environment, many different habitats and organisms can be affected by these particles. Due to the already mentioned transfer routes the plastic particles can act as vectors for alien species and diseases and may facilitate their dispersal (Barnes 2002; Barnes & Fraser 2003; Barnes & Milner 2005; Goldstein et al. 2012; Zettler et al. 2013).

Furthermore plastic in the environment can also cause adverse effects on habitats. When small plastic debris is mixed into beach sediments the physical properties of the beach can be altered, resulting e.g. in an increase in permeability or a decelerated heat transfer (Carson et al. 2011). Additionally plastic on the sea floor could prevent gas-exchange between the pore water and the sea water (Goldberg 1997).

These effects are rather scarcely investigated whereas mechanical impacts on organisms are more prominent. Indeed, for macroplastics the most obvious threats are mechanical ones like the entanglement of marine mammals or fishes or the ingestion by sea turtles or marine birds (Laist 1987; Bugoni et al. 2001; Derraik 2002; Hammer et al. 2012; Codina-García et al. 2013; Lavers et al. 2014). But it is also well known that microplastics, due to their small size, are (bio)available and ingested by a variety of organisms and are then transferred along the food web (Wright et al. 2013; Setälä et al. 2014).

Generally plastics are considered as being biochemically inert, so causing no effect when introduced into a biological tissue, but actually synthetic polymers are always carrying additives like plasticizers or flame retardants which in turn do interact with organisms (Summer 2007; Teuten et al. 2009; Hammer et al. 2012). The toxicity lies mostly in added chemicals like the widely used phthalates and bisphenol A (BPA) which are known to be endocrine disruptive substances (vom Saal et al. 2008; Oehlmann et al. 2009; Hammer et al. 2012). The type and amount of these additives depends on the type of plastic. For example PVC characteristically contains phthalates as plasticizers (Hammer et al. 2012; Fries et al. 2013). The problem is that these plasticizers are not permanently bound to the plastic and hence can leach out into the aquatic environment. This can be either the surrounding sea water or the hemolymph of an organism after uptake, thus, the toxic substances may bioaccumulate in the tissues of these organism and can be transferred up the food chain (Hammer et al. 2012).

Aside from these deliberately added chemicals there are also persistent organic pollutants (POPs), hydrophobic chemicals sorbed from the surrounding sea water (Teuten et al. 2009; Hammer et al. 2012). Owing to their relatively large surface area to volume ratio which becomes even larger due to weathering and their hydrophobic character microplastics can easily absorb and accumulate all kinds of hydrophobic contaminants like dichloro-diphenyl-trichlorethane (DDT) or polychlorinated biphenyls (PCBs). These micro-pollutants can be sorbed to the surface of plastic pellets in a 10^6 times higher concentration than present in the surrounding seawater. Marine litter resin pellets are therefore used by the International Pellet Watch for monitoring the concentration of contaminants in the environment (Ogata et al. 2009).

There is a growing awareness of this problem but it is still indeterminated how great either the physical or the chemical impact for an organism after the ingestion of microplastics is. The crucial point is that ingested microplastics potentially do cause harm to organisms and may introduce toxins at the base of the food chain which could result in bioaccumulation (Teuten et al. 2009; Cole et al. 2011). Therefore it is essential to know to which kind and concentration of microplastics the organisms are exposed to.

1.2.3 Current state-of-the-art

As previously mentioned the occurrence of microplastics in the marine environment has been already observed in the early 1970s (Carpenter et al. 1972; Carpenter & Smith 1972; Cole et al. 2011). Nevertheless, there are still few comparable data about concentrations and types of microplastic particles in the marine environment because of a plethora of methods, different size definitions for microplastic (< 1 mm vs. < 5 mm) and varying units of reference parameters used. Consequently a standardized procedure for assessing microplastic abundances in the marine environment is urgently needed (Hidalgo-Ruz et al. 2012). There are numerous studies concerning microplastic in beach sediments (Gregory 1977; 1983; McDermid & McMullen 2004; Thompson et al. 2004; Ng & Obbard 2006; Reddy et al. 2006; Ivar do Sul et al. 2009; Zurcher 2009; Costa et al. 2010; Claessens et al. 2011; Martins & Sobral 2011; Turner & Holmes 2011; Liebezeit & Dubaish 2012; Hidalgo-Ruz & Thiel 2013; Van Cauwenberghe et al. 2013a; Baztan et al. 2014; Dekiff et al. 2014) and sublittoral sediments (Van Cauwenberghe et al. 2013b; Vianello et al. 2013).

But these studies are only partially comparable because of the variety of methods used, starting with the specific zone sampled on the beach. While some studies sampled the whole beach (Ng & Obbard 2006) the majority focused on the high tide line (Baztan et al. 2014; Dekiff et al. 2014). Another disparity lies in the sample volume used, which ranges from

250 g wet sediment (Vianello et al. 2013) to 1 kg dry sediment (Reddy et al. 2006). When processing the samples, most studies used density separation but with differently dense solutions. While mostly saturated sodium chloride solutions (NaCl) with a density of roughly 1.20 g cm^{-3} were used (Thompson et al. 2004; Reddy et al. 2006; Vianello et al. 2013), a few studies applied more dense solutions like sodium polytungstate (SPT) with a density of 1.2-1.4 g cm^{-3} (Corcoran et al. 2009), sodium iodide (NaI) with a density of 1.6-1.8 g cm^{-3} (Claessens et al. 2013; Nuelle et al. 2014) or zinc chloride ($ZnCl_2$) with a density of approximately 1.7 g cm^{-3} (Imhof et al. 2012; Liebezeit & Dubaish 2012). According to the solution used different proportions of the plastic polymers in a sample can be recovered.

Usually after density separation the resulting supernatant is filtered and the last step is the identification of plastic particles which can be performed on different levels of quality. One option is the visual sorting under a dissecting microscope (Liebezeit & Dubaish 2012), other solutions are spectroscopic approaches with Fourier-transform infrared or Raman spectroscopy (Thompson et al. 2004; Claessens et al. 2011). Finally also the units of reference parameters used for the assessment of marine sediments vary and are given in number or weight of particles per m^2, m^3 or kg (dry) sediment (Hidalgo-Ruz et al. 2012).

All these dissimilarities in the methodology make it very difficult to estimate the real amount of microplastics in the marine environment and hinder a representative analysis of long-term trends. Thus the development of a standardized methodology has a high priority.

1.3 Aim

Due to the above mentioned reasons the main focus of this study was on the applicability of a replicable and effective method to access microplastic abundance and composition in environmental samples. Hence, a reliable method was developed and exemplarily applied to samples obtained from beaches and sublittoral stations to evaluate the abundance of microplastic particles in sediments of the German Coast. In general there is a scarcity of data especially for the Baltic Sea. However, such data are necessary to establish a guideline for the “good environmental status” (GES) required for the Marine Strategy Framework Directive (Zarfl et al. 2011). Due to their potential to enter the marine food web it is recommended by various working groups that the abundance, distribution and composition of microplastics in the marine environment should be recorded and monitored in order to address the MSFD (Galgani et al. 2010; Cole et al. 2011; Krause et al. 2011; Zarfl et al. 2011; Galgani et al. 2013; Nuelle et al. 2014; Setälä et al. 2014). However, the development of a replicable method in order to get insight into the current state is a prerequisite to perform monitoring.

A great proportion of the microplastics will either sink to the seabed or be washed ashore, thus, marine sediments represent an appropriate target medium because this compartment acts as a ‘memory’ by accumulating a broad range of microplastics from terrestrial and marine sources, as well as high density and low density polymers. Therefore next to beach sediments also sublittoral samples were analyzed. It is worth noting that there were more surveys concerning microplastic on beaches than on the seafloor. This is not due to a lack of relevance rather because of logistic problems, since the sublittoral is more difficult and expensive to access.

This investigation presents no monitoring program but a pilot study using a combination of two efficient, replicable and representative methods to access the amount of microplastics in the North and Baltic Sea. The extraction of plastic from the sediment was conducted by the density separation approach with the recently invented Munich Plastic Sediment Separator (MPSS) (Imhof et al. 2012) followed by the spectroscopic identification of microplastics using micro-Fourier-transform infrared (μFT-IR) analysis (Harrison et al. 2012).

For the first time this new extraction method was used for marine sediments and purification of the samples through enzymatic digestion was performed. The data gained thereby can present a baseline for future monitoring methods.

Besides the applicability of these methods, this thesis should address the question whether there is considerable microplastic pollution on beaches and sublittorals of the North and Baltic Sea in or close to protected areas and whether there is a difference in the magnitude of microplastic pollution between beach and sublittoral samples.

2 Material and methods

2.1 Sampling

Sediment samples were collected from the sublittoral as well as from beaches in the German North and Baltic Sea. The beach sample locations of this study were chosen close to natural reserves. Thereby high touristic influence and interference through beach cleanups could be excluded. The sublittoral sample stations in turn were chosen in the vicinity of the beaches sampled. Two of these beaches were situated on the Baltic Coast and another two on the North Sea Coast. All of them were part of or close to nature reserves.

2.1.1 Beach sediments

The first station was in the eastern part of the island Zingst with the coordinates 54°26'43''N and 12°55'2''E. The beach is part of the West Pomeranian Lagoon Area National Park and is exposed to wave action. The sampling took place on the 12th of October 2013 around noon while a relatively strong wind was blowing in a 70 degree north-east direction with a speed of 6 Beaufort. Samples were always taken against the wind direction to avoid contamination with plastic particles and fibers from clothes. Nine Samples were taken based on a general sampling scheme (Fig. 1) in an area of 2 m² with a stainless steel spoon out of a metal tube with 12.5 cm diameter which was put into the sediment to a depth of approximately 4 cm. The resulting sediment volume was around 0.5 l and was filled into Kautex-bottles.

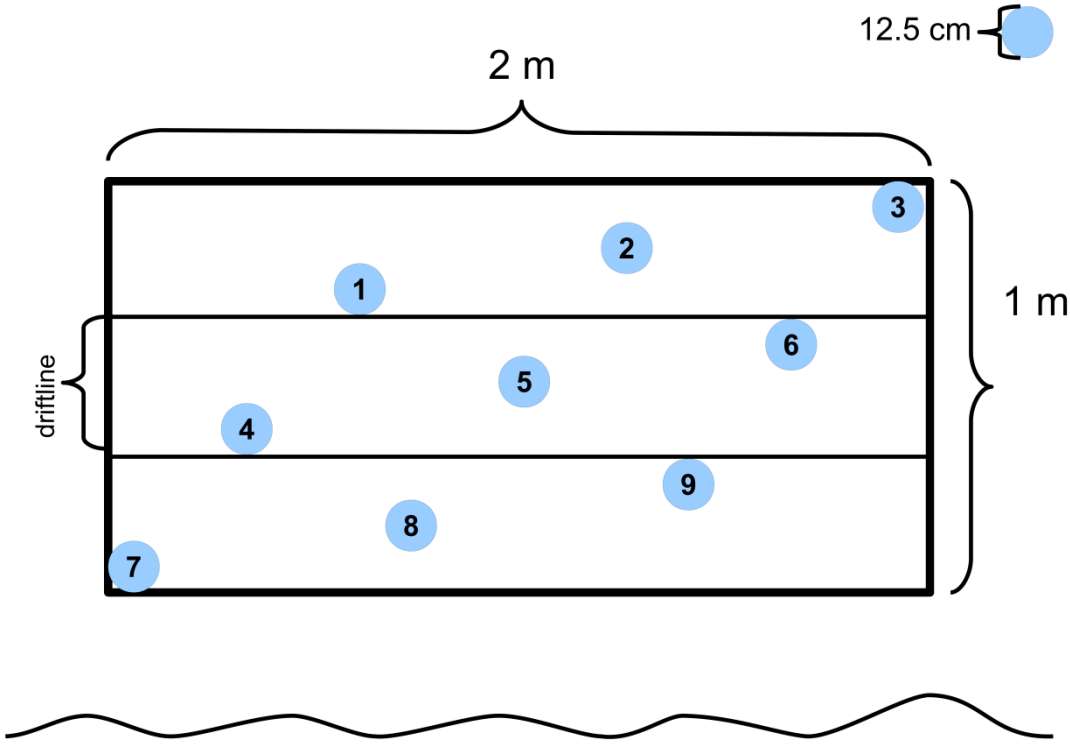

Figure 1: General sample design for beach surveys; the consecutively numbered blue circles represent the samples taken with a metal tube and a spoon each of 12.5 cm diameter and 4 cm depth

The second sampling station was close to the island Langenwerder. It is a nature reserve for seafowl and characterized as area of wind caused tidal flats, so called “Windwatt”. The station was located on the exposed site of a sandbank at 54°1’32’’N and 11°29’6’’E. While taking samples on the 15th of October 2013 around noon it was drizzling and a moderate wind was blowing from south-west. Therefore the sandbank was pretty big because water was pressed out of the cove. This made the selection of the sampling site difficult because the normal outline of the sandbank was not clearly visible. Three samples were taken out of a 1 m² area that seemed to be normally submerged. The remaining six samples were collected further westwards out of a 2 m² area where the mark of a drift line was more present then at the other site. This resulted in a divergent sampling scheme (Fig. 2).

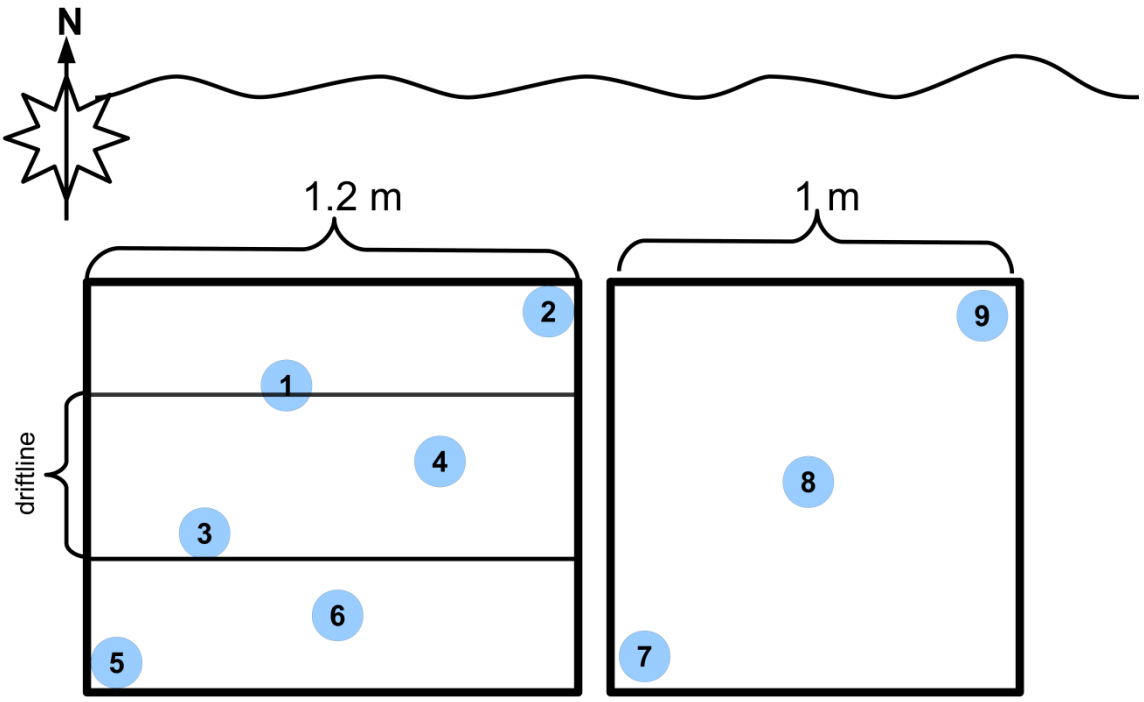

Figure 2: Differing sampling scheme for Langenwerder with one sampling area of 1.2 m² and another of 1m²; the consecutively numbered blue circles represent the samples taken with a metal tube and a spoon each of 12.5 cm diameter and 4 cm depth

The sampling sites for the North Sea coast were both on the island Sylt, at List which is in the north-west of the island, within the Schleswig-Holstein Wadden Sea National Park. One location was on the Western Beach which is exposed to heavy wave action. The other one is situated on the eastern side close to the harbor. Sampling was performed on the 12th of February 2014 in the early afternoon. During this time the average wind speed was 6 Beaufort out of 210-220° south-south-west. The sampling procedure was according to the general scheme described for Zingst and shown in Figure 1.

It is noteworthy that all the beaches contained a lot of scattered particles of macrodebris although being located in natural reserves. Especially on Sylt at the harbor sampling station a big amount auf lines and nets derived from fishing could be observed while on the western beach more packaging material and fragments were present.

2.1.2 Sublittoral sediments

Bottom samples were taken with a Van Veen Grab Sampler on 38 different sites during a microplastic sampling cruise of the research vessel “Heincke” between September and October 2013. Out of each grab approximately 1 l of sediment was transferred with a stainless steel spoon into Kautex Bottles by Dr. Martin Löder. The samples were frozen until further processing. Of the sublittoral samples five North and five Baltic Sea samples were

chosen for microplastic analyses. The locations of these stations are shown in Figure 3 next to the four different sampling stations for the beach sediment samples.

Figure 3: Sampling sites of the North Sea (a) and the Baltic Sea (b)

The beach samples were pooled according to their relative position to the driftline. For Zingst and both stations on Sylt samples 1-3, 4-6 and 7-9 and for Langenwerder 1-2, 3-4 and 5-6 were pooled resulting in three parallel samples for each beach. These twelve beach samples and the ten sublittoral samples were processed in the laboratory of the AWI in the "Biologische Anstalt Helgoland".

2.2 Sediment Characterization

The first step of the experiments in the laboratory was a determination of the water content of the sediments. This was necessary to relate the findings to the desired unit, i.e., number of particles per kg dry weight of sediment.

The first approach was drying the whole sediment in glass jars to enable the reference to the number of plastic particles found per kg dry sediment and to avoid a decrease of density of the $ZnCl_2$ solution in the separator by adding sediment plus the remaining water of the sediment. This procedure was adapted to one sample on trial. Therefore the sediment from the Kautex-bottles labeled LW 1 and LW 2 was carefully transferred into a pre-weighted glass jar. The filled glass jar was weighed again to determine the wet weight of the sediment. Afterwards it was put into an oven at 60 °C to prevent plastics from melting. The sediment was weighed every 12 hours for six consecutive days (Fig. 4).

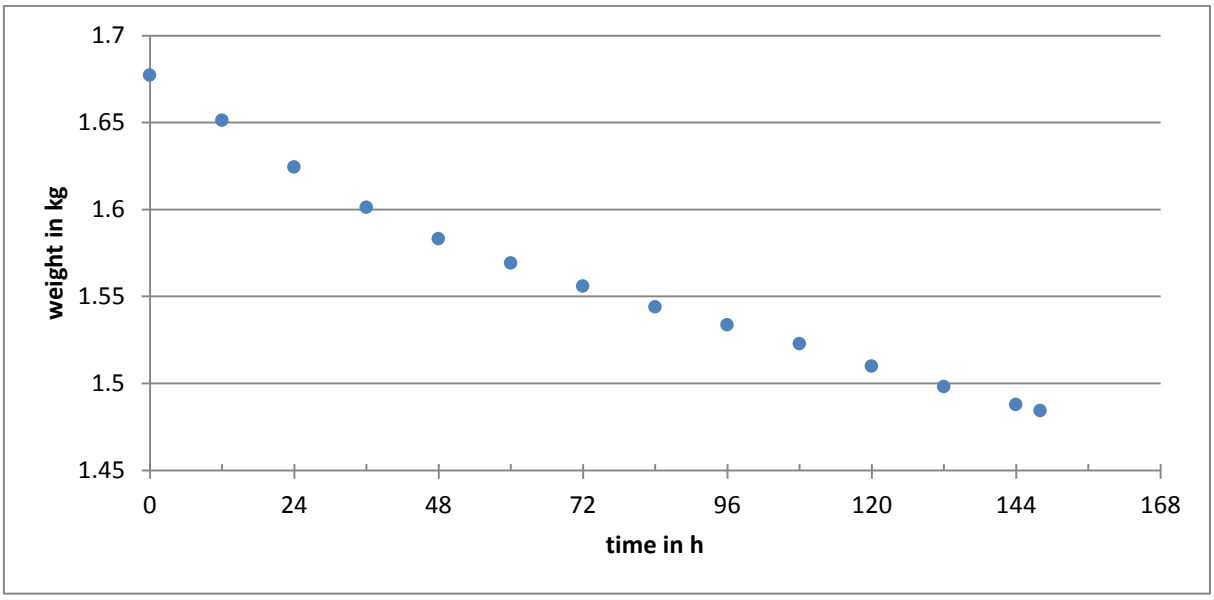

Figure 4: Drying curve of a 1.68 kg sediment sample at 60 °C

A second approach was chosen because it took more than one week to completely dry the sediment until constant weight was reached. Therefore the sediment was also filled in a pre-weighed glass jar and weighed to get the wet weight. Beforehand, five subsamples, each consisting of one teaspoon of sediment were transferred into pre-weighed glass Petri-dishes. These were then incubated at 60 °C for 48 h. The water content was calculated using the following formula:

$$\text{mass fraction} = (\text{wet weight} - \text{dry weight}) / \text{wet weight} \qquad (1)$$

Additionally basic characteristics of the sediment like coarseness, color and organic matter were recorded.

2.3 Separation

The method followed a density separation approach similar to the one described in Imhof et al. (2012) with the “Munich Plastic Sediment Separator (MPSS)” which served as model and was built by “Hydro-Bios”, Kiel (Fig. 5a).

Figure 5: Munich Plastic Sediment Separator (MPSS) built by Hydro-Bios setup (a): motor (1), sediment container (2), stand pipe (3), small cone and dividing chamber (4), controller (5); ideal filling level in the upper sight glass (b); dividing chamber in the mounting with the ball valve closed (c)

Separation with the MPSS had shown to be very efficient. Recovery rates of 95.5% even for small microplastic particles < 1mm were detected by Imhof et al. (2012). Therefore, further recovery experiments were omitted.

The first step was the preparation of approximately 30 l zinc chloride solution with a density of 1.8 g cm^{-3}. The solubility for $ZnCl_2$ is described as 4300 g l^{-1} at 20 °C (GESTIS-Stoffdatenbank 2014) and according to Imhof et al. (2012) the $ZnCl_2$ stock solution should have a concentration of 64.3 % of $ZnCl_2$. To prepare the solution three 15-l containers were stocked with 5500 g water each and put into a box with cool water. Technical zinc chloride (Carl Roth GmbH, Karlsruhe, Germany) was added in four units of 2.5 kg (which were weighed into freezer bags beforehand to make handling easier). These 2.5 kg $ZnCl_2$ were added in 3 steps into each container while stirring and measuring the temperature increase of the exothermic reaction. Ice was added to the surrounding water in the box to have a greater cooling effect so that the temperature inside the container was never above 40 °C. After the addition of each package the solution was stirred again and again and left undisturbed until all salt was dissolved.

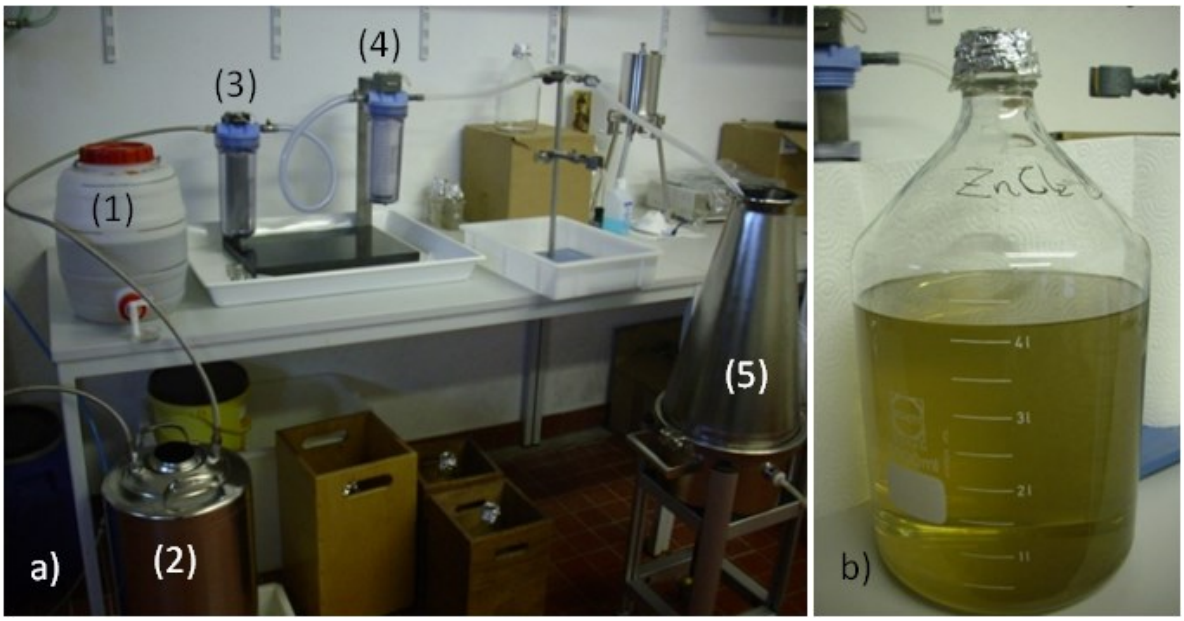

Figure 6: Pressure filtration of the prepared $ZnCl_2$ solution (a): container with $ZnCl_2$ (1), pressure vessel (2), first candle filter with 10 µm stainless steel filter (3), second candle filter with 1 µm pleated cartridge filter (4), MPSS (5); filtered $ZnCl_2$ solution (b)

Then 5 ml were transferred with a 10 ml glass pipette into a pre-weight Falcon-tube. This tube was weighed again to calculate the density of the solution (Supplementary material, Tab. S1). Final densities of all five containers with $ZnCl_2$ solution can be found in Table 1.

Table 1: Final densities in kg m^{-3} of the $ZnCl_2$ solution in the five containers

container No.	solution concentration	solution temperature °C	solution density g cm^{-3}
1	0.645	12	1.765
2	0.645	12	1.796
3	0.645	12	1.802
4	0.714	13	1.856
5	0.669	12	1.848

Before usage the prepared solutions were filtered via pressure filtration (Fig. 6a). First the solution passed over a 10 µm stainless steel filter cartridge and subsequently over a candle filter with pleated 1 µm WFPPA-filter cartridges (Wolftechnik Filtersysteme GmbH, Weil der Stadt, Germany). A pressure of 1-2 bar was sufficient to filter 25 l solution in around 10-15 minutes. After about ten runs of 30 l solution filtered each time the 1 µm filter was exchanged because filtration took significantly longer. The $ZnCl_2$ solution was still brownish yellow after the filtration but clear (Fig. 6b). Density of the solution was measured after the filtration and was still around 1.8 g cm^{-3}. Prior to the addition of sediment the first 25 l were filtered directly into the separator, its tightness was tested with water before each run. Then the small cone and the dividing chamber were mounted and the separator was filled through a funnel until the solution level became visible in the upper sight glass (Fig. 5b). The ball valve was opened and closed a few times to remove air bubbles and was then left open. The rotor of the separator was turned on at full speed for approximately 10 minutes to remove air pockets under the rotor and to disengage particles stuck to the walls of the separator. Afterwards the motor was turned off and left off for an hour in order to enable the rise of potential contaminating particles in the solution. The valve was closed again and approximately four liter of the solution were drained off through the sliding tap to enable the later addition of the sediment. The dividing chamber was dismounted and the content was rinsed into a 100 ml Schott-bottle. These samples should serve as procedural blanks for the $ZnCl_2$ preparation process and for the separation.

The motor was turned on again at low speed of 4-8 rpm. Then the sediment from the glass jar was carefully added with a tablespoon through the sediment inlet flange. The jar and the spoon were rinsed with $ZnCl_2$ into the separator as well. The stirring continued for an hour to mix the sediment and then all bigger particles floating on the surface were transferred with tweezers into a labeled Schott-bottle. Afterwards the small cone and the dividing chamber were mounted again and the separator was refilled with $ZnCl_2$ to the upper sight glass. The ball valve was closed and opened several times to remove air bubbles inside the valve and was left covered overnight. On the next day the ball valve was closed and the sliding tap was

opened to drain off the $ZnCl_2$. The chamber was demounted and fit into the mounting (Fig. 6c). A rinsed and labeled 250 ml Schott-bottle was placed under the funnel-cap, the plug removed and the valve opened to recover the sample. The chamber was rinsed carefully with deionized water and the 250 ml Schott-bottle was stored for further analysis. The drained $ZnCl_2$ solution was later recycled via pressure filtration and the density of the solution was measured after every filtration and was adjusted when decreasing below 1.70 g cm^{-3}.

2.4 Sample Purification

2.4.1 Filtration

The samples were processed in two entities consisting of 10 to 12 samples respectively. The first ten samples were filtered using a bottle top filter (Nalgene™ Thermo Fisher Scientific Inc., Waltham, USA) and a stainless steel mesh disc of 10 µm mesh size to remove the $ZnCl_2$ solution to avoid precipitation of any zinc salts in the further process. Afterwards the remaining particles on the filter were rinsed through a funnel into a new 250 ml Schott-bottle. The filters were checked for remaining particles under a dissecting microscope. When particles were found to remain on the filter, the filter was stored in a 100 ml Schott-bottle containing approximately 40 ml of 5% sodium dodecyl sulfate solution (SDS) (Sigma Chemie, Deisenhofen, Germany) and put for 3 minutes in an ultrasonic bath. After ultrasonication the filter was rinsed with ultrapure water until it was clean and the content of the bottle was added to the sample in the further process. The other twelve samples were filtered directly prior to the purification using bottle top filters and a 10 µm stainless steel mesh as well. Unlike the first entity of samples these were first poured through a tube containing a 500 µm sieve in order to remove the fraction >500 µm. This fraction was rinsed back into the sample bottle and could be investigated for larger microplastic particles under the microscope. The smaller fraction was caught in the bottle top on the 10 µm filter. The tube as well as the bottle top was also rinsed with Emprove® Ethanol (Merck HDGA, Darmstadt, Germany) that was previously diluted to a concentration of 24% by adding deionized water and then filtered over a 0.2 µm Cellulose Nitrate filter (Satorius Stedim, Göttingen, Germany) to ensure particle freeness. The intention of this rinsing step was to ensure that particles still stuck to the walls of the bottle top filters were rinsed into the bottle. Ethanol proved to be more efficient for this rinsing step because of its different surface tension compared to water.

Because the stainless steel filters started leaking during the further process they were exchanged with polycarbonate filters of the type TCTP (Merck Millipore, Darmstadt, Germany) with 10 µm pore size.

Prior to the purification another preparation step was the filtration of the detergents that were used. The technical enzymes were obtained from ASA Spezialenzyme GmbH, Wolfenbüttel, Germany and delivered in plastic containers. To remove potential contaminations with plastic particles as well as residues of the organisms which the enzymes were derived of, all the enzymes were filtered: first two times over GF/C glass fiber filters (Whatman GmbH, Dassel, Germany) to remove impurities and afterwards over Cellulose Nitrate filter of 0.2 µm pore size. A pore size of 0.45 µm was used for the filtration of Protease because of its high viscosity as well as for the SDS.

2.4.2 Enzymatic Digestion

The enzymatic digestion was performed to remove organic compounds from the sample. It followed a scheme established by Antonia Pott and Svenja Mintenig for their master's theses (Mintenig 2014). The protocol was adjusted to better fit to the needs of samples derived from sediments and can be seen in Figure 7. Instead of laundry detergents (Biozym F and SE) technical enzymes were used to achieve a better purification.

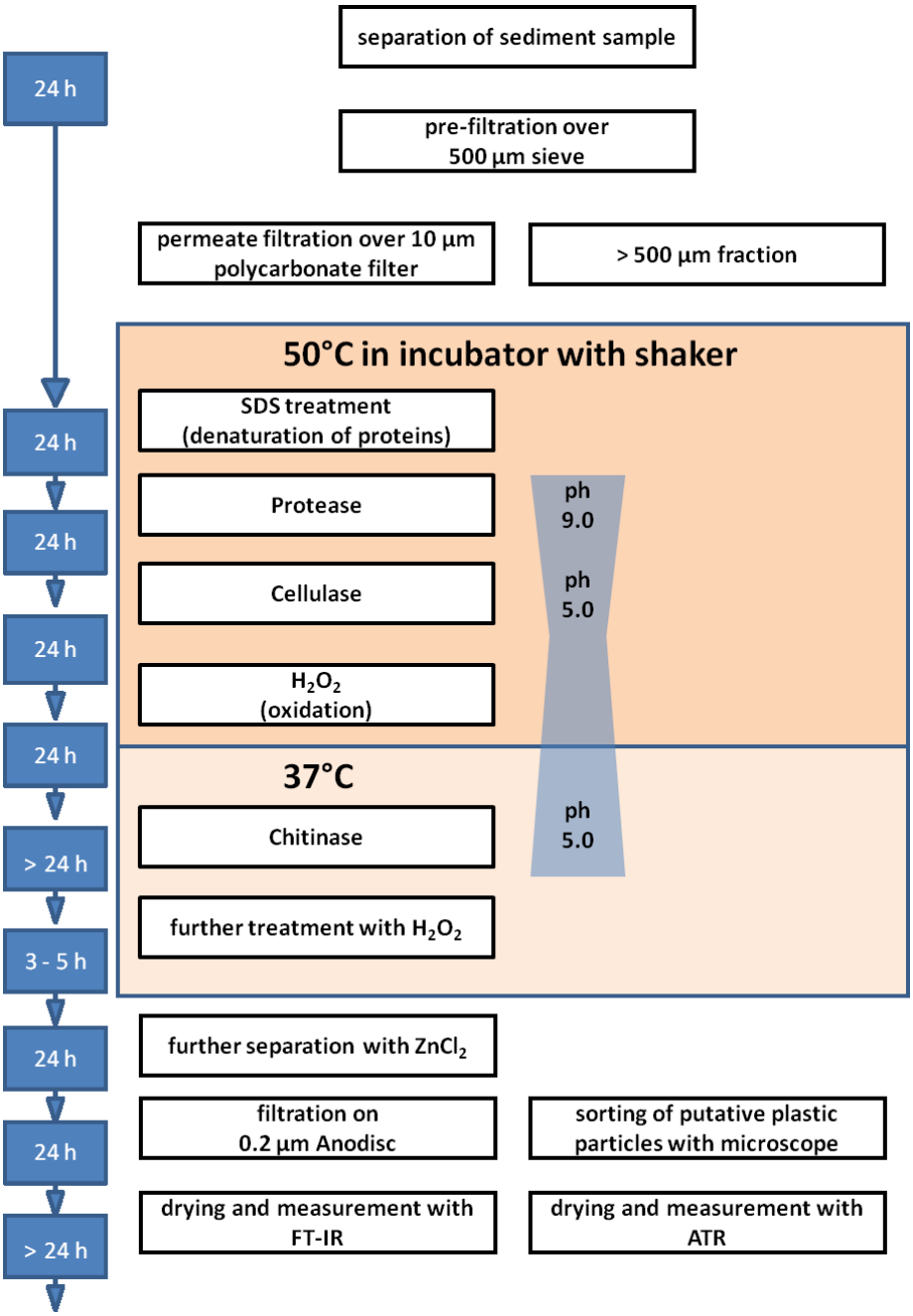

Figure 7: Sample purification scheme of enzymatic digestion

All the treatments were performed in the bottle tops with the polycarbonate filters. The bottle top was plugged and a certain amount of reagent was added onto the filter so that the sample was fully covered. After a certain application time subsamples of several samples were transferred into a glass Petri-dish using a glass pipette. The digestion progress was documented under the dissecting microscope. After each step the reagent was removed and the bottle top was rinsed carefully with ultrapure water. The incubation took place in a Multitron Incubator with a shaker (Infors HT, Bottmingen, Switzerland) at 50 °C or 37 °C. The first compound used was SDS, an anionic surfactant to denaturize proteins that makes the cell structure accessible for the subsequently applied enzymes.

Approximately 30 ml of a SDS solution (4-5 w/w%) was added onto each filter and left for 24 h in the incubator at 50 °C while shaken with 40 rounds per minute.

For the following enzymatic steps a 1% Phosphate-buffered saline (PBS) with an adjusted pH of either 5.0 or 9.0, according to the pH optimum of the particular enzyme, was used to create ideal conditions for the enzymes to be most efficient. At first 25 ml PBS with a pH of 9.0 and 5 ml Protease A-01 (EC 3.4.21.62), a serine endopeptidase, were added onto each filter and left for at least 24 hours at 50 °C in the incubator. For the digestion of cellulose 5 ml Cellulase TXL (EC 3.2.14) was applied with 25 ml of PBS with pH 5.0 and left for at least 24 hours at 50 °C in the incubator as well. Prior to the last enzymatic step a pretreatment with H_2O_2 was done. Although H_2O_2 is often applied and highly efficient to remove biogenic organic matter, it showed also to be destructive to synthetic polymers when applied for a long time (seven days) in a high concentration (Nuelle et al. 2014). Therefore the utilization of H_2O_2 was reduced to a minimum. During the first application it was used to dissolve residues left by Protease and Cellulase and to remove the organic film on the carapaces to make chitin accessible for the Chitinase for the second time only briefly to remove biogenic organic matter mostly proteins released by eliminating chitin. Therefore 30 ml of H_2O_2 (Carl Roth GmbH, Karlsruhe, Germany) was added onto the filter and left for 24 h at either 37 °C or 50 °C. The last enzyme used was Chitinase (EC 3.2.1.14) (ASA Spezialenzyme GmbH, Wolfenbüttel, Germany. In this case 1 ml Chitinase and 15 ml PBS with a pH of 5.0 was added to the sample and the bottle top was incubated at 37 °C for approximately three days.

Subsequently H_2O_2 was added for a second time in order to oxidize the residues of the enzymatic digestion. Therefore about 15 ml 35 % H_2O_2 were added and left usually for 3-5 hours until no further reaction apparent from the gas development could be observed. After removing the H_2O_2 again by filtration the bottle top walls were rinsed carefully with ultrapure water and diluted ethanol to ensure that all particles were rinsed onto the filter. Then the bottle top was dismounted and the residues on the filter were rinsed with $ZnCl_2$ of 1.7 g cm^{-3} density into a separating funnel to separate residues of heavier particles. In case there were still particles stuck to the filter it was left in $ZnCl_2$ for a few hours and if necessary ultrasonication was applied for 3 minutes. The funnel was left mounted on a laboratory stand overnight allowing the rest of the more dense sediment and other particles to settle in the funnel while the less dense particles were buoyant on the surface. Afterwards the sediment and the overlying $ZnCl_2$ column were drained until just 6-10 ml of the liquid surface layer containing all the less dense particles was left in the funnel. Next to the purpose of removing remaining sediment grains, shells and diatoms from the sample its volume could be reduced drastically during this step in order to shorten the time needed for the subsequent filtration onto the filter for the analysis. Therefore the sample was filtered through an acrylic glass tube

onto a 25 mm aluminium oxide filter with 0.2 µm pore size (Anodisc, Whatman GmbH, Dassel, Germany) in order to concentrate the particles on a filter area of 9 mm diameter. The funnel as well as the tube was rinsed several times with ethanol (24%) and ultrapure water to ensure that no particles were lost. Finally the filter could be carefully removed from the filtration unit, transferred into a Petri-dish, dried overnight in an incubator at 40 °C and stored at room temperature until the subsequent spectroscopic analysis. To assess the background contamination a blank was run as well. Therefore 250 ml of ultra pure water were processed like the samples with the second entity of the environmental samples and documented as well.

2.5 FT-IR Analysis

2.5.1 Focal Plane Array-based µFT-IR mapping

For the quantification of microplastic particles present in the purified samples as well as the determination of the plastic polymer types of the particles Fourier Transform Infrared Spectroscopy (FT-IR) was applied. µFT-IR is a combination of the spatial specificity of microscopy and the chemical specificity of spectroscopy (Bhargava et al. 2003). This analyzing method is based on the fact that due to excitation by infrared light certain chemical bindings show bending and stretching motions. These resulting vibrations lead to a certain degree of absorbance of the infrared light which can be measured. The signals are captured by a detector and a subsequent Fourier transformation converts them into an absorbance spectrum plotted as a function of wavenumber. The location and the intensities of the absorbance peaks in the spectrum are a consequence of the presence and absence of certain functional groups. The range of infrared radiation particularly suited for polymer analyses is the information-rich and well-characterized mid-infrared region of the spectrum (Bhargava et al. 2003). Because every polymer has a specific combination of functional groups it shows characteristic absorption bands and can therefore be identified accordingly (Fig. 8).

Usually single detectors are used, but with such detectors only one signal derived from one point of the sample can be detected at once. To measure a sample stretching over a larger area a great amount of time is necessary when measuring with single detectors point by point. That is where the Focal Plane Array (FPA) detectors come into play. In a FPA detector thousands of single detectors are arranged in a grid. This allows for the simultaneous detection of a multitude of spectra within one measurement, thus the mapping of an area is possible in a fraction of time when compared to one single detector (Bhargava et al. 2000).

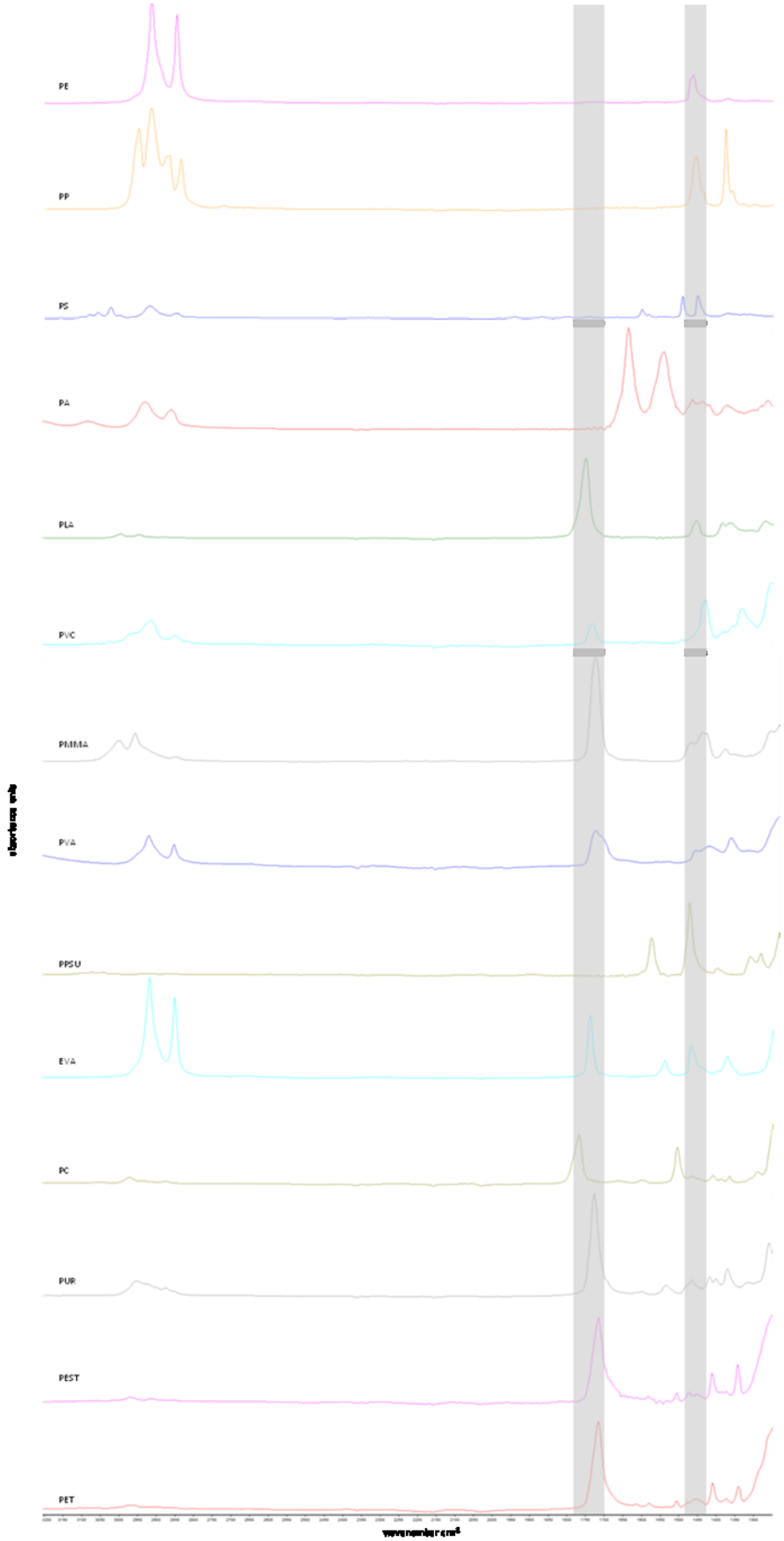

Figure 8: IR Spectra of some main plastic types with the two integrating areas from 1790-1700 cm^{-1} and 1480-1430 cm^{-1} highlighted

2.5.2 Analysis and evaluation

The measurement was done by using a Hyperion 3000 microscope (Bruker Optics, Ettlingen, Germany) (Fig. 9) with a Focal Plane Array (FPA) detector containing 64x64 detector elements. In order to enable the mapping of a filter area of 64 mm² (9 mm in diameter) various measurement settings had to be optimized which was elaborated in previous works (Kuczera 2013; Mintenig 2014).

Initially the filter was placed on a Calcium Fluoride (CaF_2) window between two FT-IR sample holders. The sample holder was mounted onto the microscope stage.

Before the mapping of the actual filter a measurement of the background spectrum was necessary. Normally this should be conducted on the same filter in a particle free area at the edge. Since this was not possible for most of the filters the background was measured on a separate new Anodisc filter. At first the whole filter was photographed through a visual objective with 4 times magnification and subsequently with an objective for the infrared radiation with a 15 times magnification, resulting in a final magnification of 40 times and 150 times, respectively. These pictures served later as reference for the chemical imaging. The measuring area was selected containing the area the sample had been concentrated on consisting of usually 63 x 63, thus 3969 fields.

In contrast to Harrison et al. (2012), who used the reflectance mode for µFT-IR mapping the measurement was conducted in transmission mode in an IR range of 3200 cm^{-1} to 1250 cm^{-1}. Harrison et al. (2012) reported the reflectance procedure to be of limited feasibility because of spectral distortion resulting from irregular shaped surfaces of environmental microplastic particles. The lower boundary of the wavenumber range was defined by the use of aluminium oxide filters that do not transmit radiation below 1300 cm^{-1}. The upper one could be set because plastic polymers show no characteristic peaks above it.

Resolution was set to 8 cm^{-1}, so that every 8^{th} wavenumber was measured, and the number of co-added scans reduced to 6 per field. This turned out to be sufficient in a previous study and reduced the time needed for the measurement drastically. With these settings applied the measurement took still 10-12 hours. During this time the detector had to be cooled constantly with liquid nitrogen (set value: 85.4 °K) to ensure the functioning of the detector and the production of high quality spectra.

Figure 9: Hyperion 3000 (Bruker Optics, Ettlingen, Germany) FT-IR microscope

The recording and evaluation of the data was performed using OPUS 7.2 Software (Bruker Optics, Ettlingen, Germany). To specify the results the resulting spectra were integrated over two wavenumber regions, which were used as proxys for plastic polymers. One is ranging from 1790-1700 cm^{-1} and is characteristic for the presence of carbonyl groups. The other is ranging from 1480-1430 cm^{-1}, which is characteristic for the bending of C-H bounds, where most plastic polymers show a peak. The second region is intrinsic for e.g. PE, which is a ubiquitous used (Harrison et al. 2012; PlasticsEurope 2013). These ranges were also used in previous studies at the BAH (Kuczera 2013; Mintenig 2014) and as can be seen in Figure 8 every common polymer has at least one prominent peak in one of the integrating ranges. The data obtained after the integration was visualized in a false color plot using a rainbow color scheme for each integrated wavenumber range separately. All parts of the measured area that were colored dark blue showed no prominent absorbance signal in the integrated wavenumber range while red or pink colored areas of the picture showed the prominent peak and were investigated more closely.

This close investigation had to be conducted manually. Every particle showing a noticeable signal in at least one of the two ranges could potentially be plastic and thus the particles were consecutively numbered and their characteristic spectra were extracted for a search in the spectra database. The spectra were compared to reference spectra to verify if they match to any plastic polymer spectrum, i.e., if they could not be assigned to a polymer type in an instant.

3 Results

3.1 Sediment Characterization

Compared to beach sediment samples, the sublittoral sediments revealed generally higher water contents and exhibited a finer grain size. Especially the sublittoral samples from the Baltic Sea appeared to have a greater mud proportion than the ones from the North Sea. Concerning the color, the sediments from the Baltic coast were more grayish and the ones from the North Sea more brownish. The determined water content as mass fraction and the resulting dry weight of the sediment used for the further analysis is presented in Table 2 next to a description of the sediment.

Table 2: Sediment characterization of all the beach and sublittoral sediments used, calculated water contents and resulting dry weight, North Sea samples shaded in gray

sample	"water content" [weight %]	sediment weight [kg dryweight]	sediment characterization
LW_12_1	16.70%	**1.3971**	sand with fragments of shells and few small pebbles (~ 2 mm)
LW_34_2	11.45%	**1.5222**	sand with fragments of shells and few small pebbles (~ 2 mm)
LW_56_3	11.93%	**1.5490**	sand with fragments of shells and few small pebbles (~ 2 mm), organic fluff
Z_123_4	7.40%	**1.4647**	drier and brighter sand then LW, organic content mostly seaweed
Z_456_5	5.68%	**1.6608**	drier and brighter sand then LW
Z_789_6	5.73%	**1.6480**	drier and brighter sand then LW
SH_123_12	3.04%	**2.6002**	brownish sand relatively coarse, homogen
SH_456_13	4.07%	**2.6973**	brownish sand relatively coarse, homogen, containing a few pieces of wood
SH_789_14	3.68%	**2.7572**	brownish sand relatively coarse, homogen, containing pieces of shell and wood
SW_123_15	3.66%	**2.2958**	brownish sand relatively coarse, homogen, brighter than SH
SW_456_16	3.25%	**2.3232**	brownish sand relatively coarse, homogen, brighter than SH
SW_789_17	4.05%	**2.3725**	brownish sand relatively coarse, homogen, brighter than SH
H15_18	28.10%	**1.1761**	brownish sediment, like small flakes, containing a lot of organisms
H16_19	20.84%	**1.4147**	light brownish sediment, relatively coarse containing lots of worm tubes
H17_20	16.73%	**1.5742**	brownish sediment, fine sand, similar sorted, a few organisms
H18_21	20.66%	**1.6319**	brownish very fine but sandy sediment containing polychaets and organic fluff
H19_22	19.42%	**1.6997**	brownish, fine sand, containing a few worms and their tubes
H29_7	59.08%	**0.5073**	black, fine sediment, anoxic, containing shells, 1 cm water layer on top
H30_8	70.80%	**0.3371**	black, very fine sediment, sticky, anoxic, 2 cm water layer over the sediment
H31_9	18.94%	**1.6023**	fine sediment mostly brownish but containing anoxic spots and shells
H32_10	23.49%	**1.4038**	relatively sandy sediment, brownish black muddy sediment, slightly anoxic
H33_11	33.76%	**1.1883**	relatively fine, olive tainted dark grey sediment, just slightly anoxic

3.2 Separation of microplastic from sediment

All the 22 samples selected were successfully separated using the MPSS. Figure 10 exemplarily shows the initial sediment sample of approximately 1 l volume of three samples processed stored in a glass jar and the result of the separation with all buoyant material visible in the upper sight glass of the dividing chamber. It can be seen that after the separation with the MPSS the sample volume was clearly reduced to a smaller amount of material with a lower density than 1.7 g cm^{-3}. Next to coarse and fine sediment also bivalves and shell fragments were left in the separator after draining the $ZnCl_2$. Nonetheless, there was also non-synthetic debris derived of organisms like worm tubes (Fig.10d + e) left in the

separated samples. Therefore an efficient enzymatic digestion of the samples was necessary.

Figure 10: Initial sediment samples in the upper row and below the resulting sample volume in the dividing chamber after the separation with the MPSS: sample H17_20 (a + d), H19_22 (b + e) and H30_8 (c + f)

3.3 Sample Purification via enzymatic digestion

Optical changes of the samples as a consequence of the enzymatic purification treatment could already be observed on the filters in the bottle tops. As it can be seen in Figure 11, the content on the filter visually changed during the enzymatic digestion because organic matter was macerated. This could also be perceived via the duration of the filtration steps. The first filtration of the sample onto the filter mostly took a few hours. With every treatment the filtration was done more quickly and the last filtration step was normally done in a few minutes, at least in less than one hour.

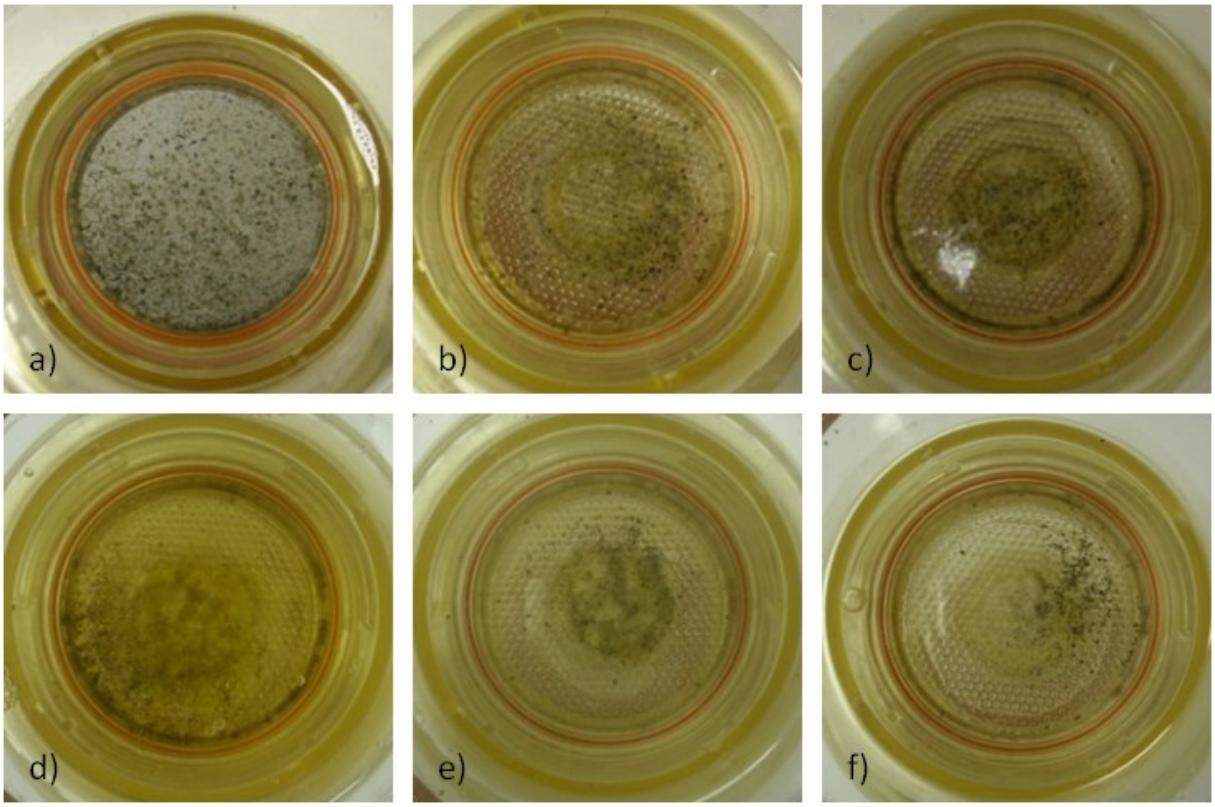

Figure 11: Enzymatic digestion progress of sample H30_8: before the treatment started (a), after 24 h SDS (b), 30 h Protease (c), 24 h Cellulase (d), 24 h H2O2 (e) and 48 h Chitinase (f)

The progress of the enzymatic digestion was documented in more detail using a dissecting microscope with a camera (Olympus SZX 16). The results are illustrated in Figure 12. In the initial situation, depicted in Figure 12a, organisms are sticking to each other, to particular organic matter and sediment, while after the 24 hours treatment with SDS (5 %) at 50 °C particles and organisms were found more solitary and discolored (Fig. 12b). As a result of the 30 hours Protease incubation particle aggregates were mostly dissolved and smaller (Fig. 12c). The subsequent treatment with Cellulase for 24 h showed that biogenic particles had become thinner and at least partially transparent (Fig. 12d). After a 24 hour treatment with H_2O_2 the sample showed clearly that organic matter had dissolved or at least was discolored and partly dissolved (Fig. 12e). Chitinase was applied for 48 hours and after this mostly minerals and resuspended sediment were left (Fig. 12f). These remaining materials were removed in the following separation.

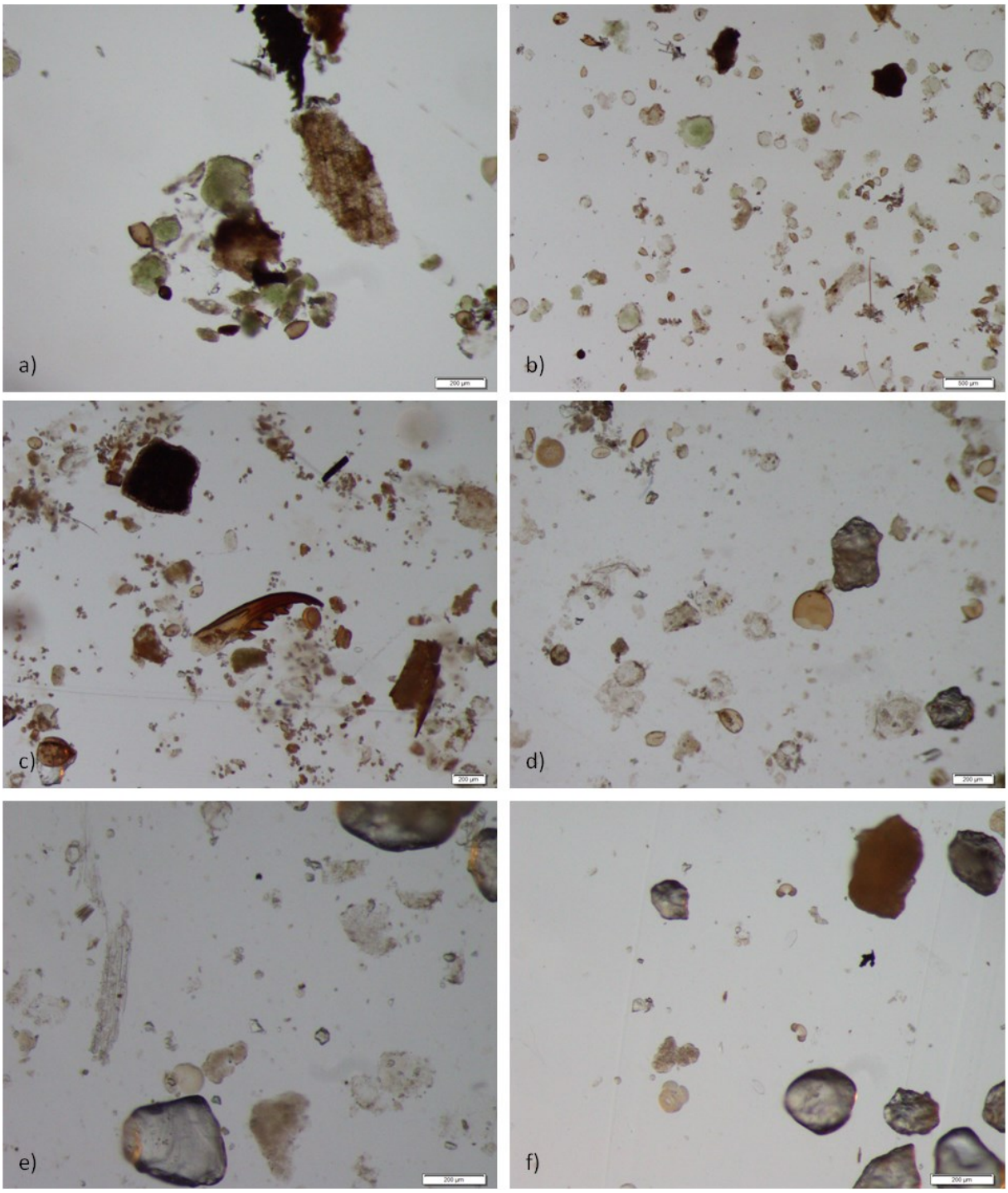

Figure 12: Enzymatic digestion progress of sample LW_12_1: before the treatment started (a), after 24 h SDS (b), 24 h Protease (c), 24 h Cellulase (d), 24 h H_2O_2 (e) and 48 h Chitinase (f), pictures were taken with a dissecting microscope in 2.5-8 times magnification

3.4 Detection of microplastic via µFT-IR spectroscopy

3.4.1 Filters for µFT-IR- mapping

The extraction and purification process consisted of several steps that could not be done for all the samples simultaneously. Consequently, the whole procedure was relatively time-consuming and thus not all of the samples that were initially separated with the MPSS could be measured via µFT-IR. However, twelve samples could be concentrated on 0.2 µm Anodisc filters. The ones (5) that could not be measured via µFT-IR due to a lack of time or because of a too thick filter cake layer are shown in Figure S1 in the part “Supplementary material”. In total eight filters consisting of 7 environmental samples (three beach, four sublittoral samples) and one blank were measured via µFT-IR spectroscopy. The results of the µFT-IR spectroscopy are presented as screenshots of the OPUS Software. Hereby on the left side the chemical mapping derived from the two selected integrating areas is shown and on the right side the image of the filter while the upper filter picture was taken with the Hyperion microscope and the one below using a dissecting microscope (Olympus SZX 16) (Fig. 13- 20).

Most of the samples showed also particles outside of the concentrated area. These particles were almost exclusively very fine sediment grains but in case of sample H19_22 (Fig. 13), H16_19 (Fig. 15) and H30_8 (Fig. 18) also larger particles were found outside the original concentration filter area.

Another noticeable feature was that especially at the edge of the concentrated sample area total absorbance at values of 1.5-2.0, was observed. This was also true for black particles in general.

Figure 18 illustrates an example of a filter cake layer which was too thick on the filter and was therefore not suitable for a µFT-IR analysis. Due to the thickness it was difficult to select a layer on which the infrared light should focus during the measurement. Thus, in most parts of the filter no precise spectra could be achieved due to total absorbance by the thick filter cake. Additionally the sample contained a high amount of dark particles that led to total absorbance as well.

Another filter which derived from one of the sediment samples taken on Zingst (Fig. 19) unfortunately showed a matrix layer that was covering most particles. Due to this layer an evaluation was not possible. Most likely this layer stem from a precipitate in the new ethanol charge, which unfortunately contained denaturants.

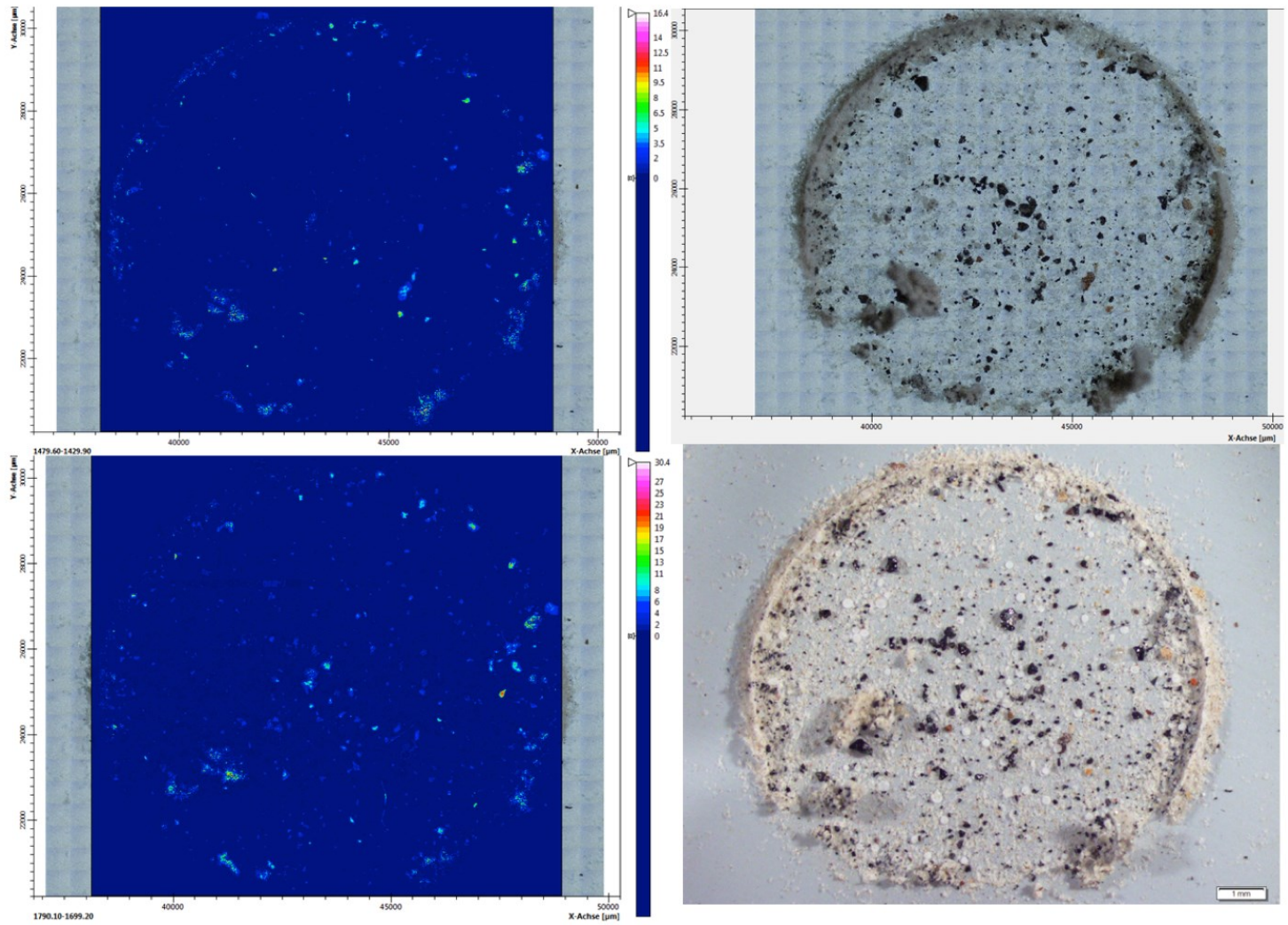

Figure 13: Left side: false color plots, the upper panel shows the result of the integration in the range 1480-1430 cm^{-1}, the lower panel in the range 1790-1700 cm^{-1}, putative microplastic particles are highlighted in light blue to white; right side: image of the filter of sample H19_22 upper panel picture from the FT-IR microscope, lower panel picture from the dissecting microscope

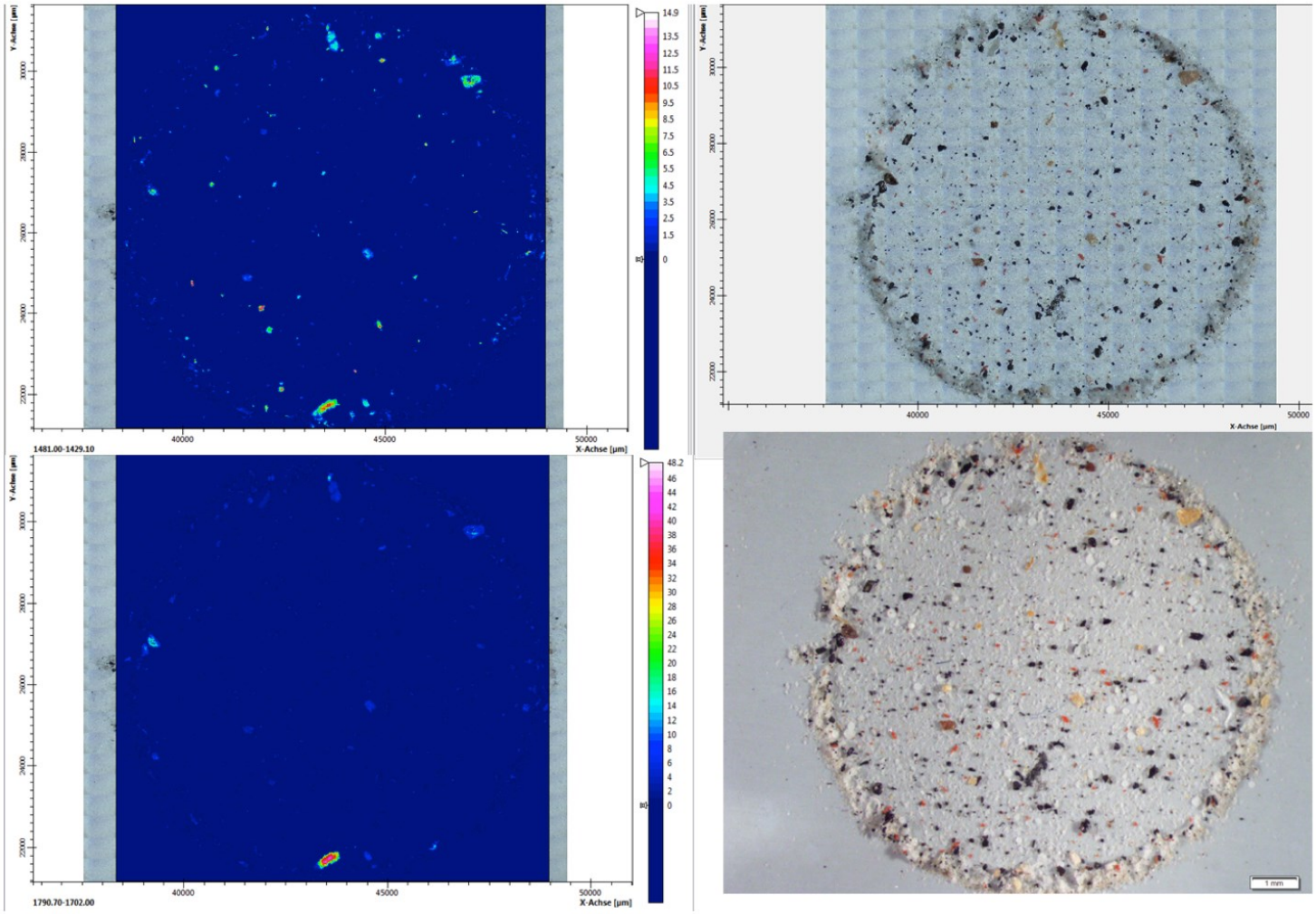

Figure 14: Left side: false color plots, the upper panel shows the result of the integration in the range 1480-1430 cm^{-1}, the lower panel in the range 1790-1700 cm^{-1}, potential microplastic particles are highlighted in light blue to white; right side: image of the filter of sample H18_21 upper panel picture from the FT-IR microscope, lower panel picture from the dissecting microscope

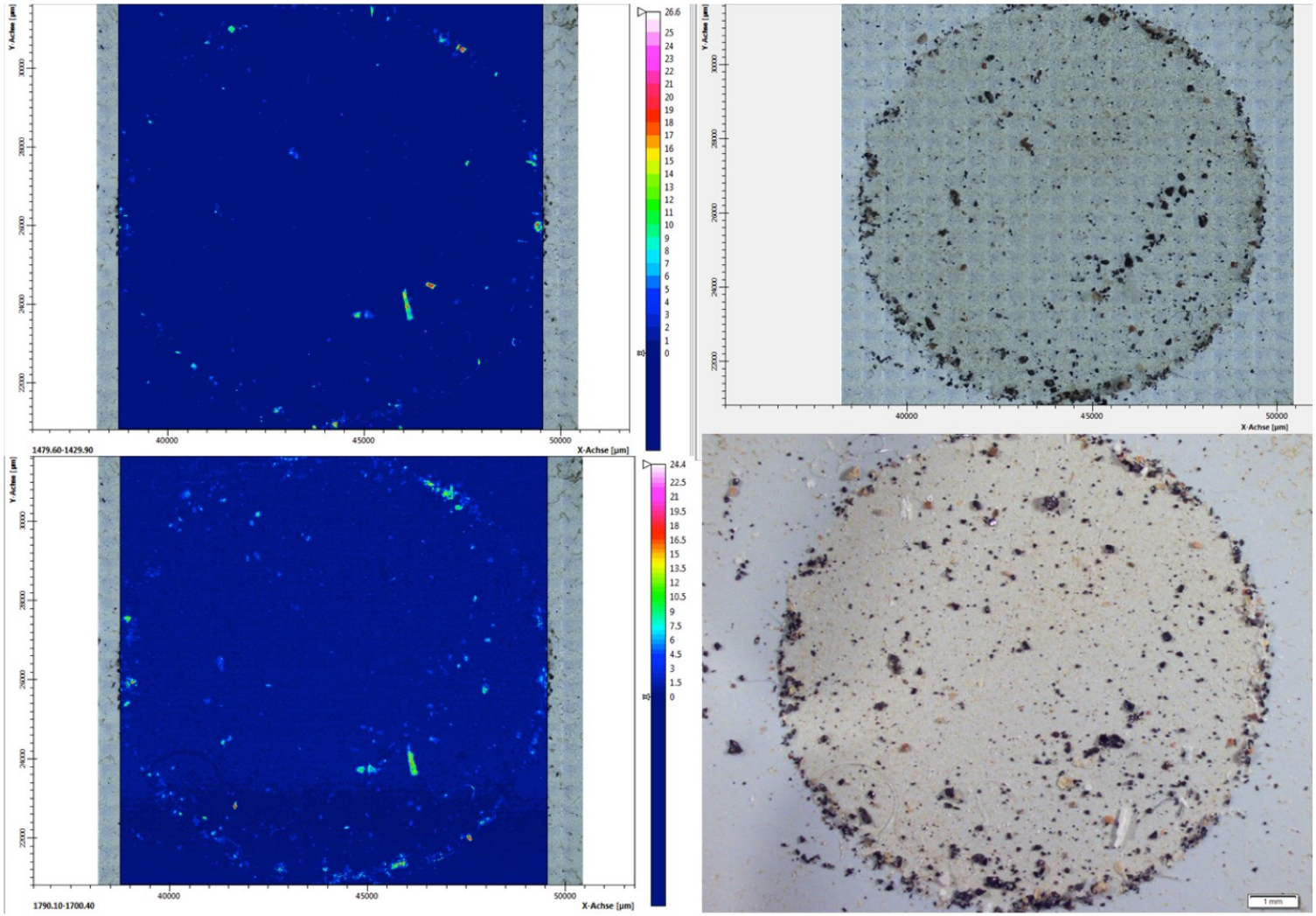

Figure 15: Left side: false color plots, the upper panel shows the result of the integration in the range 1480-1430 cm^{-1}, the lower panel in the range 1790-1700 cm^{-1}, potential microplastic particles are highlighted in light blue to white; right side: image of the filter of sample H16_19 upper panel picture from the FT-IR microscope, lower panel picture from the dissecting microscope

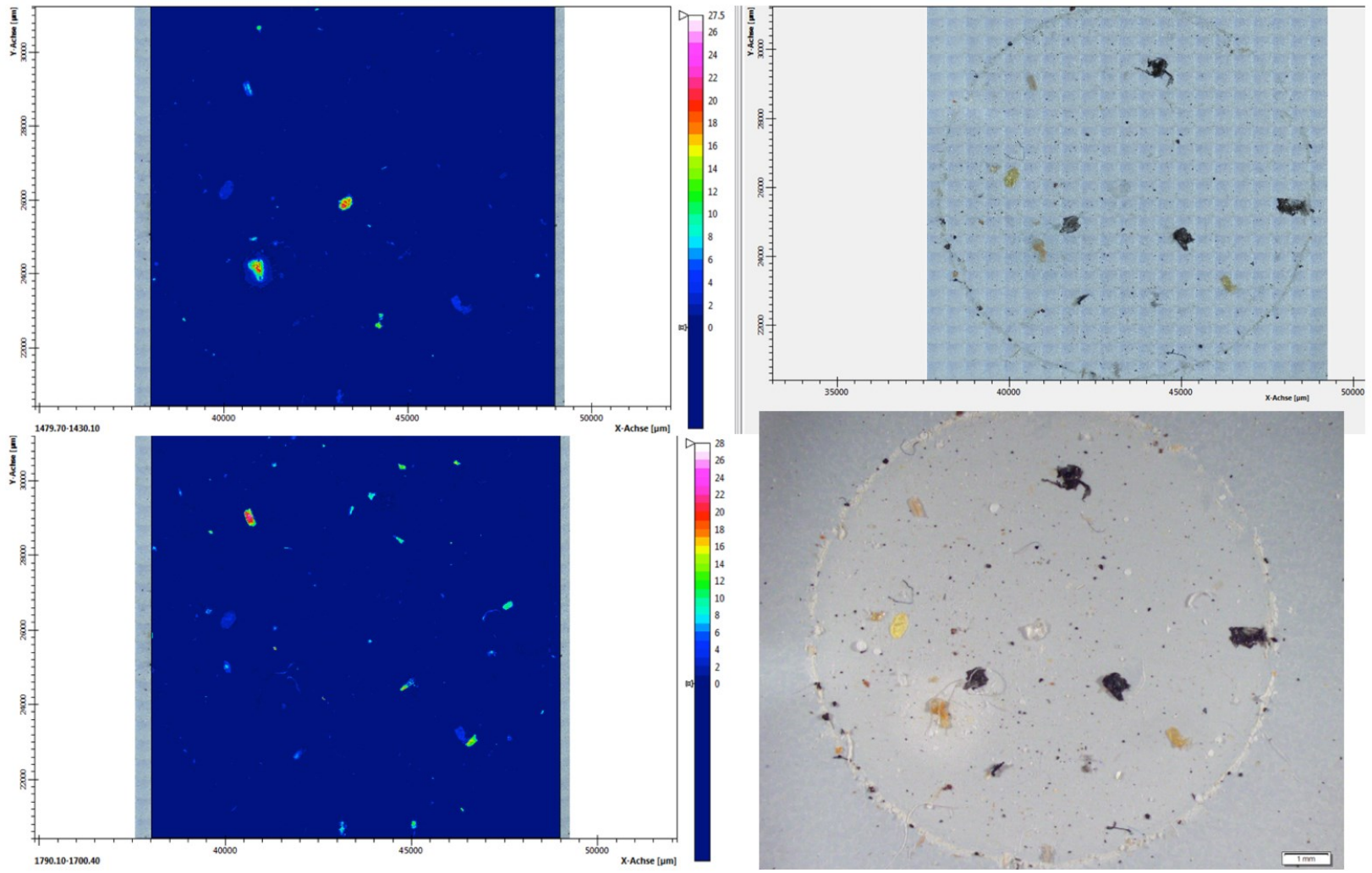

Figure 16: Left side: false color plots, the upper panel shows the result of the integration in the range 1480-1430 cm^{-1}, the lower panel in the range 1790-1700 cm^{-1}, potential microplastic particles are highlighted in light blue to white; right side: image of the filter of sample SW_456_16 upper panel picture from the FT-IR microscope, lower panel picture from the dissecting microscope

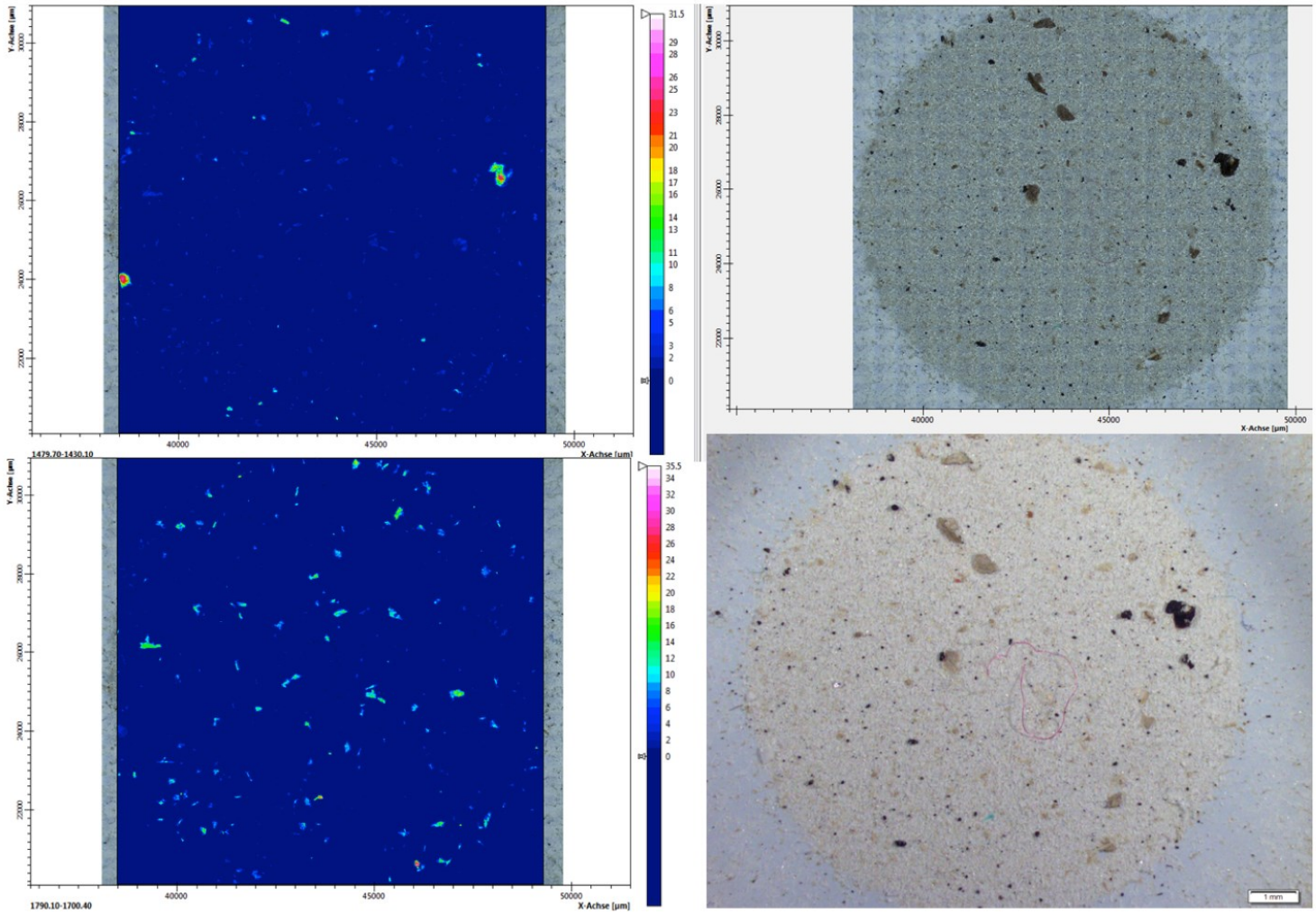

Figure 17: Left side: false color plots, the upper panel shows the result of the integration in the range 1480-1430 cm^{-1}, the lower panel in the range 1790-1700 cm^{-1}, potential microplastic particles are highlighted in light blue to white; right side: image of the filter of sample SH_456_13 upper panel picture from the FT-IR microscope, lower panel picture from the dissecting microscope

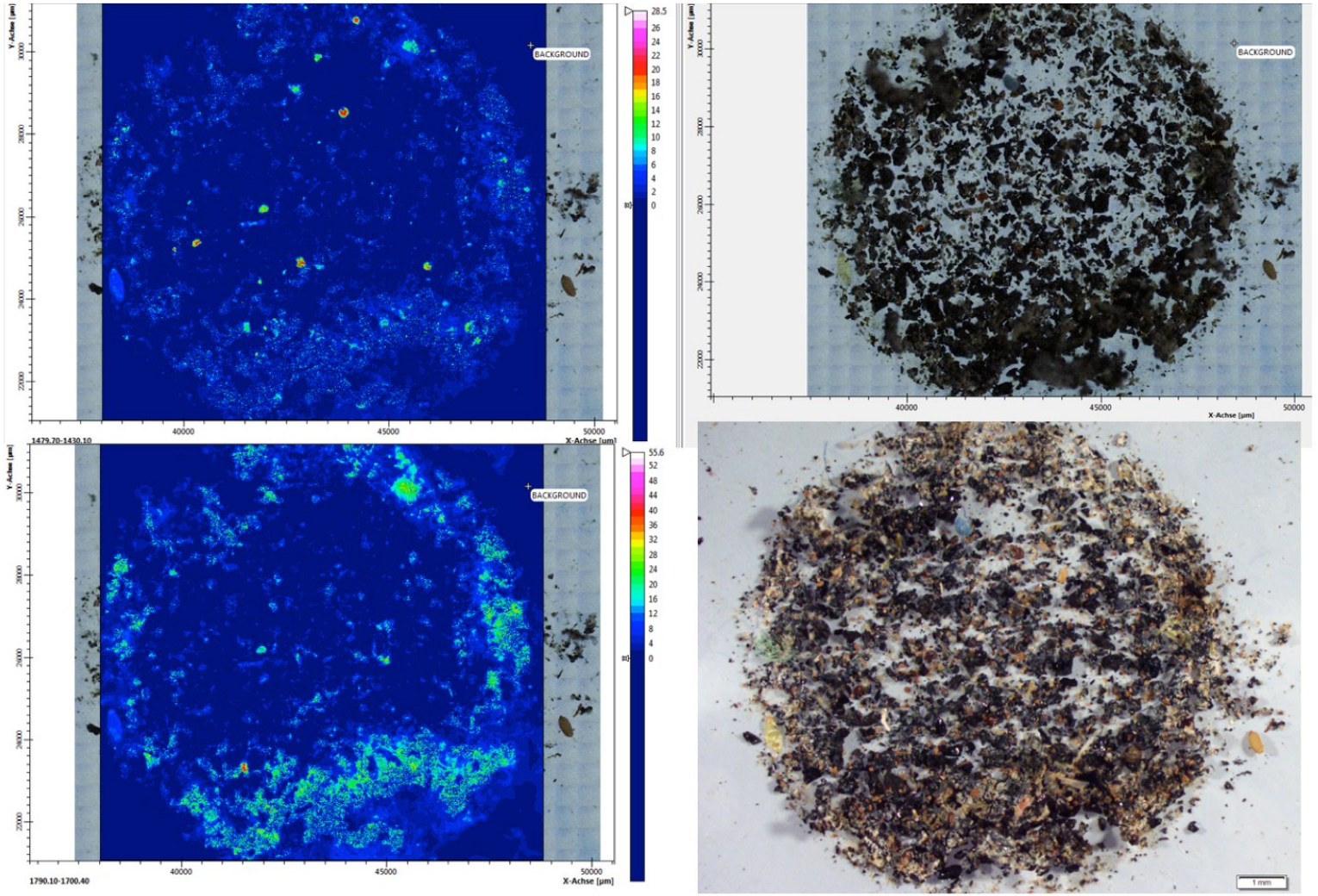

Figure 18: Left side: false color plots, the upper panel shows the result of the integration in the range 1480-1430 cm^{-1}, the lower panel in the range 1790-1700 cm^{-1}, potential microplastic particles are highlighted in light blue to white; right side: image of the filter of sample H30_8 the upper panel picture from the FT-IR microscope, lower panel picture from the dissecting microscope

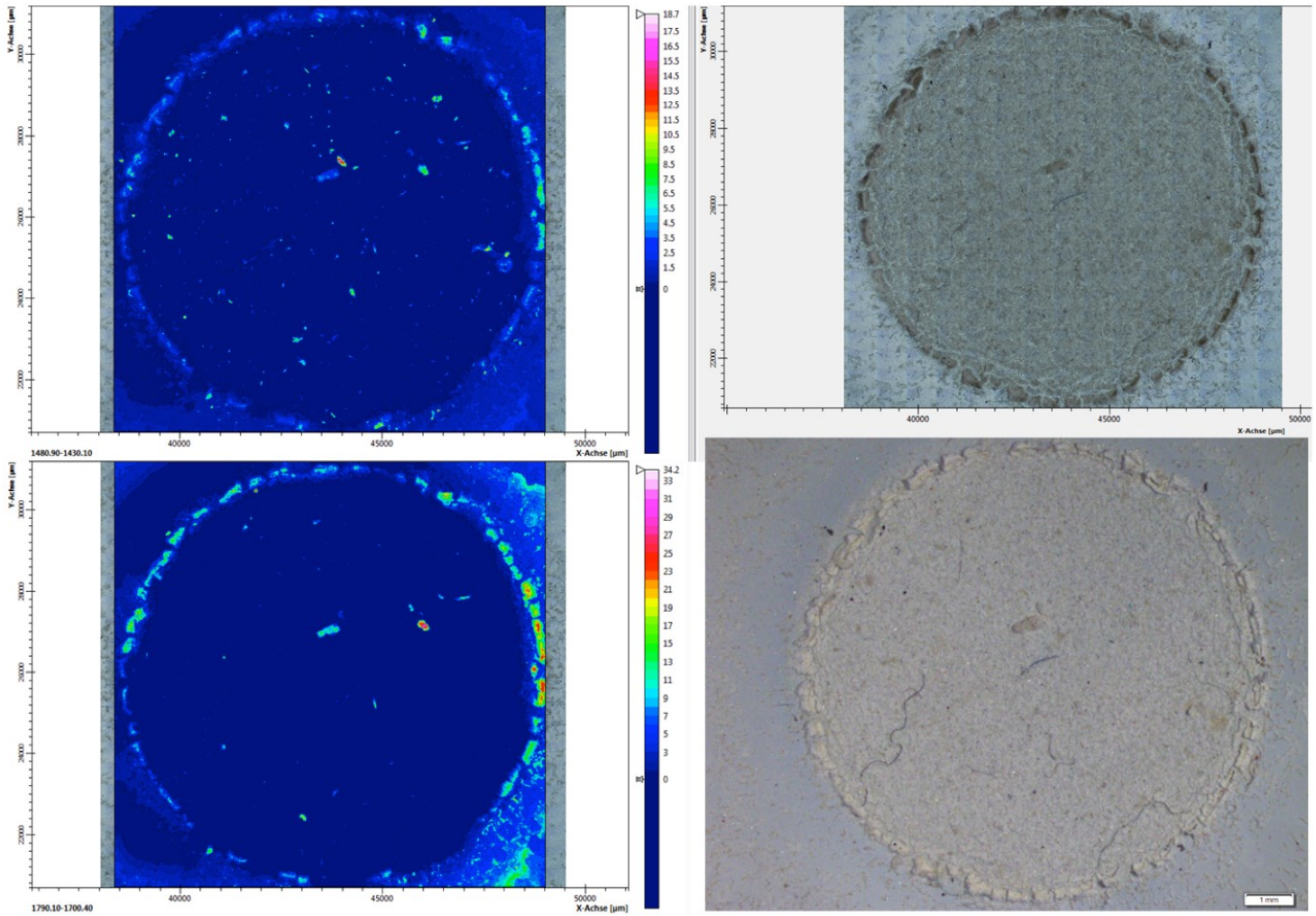

Figure 19: Left side: false color plots, the upper panel shows the result of the integration in the range 1480-1430 cm^{-1}, the lower panel in the range 1790-1700 cm^{-1}, potential microplastic particles are highlighted in light blue to white; right side: image of the filter of sample Z_456_5, the upper panel picture from the FT-IR microscope, lower panel picture from the dissecting microscope

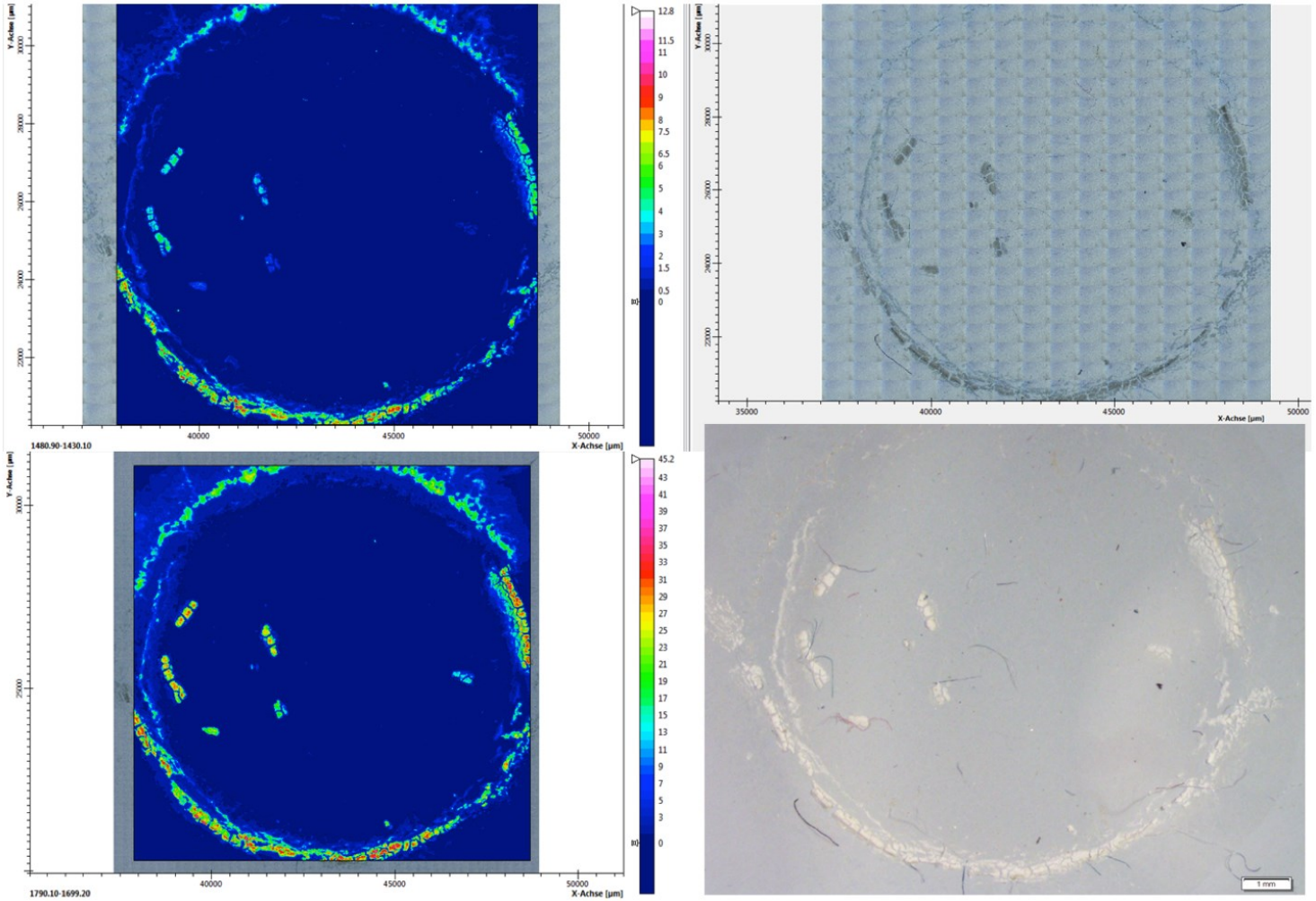

Figure 20: Left side: false color plots, the upper panel shows the result of the integration in the range 1480-1430 cm^{-1}, the lower panel in the range 1790-1700 cm^{-1}, potential microplastic particles are highlighted in light blue to white; right side: image of the filter of blank "Bl" the upper panel picture from the FT-IR microscope, lower panel picture from the dissecting microscope

As can be seen in Figure 20 this contamination with a precipitate affected the blank “Bl” processed with the samples of the second entity as well. Therefore this filter was not precisely analyzable after µFT-IR mapping. Still, in the center of the filter were a very few particles marked in the false color plot and most of them showed no synthetic polymer spectrum. But it is noteworthy that there was a clearly visible contamination with fibers quite likely originating from clothes although all the samples were covered most of the time.

3.4.2 Plastic types analyzed via µFT-IR

Since functional groups of polymers are detected when using FT-IR spectroscopy, classes of polymers can be identified precisely and fast. One of these polymer types identified was polystyrene (PS) and was detected in all of the analyzed samples. PS was found mainly in its unexpanded form as colorless spherule. Exemplarily an optical image of the corresponding particle obtained from sample H18_21 is shown in Figure 21. Hereby the original particle on the filter, a pinkish white colored spherule approximately 143 µm in size, is shown on the right side, the matching false color plot on the left side and at the bottom the extracted spectrum and the matching ATR-spectrum from the reference database. For every other polymer type identified these Figures S2-S11 can be found in the part “Supplementary material” in an alphabetical order.

It is worth noting that the majority of the particles, independently from the plastic type, were transparent. Most particles could clearly be classified because of their well-defined, highly specific and sharp absorbance bands resulting from their repetitive structure in contrast to the more complex range of signals derived of other organic material (Harrison et al. 2012). However, some spectra showed slight variations due to organic residues on the particles. These ambiguous plastic particles were counted as well but put into a different category (Supplementary material, Tab. S2).

There were also a few particles that could not be identified because there were no comparable spectra in the database. Most likely most of them were of an organic origin but especially one spectrum could be found 35 times in sample H16_19 and 3 times in SH_456_13 and the distinct peaks could also point to a synthetic polymer (Supplementary material, Fig. S12).

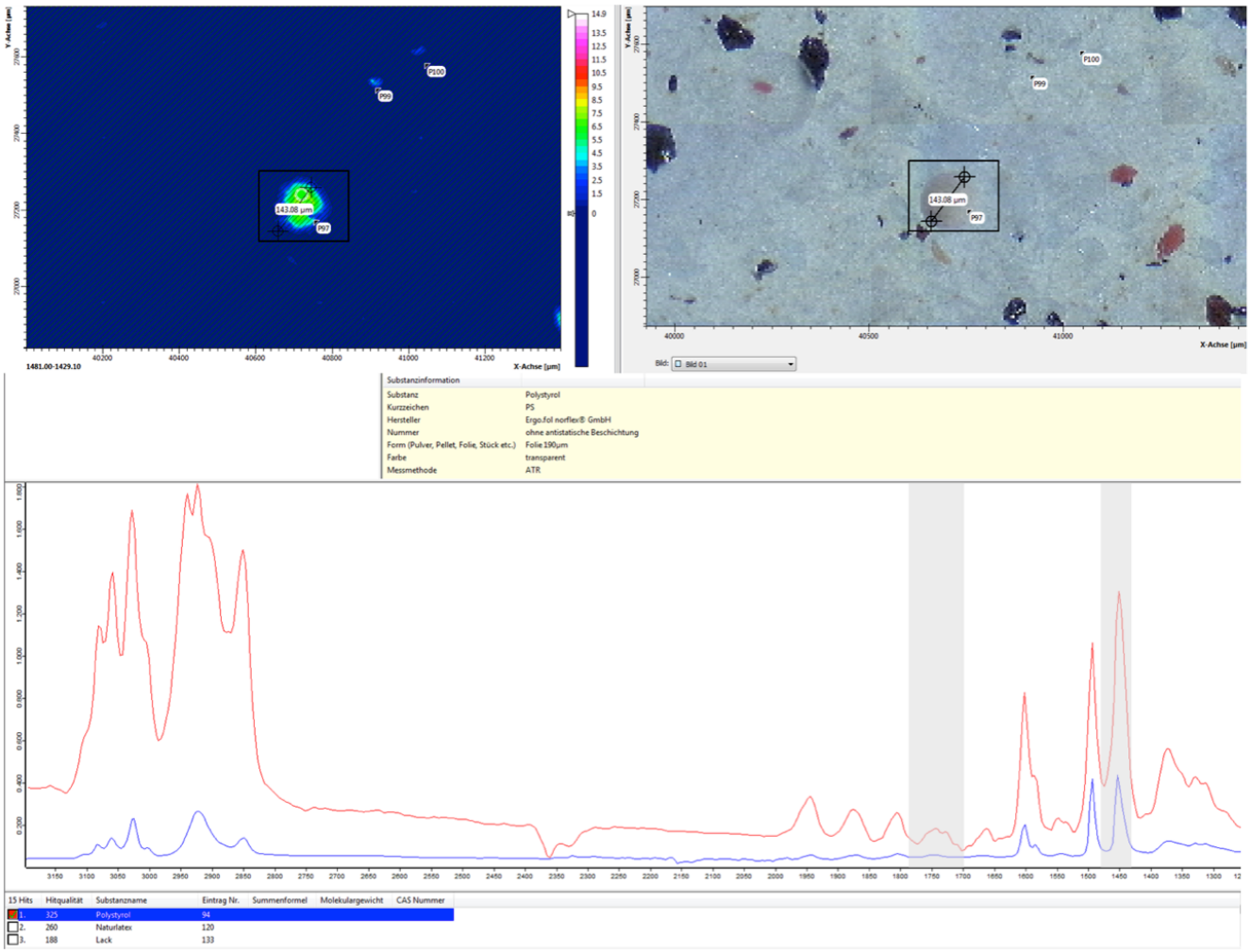

Figure 21: Spectrum ascribed to polystyrene (PS) extracted from sample H18_21: The optical image on the right showing the surface of the Anodisc filter with a rose-white spherule (black frame) approximately 143 µm in size on it and the matching false color plot on the left. Below the extracted spectrum and the matching PS-spectrum obtained from the spectra database

3.4.3 Occurrence of microplastic in sediment samples

The particles that showed a distinct spectrum were counted and the results are summarized in Table 3. Samples that could not be evaluated as precisely as the others were marked with * (Tab. 3) and are not presented in Figure 22 and were hence excluded from the calculation in Table 4.

Table 3: Abundance and distribution of plastic types in the samples

station	H19_22	H18_21	H16_19	SW_456_16	SH_456_13	H30_8*	Σ
polymer type							
EVA	1	4	1				6
PA	1		1	2			4
PC				1			1
PE		7	1	13	6	1	28
PEST	1		7	1		1	10
PP	54	78	91	52	76	8	359
PPSU				2			2
PS	1	11	1	3	2		18
PVA					2		2
PVC	1	1	2	4			8
PMMA	4	4		13	7		28
Σ	63	105	104	91	93	10	

Sample SW_456_16 showed the greatest range of plastic types with nine polymers out of eleven. All samples showed a very high prevalence of PP followed by PE and PMMA. It is also noteworthy that next to PP merely PS was detected in all the samples and this polymer is on the third rank concerning the frequency. The highest number of plastic particles, 105, was found in sample H18_21 followed by 104 counts in sample H16_19.

Due to the previously described deficiency of the samples from the Baltic Sea only the samples of North Sea sediments were used for the following evaluation.

To show the results presented in Table 3 in a spatial context, the relative abundances of plastic types detected in the samples are illustrated as pie diagrams in a map of the German North Sea (Fig. 32).

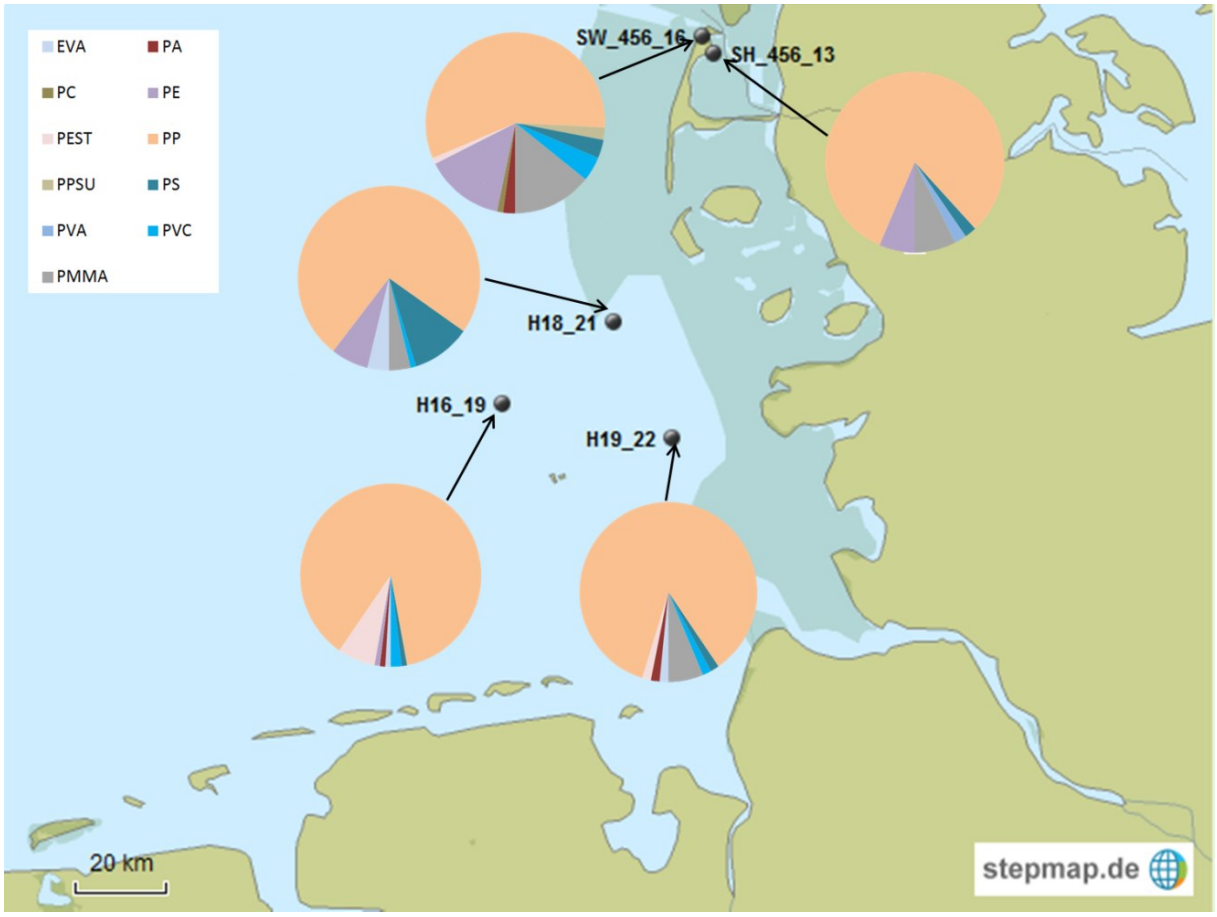

Figure 13: Relative abundance of several plastic types (pie diagrams) in samples from the North Sea obtained by analysis with µFT-IR spectroscopy. The map was created using StepMap on www.stepmap.de

Most striking is the high proportion of PP while the other plastic types, even PE, play a minor role. When taking the initial amount of sediment used for the analysis into account the quantity of microplastic particles detected in the two beach samples from Sylt was 34 and 39 items per kg dry weight sediment similar to the result of 37 for one of the sublittoral samples H19_22 (Tab. 4). The results for the other two sublittoral samples were higher. With 74 items per kg dry weight sediment in sample H16_19 even twice as high. However, whether these results are significant could not be determined due to the small sample quantity.

Table 4: Abundance of microplastic particles in the samples taken in the North Sea expressed as number of plastic items per kg dry weight sediment calculated from the actually detected number of plastic items per sample and the corresponding sediment weight

station	sediment weight (kg dry weight)	number of plastic items identified via µFT-IR	plastic items per kg sediment (dry weight)
SH_456_13	2.697	93	**34**
SW_456_16	2.323	91	**39**
H16_19	1.415	104	**74**
H18_21	1.632	105	**64**
H19_22	1.700	63	**37**

4 Discussion

4.1 Methods

While the identification of plastic particles >500 µm is feasible using dissecting microscopes this approach is not suitable for smaller particles.

Hence, the focus of this study lies on particles <500 µm because they are normally not accessed in other studies. Since the main aim of this study was the development of a replicable method capable of the detection of microplastic in sediment not visible to the naked eye, advantages, limitations and potential improvements of the individual methodological steps will be discussed in this section.

The stations selected for sampling seemed to be very suitable. All samples measured contained a noticeable amount of microplastic and the filters that could not be measured contained also visible particles which seem to be of synthetic origin (Supplementary material, Fig S1b + e). The sampling protocol used here could be also used by further studies. However, as stated by other authors like Nuelle et al. (2014) the distribution of microplastics in sediments is expected to be very heterogeneous and potentially low in some areas. Therefore, it is favorable to investigate a large sample quantity. So it may be a good or even better alternative to pool all the samples taken at one beach inside a 1 or 2 m² area. Such a procedure seems to be reasonable as the MPSS facilitates the separation of a great amount of sediment (6 l) in a single run (Imhof et al. 2012). However, the sample volume of roughly one liter sediment as used for this study appeared to be sufficient since a representative amount of microplastic has been detected in the samples.

The subsequent extraction method with the MPSS and the use of $ZnCl_2$ with a density of at least 1.7 g cm^{-3} can be considered as successfully applied since not just low density polymers like PE und PP were identified in the samples but also more dense polymers like PVC. Due to the time needed for testing the tightness of the mounted separator, the filtration of the $ZnCl_2$, the settling duration of the sediment and the cleaning of the separator afterwards, 24 hours should be considered for the separation of one sample.

The mixing of the $ZnCl_2$ solution proved to be difficult since no tables on the added amount of $ZnCl_2$ and the resulting density of the solution were easily available. Therefore it was necessary to calculate the amount of $ZnCl_2$ needed to obtain a certain volume of a solution with the desired density. Additionally, the mixing was time and work consuming. The $ZnCl_2$ had to be added in small amounts to a manageable volume of water and stirred quite a long time, furthermore, there was also a lot of waiting time for the solution to cool down because of the exothermic reaction. A disadvantage of $ZnCl_2$ is that this salt is ten times more expensive than NaCl and also toxic to aqueous organisms. Therefore spillage should be

prevented and the solution should be reused multiple times. Despite the large amount of $ZnCl_2$ that was needed for the separation with the MPSS it could be recycled with relatively little loss (about 500 ml per run consisting of 200 ml for the sample and the $ZnCl_2$ blank and 200 ml rest in the filtration unit and maybe 100-200 ml left in the sediment after separation). The decrease in density was not very high, about 0.007 g cm^{-3} (±0.01) (= 7 mg cm^{-3}) per run, so that an adjustment of density was only necessary after a couple of separation runs.

It is also worth noting that the sediment should not be completely dry when filled into the separator. It could be observed in a trial run that dry sediment does not sink sufficiently but form bubbles filled with air and sediment at the surface because of the high surface tension of $ZnCl_2$ solution. Even stirring did not eliminate these bubbles but just reduced them in size. It took more than 24 hours for the sediment to get soaked in $ZnCl_2$ and to sink to the bottom.

The sample volume reduced by the separation contained additionally to plastic particles also organic debris and phytoplankton like diatoms. Furthermore, frequently fine sediment like clay particles were found especially in the samples derived from the sublittoral sediments. Since an optical change of the samples during the purification process could be observed, the enzymatic digestion protocol used in this study was mostly very successful and can thus be considered to be highly promising for future analyses. Nevertheless, the reaction times should be adjusted according to the individual sample conditions. In this context the enzyme activity should be measured exemplarily for a few samples containing a high amount of biogenic matter like proteins and cellulose to adjust the appropriate amount of enzyme and corresponding buffering solution used and the application time for a sufficient maceration. Due to its high efficacy in removing biogenic organic matter H_2O_2 should not be excluded from the protocol but application time should be reduced to a minimum in order to minimize a potential oxidation of the synthetic polymers (Nuelle et al. 2014). Furthermore the incubation with H_2O_2 should be done at room temperature because some of the polycarbonate filters showed visible changes after the treatments with H_2O_2 at 50 °C (Supplementary material, Fig. S13). For future studies the use of stainless steel filters is recommended because they are more durable, pose no risk of contamination, are more easy to clean and reusable. Of course it has to be ensured that they are not leaking which was the reason for exchanging them during this study.

In a recent study by Cole et al. (2014) the enzymatic digestion using Proteinase-K, proved to be capable of digesting more than 97% (by weight) of the biogenic organic matter present in a biota-rich sample. Proteinase-K is a proteolytic enzyme and similar to Protease-A01 used in this study. The extension of an enzymatic digestion protocol using only Protease with the additional application of Cellulase and Chitinase as proposed here is therefore very

promising and addresses samples of different biological background. With the enzymatic approach tested here it was possible to purify environmental samples without the use of highly acid or alkaline solutions like HCl or NaOH which has been shown recently to be also less effective (Cole et al. 2014) and additionally aggressive to plastic polymers (Claessens et al. 2013).

High quality easy to identify IR-spectra without impurities proved that the protein digestion worked very well. These impurities that can influence the spectra could be residues of biogenic organic matter or also plastic-associated pollutants like PAHs (Endo et al. 2005; Rios et al. 2007; Harrison et al. 2012). Especially residues of proteins e.g. a protein matrix can be a big problem as shown during previous studies (Mintenig 2014). This proves that the use of technical Protease with a high activity and the additional treatment with H_2O_2 after the enzymatic digestion was very effective to remove residues of biogenic organic matter. It has been agreed upon by a number of authors that such effective purification methods are necessary when using µFT-IR analysis (Harrison et al. 2012; Hidalgo-Ruz et al. 2012; Vianello et al. 2013).

Harrison et al. (2012) stated furthermore that when using the µFT-IR mapping technique there is a need for efficient and reproducible methods to separate microplastics from sediments. The density separation which was implemented in the purification protocol subsequent to the enzymatic digestion was capable of removing quartz residues and most of the resuspended sediment. Unlike in a previous study (Mintenig 2014) diatoms, mostly just their empty valves, could not be fully eliminated. However, the silica spectrum was not interfering with the synthetic polymer spectra and did therefore pose no problem to a proper analysis.

In contrast to this the thickness of the concentrated sample on the filter did sometimes impair the measurement in transmission mode due to the occurrence of total absorption. So as to prevent such non-analyzable filters, samples with a high amount of floating particles should be split and filtered onto more than one Anodisc filter. This increases the time needed for the evaluation of a sample but improves the potential results considerably. The thickness of the layer on the filter is negligible when using µFT-IR in reflectance mode but as stated in other studies this mode is highly susceptible to refractive errors (Harrison et al. 2012; Vianello et al. 2013). The reflectance mode is thus not really appropriate for environmental samples since the surface of microplastic particles derived from environmental samples is not plain because of degradation processes resulting in spectral distortion and low quality IR-spectra (Corcoran et al. 2009). Another limitation to µFT-IR in transmission mode is the presence of black particles which often cannot be measured due to the occurrence of total absorption.

These particles could be assigned to residues from combustion like carbon black but also to black synthetic polymers like e.g. PE. To overcome this limitation black particles should be counted with the dissecting microscope and the biggest particles could be measured exemplarily using the Attenuated Total Reflectance (ATR) another kind of IR spectroscopy technique suitable for bigger particles. Additionally, the > 500 µm fraction should be sorted under the dissecting microscope and potential plastic particles should be measured with ATR in order to assess the amount of visible microplastics in a sediment sample. By the inclusion of such a step a comparison with other studies, which focus often on these size class of plastic is more feasible (Hidalgo-Ruz et al. 2012). It was planned to include such an ATR analysis of the fraction < 500 µm in this study, too, and thus a 500 µm pre-screening of the samples was conducted. However, this analysis was not possible due to the lack of time.

Despite its limitations the µFT-IR mapping technique exhibits many advantages for the microplastic analysis. On the one hand the necessity of pre-sorting very small potential microplastic particles can be omitted. The fact that most of the particles, identified using µFT-IR, were transparent and thus potentially missed in a visual analysis emphasizes that this method is highly recommendable when identifying small particles when they are barely visible. Moreover the measurement runs mostly automatically and just the evaluation has to be done manually. Additionally, it is a non destructive method in contrast e.g. Pyrolysis-gas chromatography-mass spectrometry (Pyr-GC/MS) used in some other studies (Fries et al. 2013; Nuelle et al. 2014). Finally the self-generated IR spectra database proved to be well equipped since most particles that showed a distinct spectrum could be assigned to a kind of polymer. Considering the distinct spectrum several times found in the samples but not in the IR spectra database (Supplementary material, Fig. S12) shows that the database should still be extended.

If not stated explicitly non-plastic materials were used during the whole sample processing to minimize the contamination risk. However, it was almost impossible to fully avoid the use of plastic objects in the laboratory. Since the procedural blank was not precisely analyzable a background contamination could not be fully excluded. Nevertheless, the samples measured here showed a different composition of plastic types which makes a contamination unlikely. The only striking overlap is the high proportion of PP particles but a contamination of that extent would have been noticeable in the blank as well.
However, the above mentioned contamination with fibers is a known problem when examining samples for microplastic (Nuelle et al. 2014). The detection of fibers in samples is anyway problematic since only fibers with a diameter of at least 10 µm can be analyzed using µFT-IR (Bhargava et al. 2003). It is difficult to fully prevent such an airborne

contamination but it can be reduced by covering the sample and all the materials used when not actually working with them. Since this was done in this study the contamination with fibers originated most likely from the photo documentation of the subsamples although the pipettes and Petri-dishes used were always rinsed with ultrapure water. To overcome this source of contamination the materials used should be wiped with ethanol additionally to remove eventual fatty residues to which fibers could stick. Furthermore, the documentation time as well as the amount of samples used for documentation should be reduced to a minimum.

4.2 Results

The methodology applied in this study facilitated the concentration of one sediment sample that was extracted and purified via enzymatic digestion on a 64 mm² filter area. This therefore allowed for a mapping of this area using µFT-IR spectroscopy resulting in the detection of all small microplastic particles of a large sample volume at once.

The evaluation of the filters measured with µFT-IR spectroscopy revealed a considerable abundance of microplastics in the three sublittoral samples and the two beach samples from the North Sea. The detected microplastic concentrations for the sublittoral samples are comparable to those found by Thompson et al. (2004) and Claessens et al. (2011). In contrast the observed concentrations for the beach sediments in this study were either higher (Thompson et al. 2004) or three times lower (Claessens et al. 2011) than in the literature. On the contrary another study using also µFT-IR reported much higher concentrations of microplastics in the Lagoon of Venice which is strongly impacted by human activities, but these numbers were not obtained by measuring a whole filter but by extrapolating several measured areas to a whole filter (Vianello et al. 2013). However, this highlights that the discrepancy in methodology exacerbates the comparison of detected abundances with other studies.

In contrast to this restriction, the comparison of the composition of microplastic regarding the plastic type is more feasible. The polymer types found in this study correlate with results reported by other studies. Most studies report the high proportional presence of PP and PE in accordance with the high demand of these lightweight plastics (PlasticsEurope 2013; Vianello et al. 2013). Another congruity is the presence of PS spherules (Reddy et al. 2006; Claessens et al. 2011; Vianello et al. 2013). Additionally, other studies detected PVC, PA and PEST in sediment samples even in higher percentages (Browne et al. 2010).

Some of the particles, mostly potential PE and PP, that were assigned to the second category and excluded from the assured results showed an unusual peak in the range

around 1710 cm^{-1} that made the spectra less distinct. But since this peak is characteristic for carbonyl groups it was assigned to photo-oxidation of the polymers in a recent study (Zbyszewski et al. 2014). This could imply an underestimation of the microplastic contamination detected in the present study. Since not all particles assigned to the second category showed this peaks and the number of second grade particles was already low the detected and reported numbers of plastic particles per kg dry weight sediment can be considered plausible. However, the alteration of the spectrum due to this peaks indicating photo-oxidation should be considered in further studies.

By analyzing more filters in future studies a spatial comparison between sublittoral and beach samples as well as between North and Baltic Sea regarding the scale of microplastic pollution should be addressed. Due to the small quantity of samples measured using µFT-IR in this study no comparison of North and Baltic Sea in terms of microplastic pollution is possible. However, as could be seen by reference to the one filter measured (H30_8) where PP, PE and PEST could be detected and the microscopic images of the other filters derived from samples of the Baltic Sea (H33_11, H32_10, LW_12_1 and Z_456_5) (Supplementary material, Fig. S1) there is a contamination in the Baltic Sea as well. The extent of this microplastic contamination should be a main focus in future studies due to a lack of data especially for the Baltic Sea (UNEP 2009).

Nevertheless, with the data at hand it is already apparent that there is considerable microplastic pollution in the North Sea regarding sublittoral and beaches. It is worth noting that microplastics also occurred in sediments from a National Park. Since these nature reserves represent areas with vulnerable habitats protecting these areas against their contamination with plastic debris should be of particularly concern.

5 Conclusion

The presence of small microplastic particles in the marine environment has been already mentioned in the literature (Ivar do Sul & Costa 2014). Nonetheless, concrete and comparable data on the abundance and distribution of microplastics is still scarce mostly due to differences in methodology. Since the MSFD was implemented addressing the contamination of the marine environment with microplastics, the need to determine a good environmental status and develop monitoring strategies has ever increased.

To achieve the goal of an appropriate monitoring standardized methods concerning the various compartments of the marine environment e.g. water column, plankton and sediments are highly required.

For the detection of microplastics in marine sediments a methodological setup was developed in this study.

The methodological setup implemented here consisted of a density separation with the recently invented MPSS and $ZnCl_2$ with a density of at least 1.7 g cm^{-3}, a consecutive purification of the extracted sample via enzymatic digestion and a final analysis of the concentrated sample via µFT-IR spectroscopy. Next to a considerable number of microplastics also various types of plastic polymers, whereby PP was by far most prevalent, could be recorded using these methods. Therefore, the setup was confirmed to be highly suitable for the detection of small microplastics < 500 µm in environmental sediment samples. For future studies just a few suggested adjustments have to be done regarding the particular sample conditions.

Regarding the time effort the analysis of a sample starting with the density separation and ending with the evaluation of the mapped filter should be considered to take about 14 days by adopting the minimal application times for the enzymes (Fig. 7). The processing of four to five samples in parallel should be feasible.

The combination of the methods presented in this study allowed for an insight into the contamination of the German North Sea sediments with microplastics. The detected abundance of microplastics ranged from 34 to 74 particles per kg dry weight sediment. Additionally, this study showed the occurrence of small microplastics within the Schleswig-Holstein Wadden Sea National Park. Based on this promising methodological setup, further studies are required to assess the magnitude of microplastic pollution in order to evaluate the spatial distribution of microplastics in sediments of the North and Baltic Sea.

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Appendix

List of abbreviations

BAH	Biologische Anstalt Helgoland
BPA	Bisphenol A
DDT	Dichloro-diphenyl-trichlorethane
EVA	Ethylene vinyl alcohol
FPA	Focal Plane Array
GES	Good environmental status
FT-IR	Fourier Transform Infrared Spectroscopy
LDPE	Low Density Polyethylene
MPSS	Munich Plastic Sediment Separator
MSFD	Marine Strategy Framework Directive
PA	Polyamide
PBS	Phosphate buffered saline
PC	Polycarbonate
PCB	Polychlorinated biphenyl
PE	Polyethylene
PEST	Polyester
PET	Polyethylene terephthalate
PMMA	Polymethylmethacrylate
POP	Persistent organic pollutant
PP	Polypropylene
PPSU	Polyphenylsulfone
PS	Polystyrene
PSU	Polysulfone
PUR	Polyurethane
PVA	Polyvinyl acetate
PVC	Polyvinyl chloride
PyrGC/MS	Pyrolysis-gas chromatography-mass spectrometry
SDS	Sodium dodecyl sulfate

Supplementary Material

Table S1: Densities of the $ZnCl_2$ solutions of different concentrations

amount of water	added $ZnCl_2$	concentration of the soln.	temperature of the soln.	weight of the soln.	volume of the weighed soln.	density of the soln.
g	g		°C	g	ml	g ml^{-1}
5500	2500	0.3125	30	6.3347	5.00	1.2669
5500	5000	0.4762	33	7.4952	5.00	1.4990
5500	7500	0.5769	35	8.2228	5.00	1.6446
5500	10000	0.6452	20	8.8745	5.00	1.7749
5500	10000	0.6452	12	8.8634	5.00	1.7727
5500	10000	0.6452	**12**	8.8268	5.00	**1.7654**
5500	2500	0.3125	23	6.3909	5.00	1.2782
5500	5000	0.4762	26	7.4637	5.00	1.4927
5500	7500	0.5769	21	8.3009	5.00	1.6602
5500	10000	0.6452	**12**	8.9776	5.00	**1.7955**
5500	2500	0.3125	26	6.2705	5.00	1.2541
5500	5000	0.4762	29	7.5029	5.00	1.5006
5500	7500	0.5769	22	8.2572	5.00	1.6514
5500	10000	0.6452	**12**	9.0088	5.00	**1.8018**

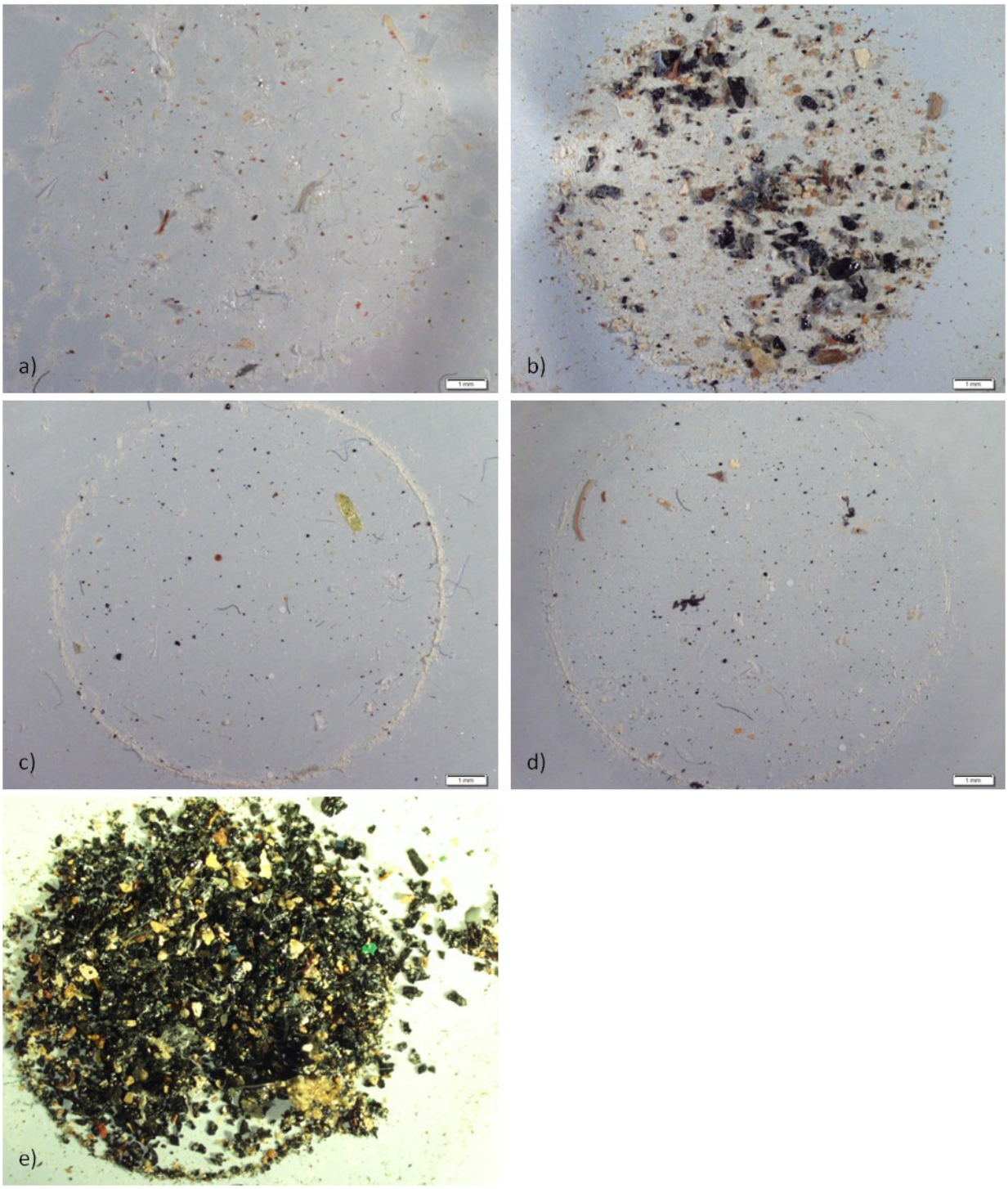

Figure S1: On Anodisc concentrated samples: LW_12_1 (a), H32_10 (b), H17_20 (c), SW_789_17 (d), H33_11 (e); not measured

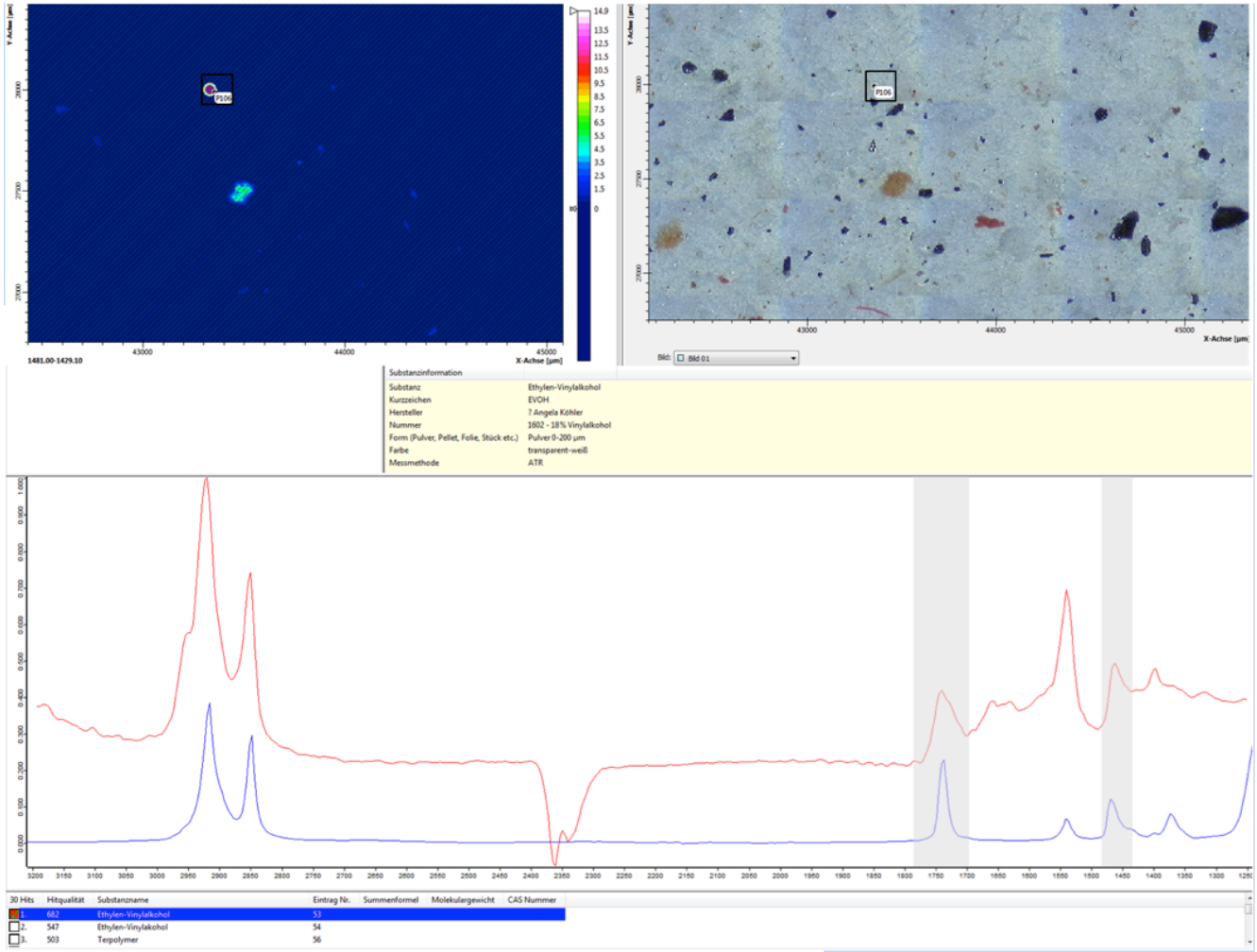

Figure S2: Spectrum ascribed to EVA extracted from sample H18_21: The optical image on the right showing the surface of the Anodisc filter with a transparent fragment (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching EVA-spectrum obtained from the database

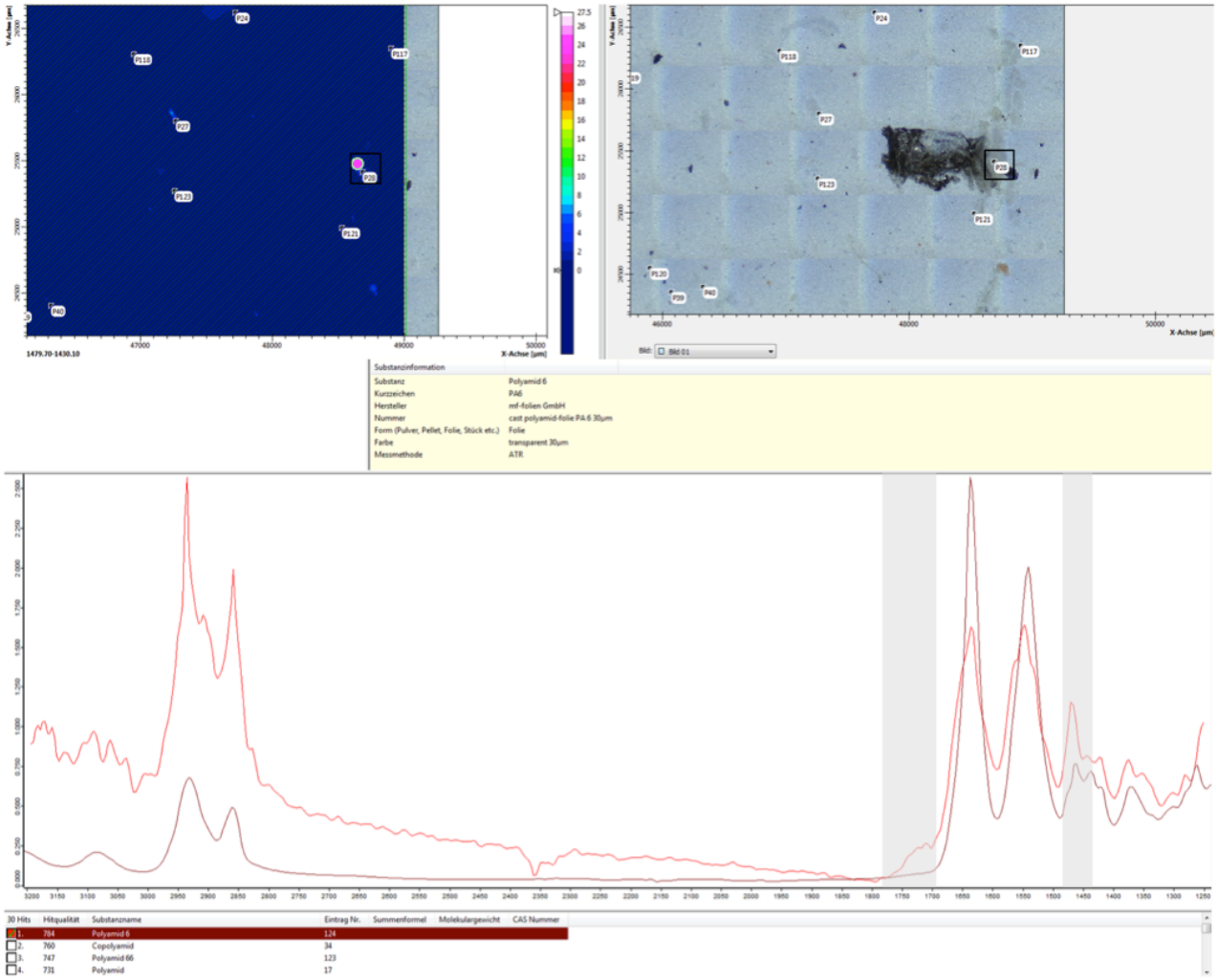

Figure S3: Spectrum ascribed to PA extracted from sample SW_456_16: The optical image on the right showing the surface of the Anodisc filter with a transparent fragment (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching PA-spectrum obtained from the database

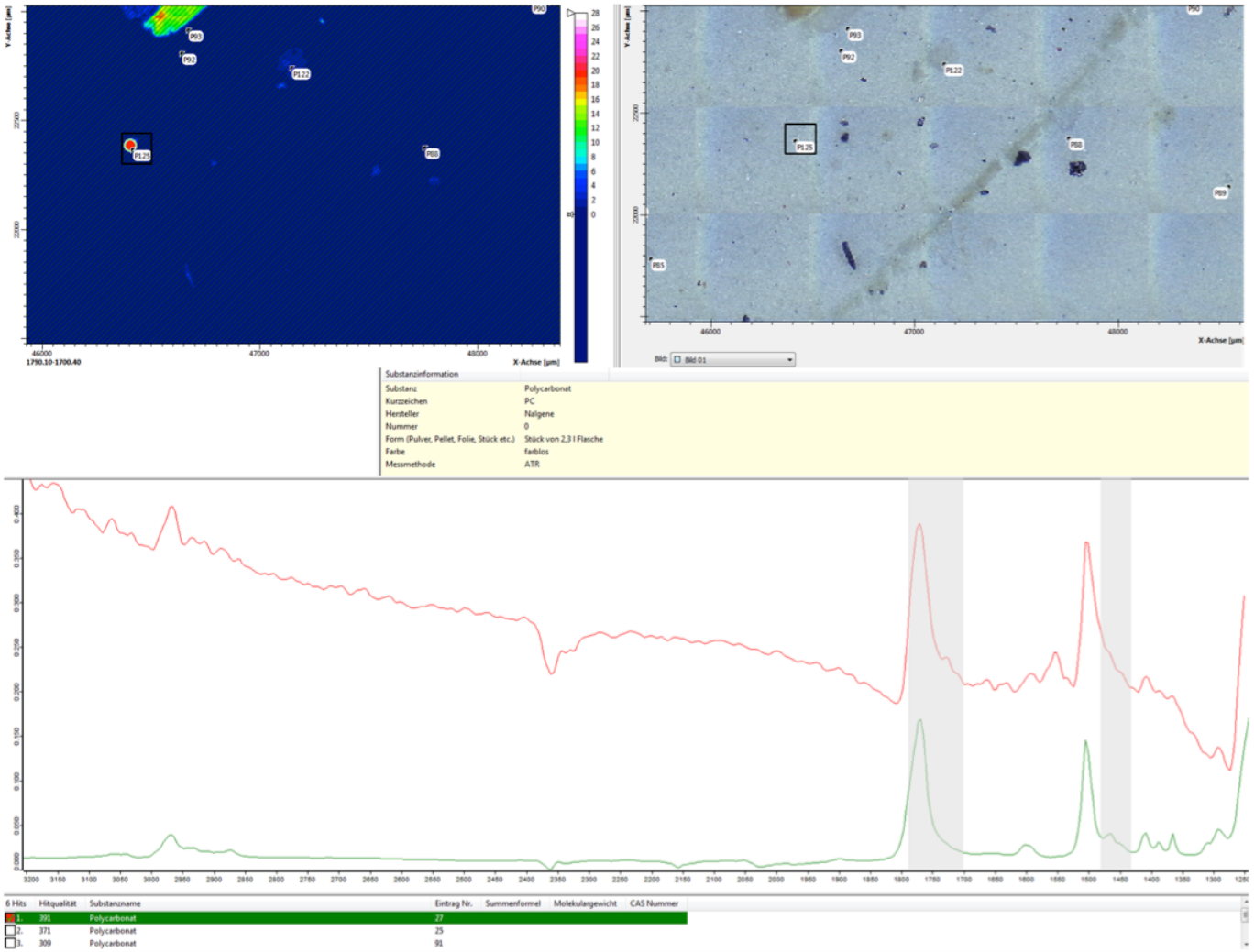

Figure S4: Spectrum ascribed to PC extracted from sample SW_456_16: The optical image on the right showing the surface of the Anodisc filter with a transparent fragment (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching PC-spectrum obtained from the database

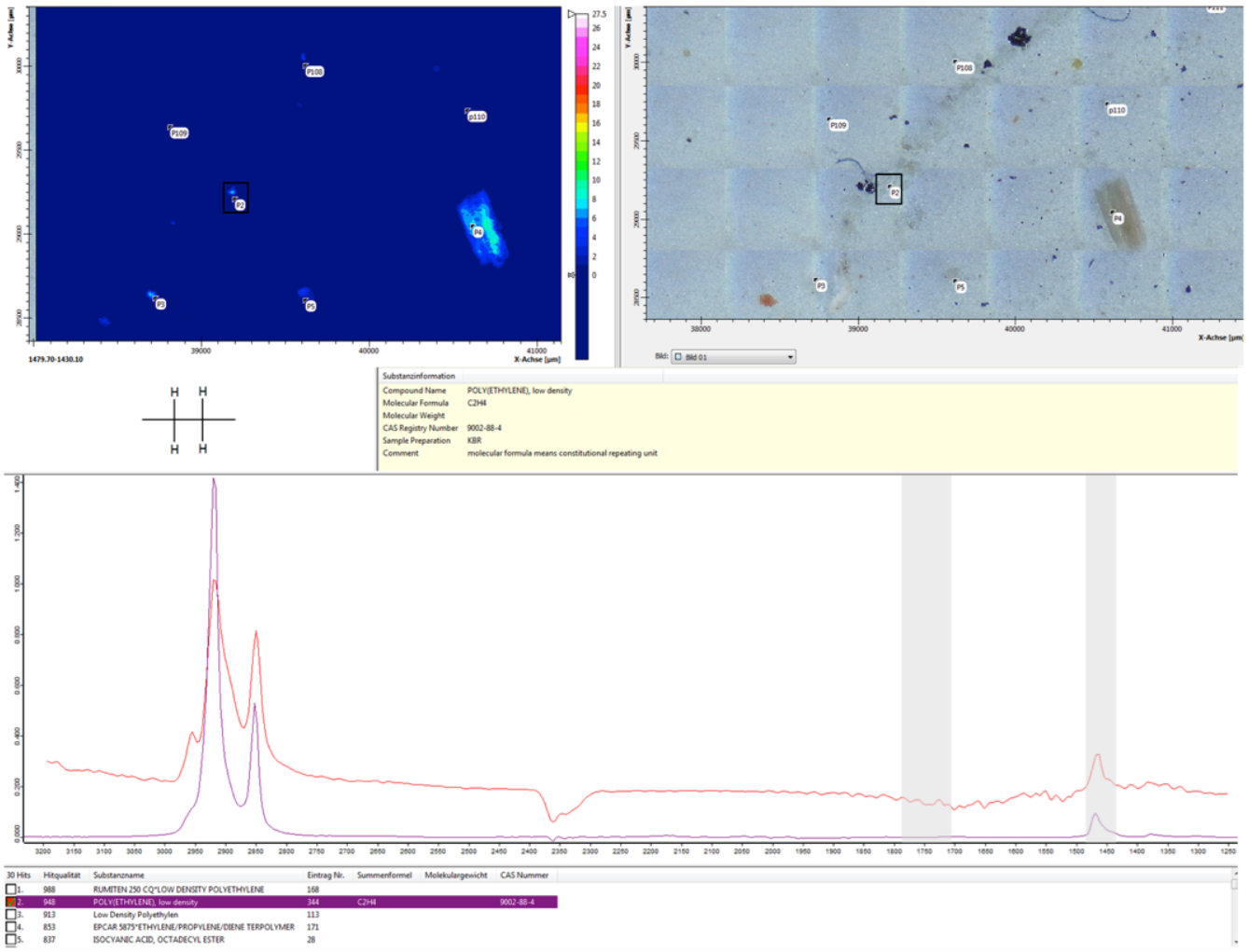

Figure S5: Spectrum ascribed to PE extracted from sample SW_456_16: The optical image on the right showing the surface of the Anodisc filter with a tiny transparent fragment (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching PE-spectrum obtained from the database

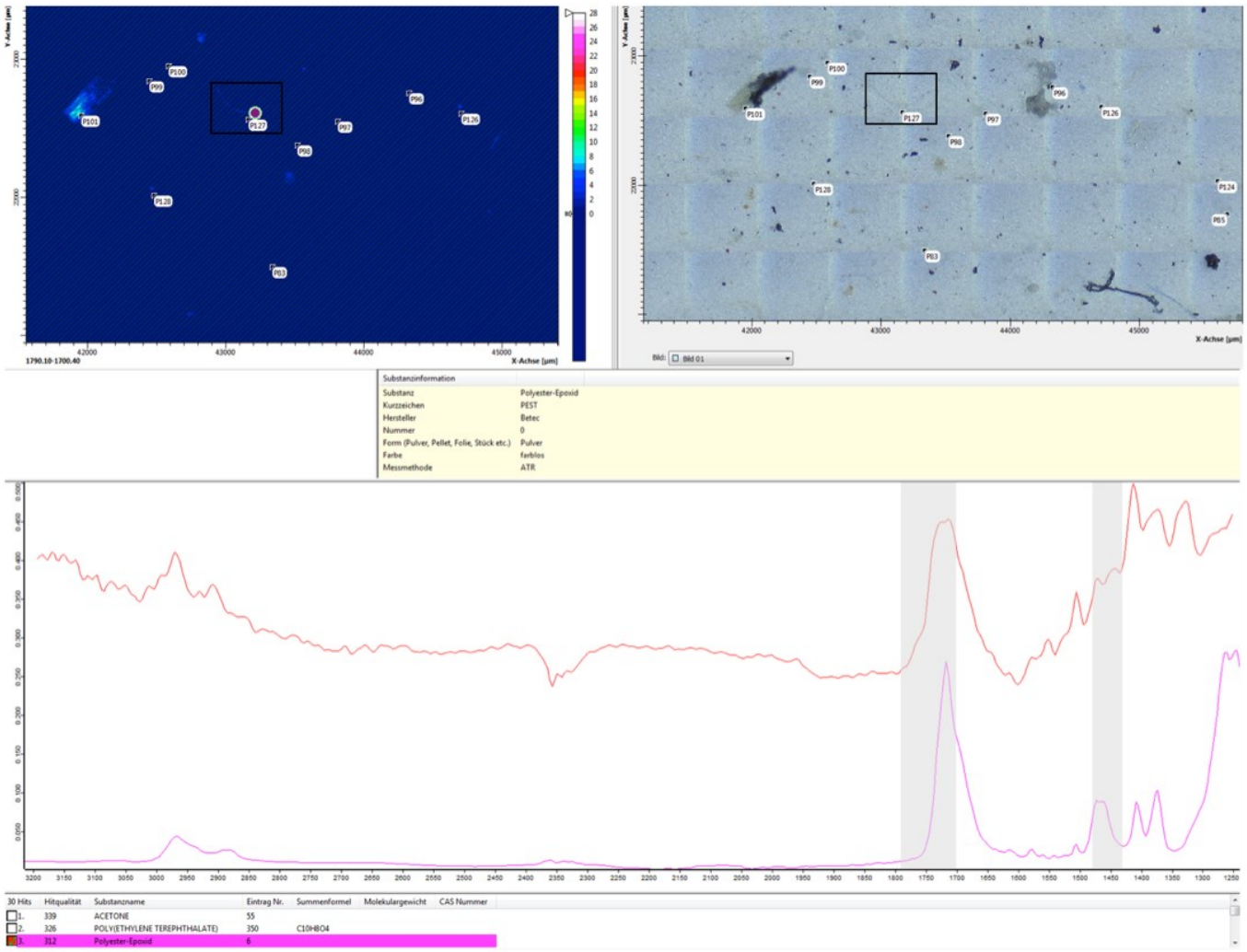

Figure S6: Spectrum ascribed to PEST extracted from sample SW_456_16: The optical image on the right showing the surface of the Anodisc filter with a colorless fiber (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching PEST-spectrum obtained from the database

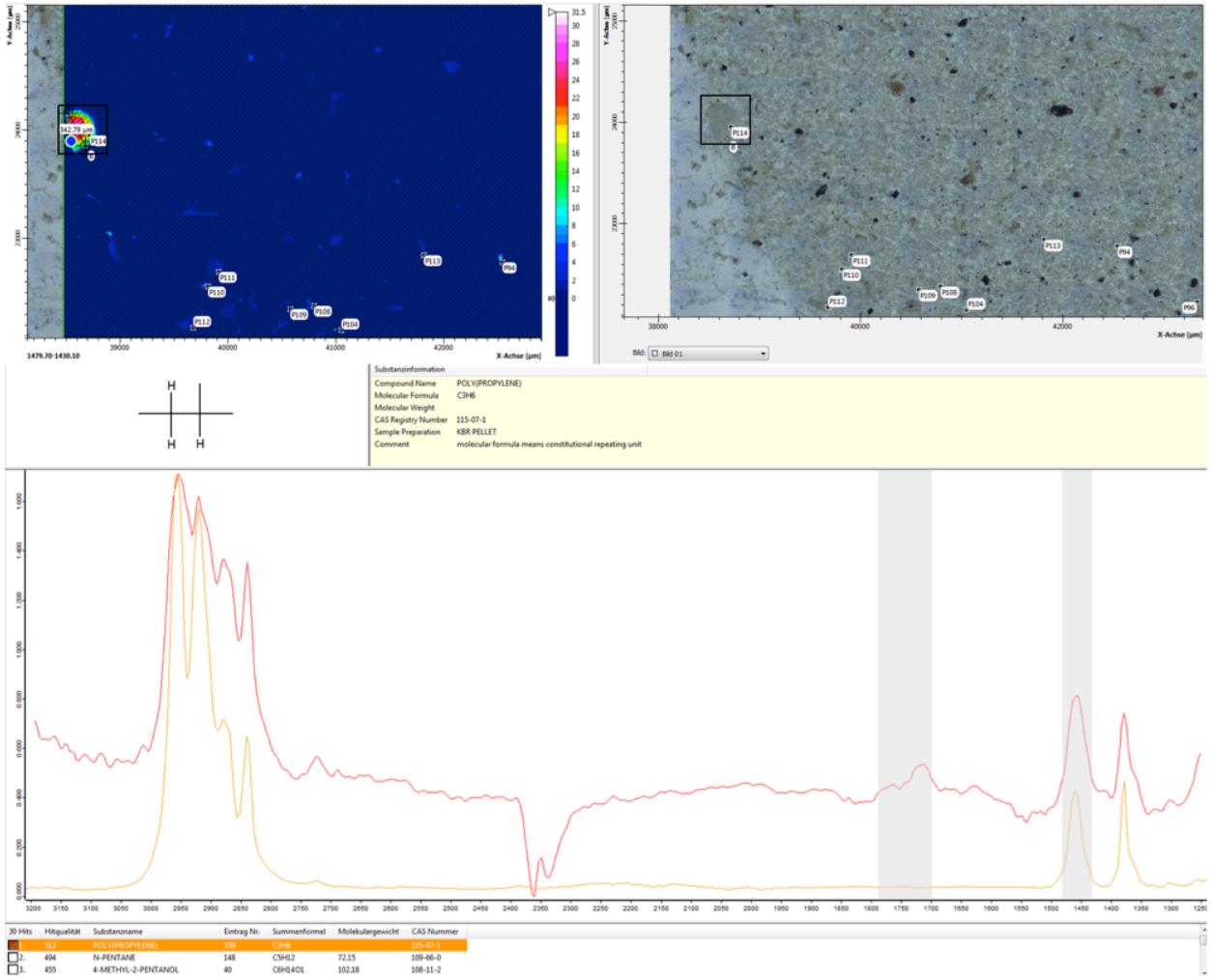

Figure S7: Spectrum ascribed to PP extracted from sample SH_456_13: The optical image on the right showing the surface of the Anodisc filter with a colorless fragment of approximately 343 µm in size (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching PP-spectrum obtained from the database

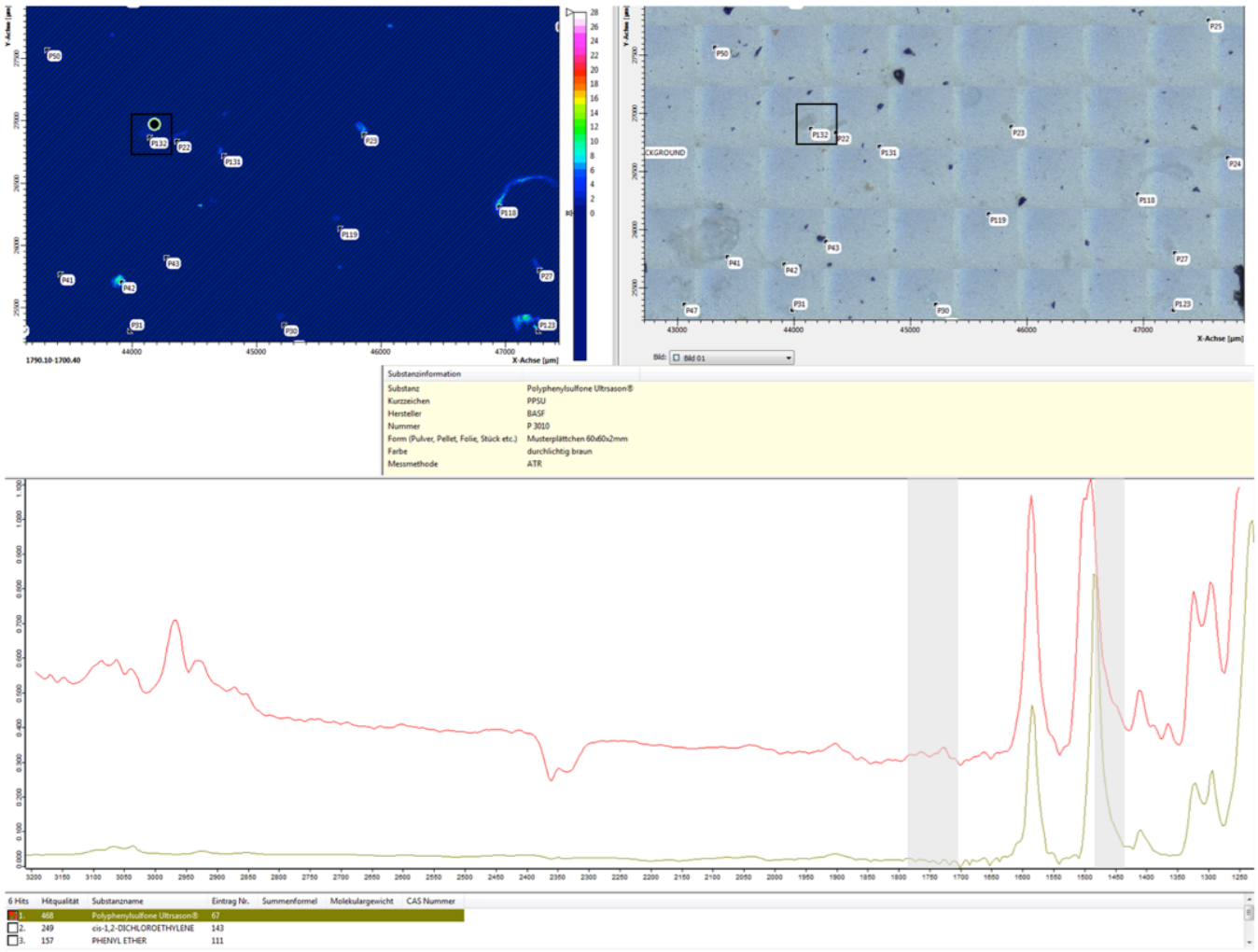

Figure S8: Spectrum ascribed to PPSU extracted from sample SW_456_16: The optical image on the right showing the surface of the Anodisc filter with a lightly beige fragment (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching PPSU-spectrum obtained from the database

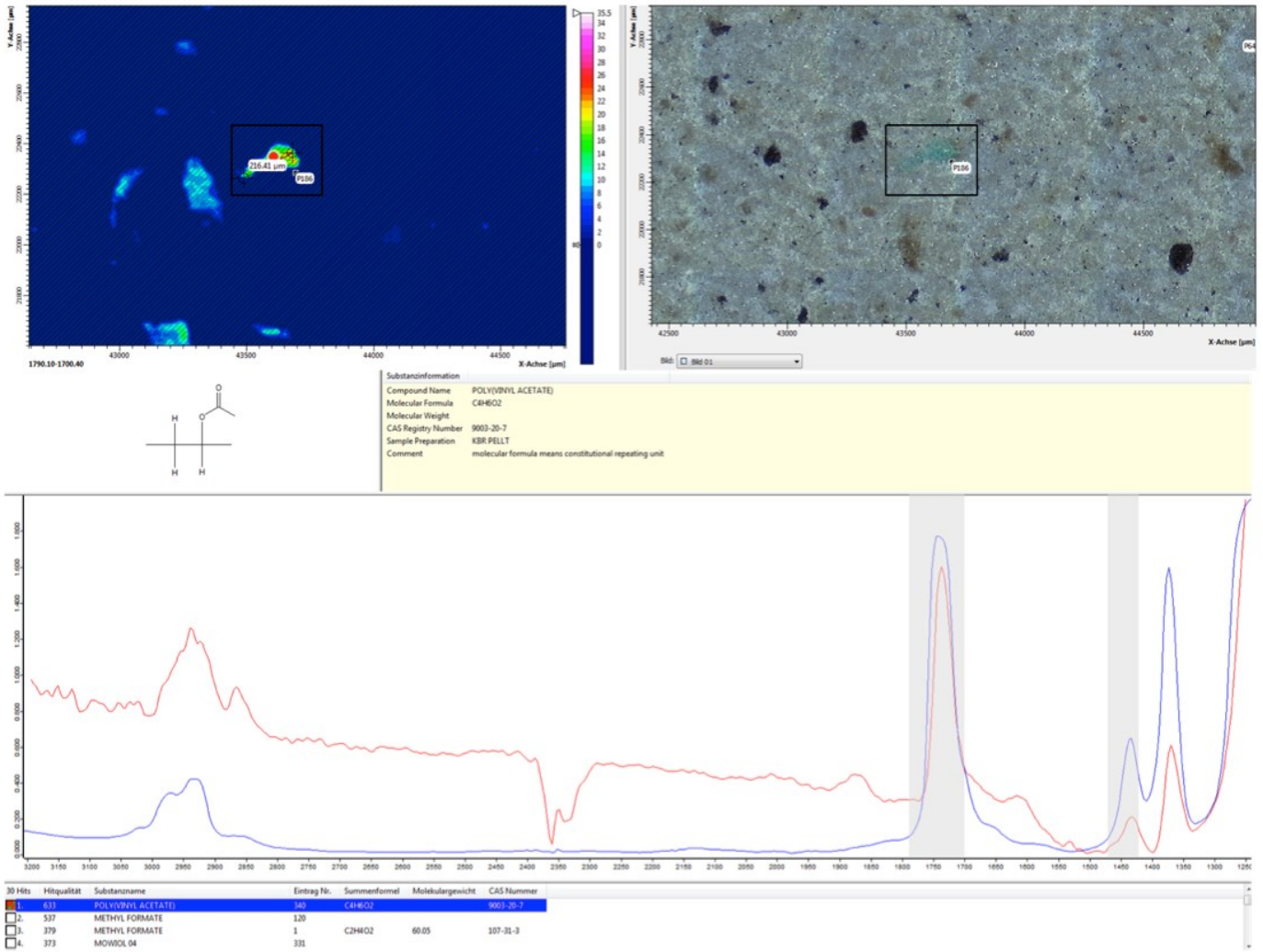

Figure S9: Spectrum ascribed to PVA extracted from sample SH_456_13: The optical image on the right showing the surface of the Anodisc filter with a greenish blue fragment of approximately 216 µm in size (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching PVA-spectrum obtained from the database

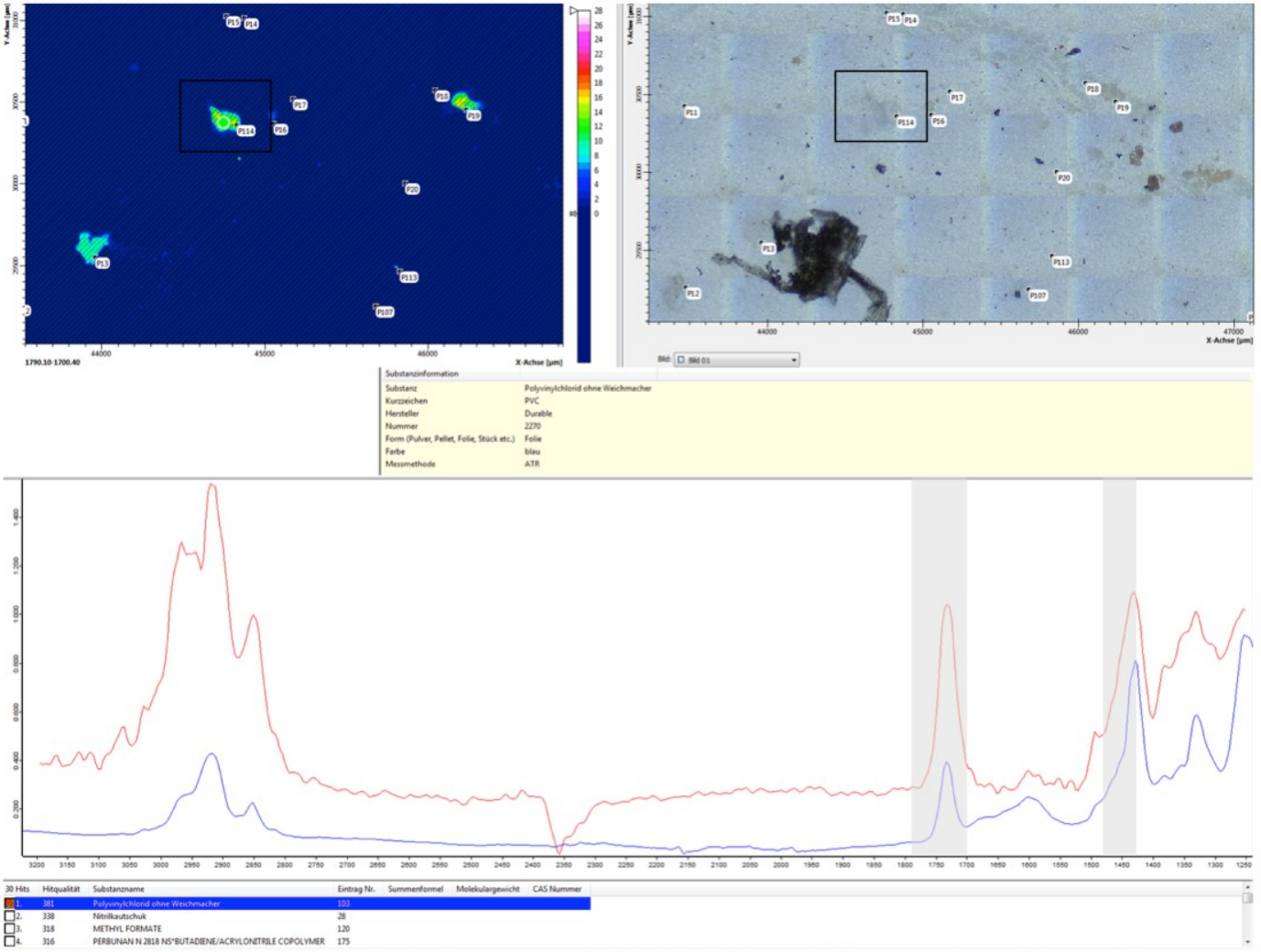

Figure S10: Spectrum ascribed to PVC extracted from sample SW_456_16: The optical image on the right showing the surface of the Anodisc filter with a colorless fragment (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching PVC-spectrum obtained from the database

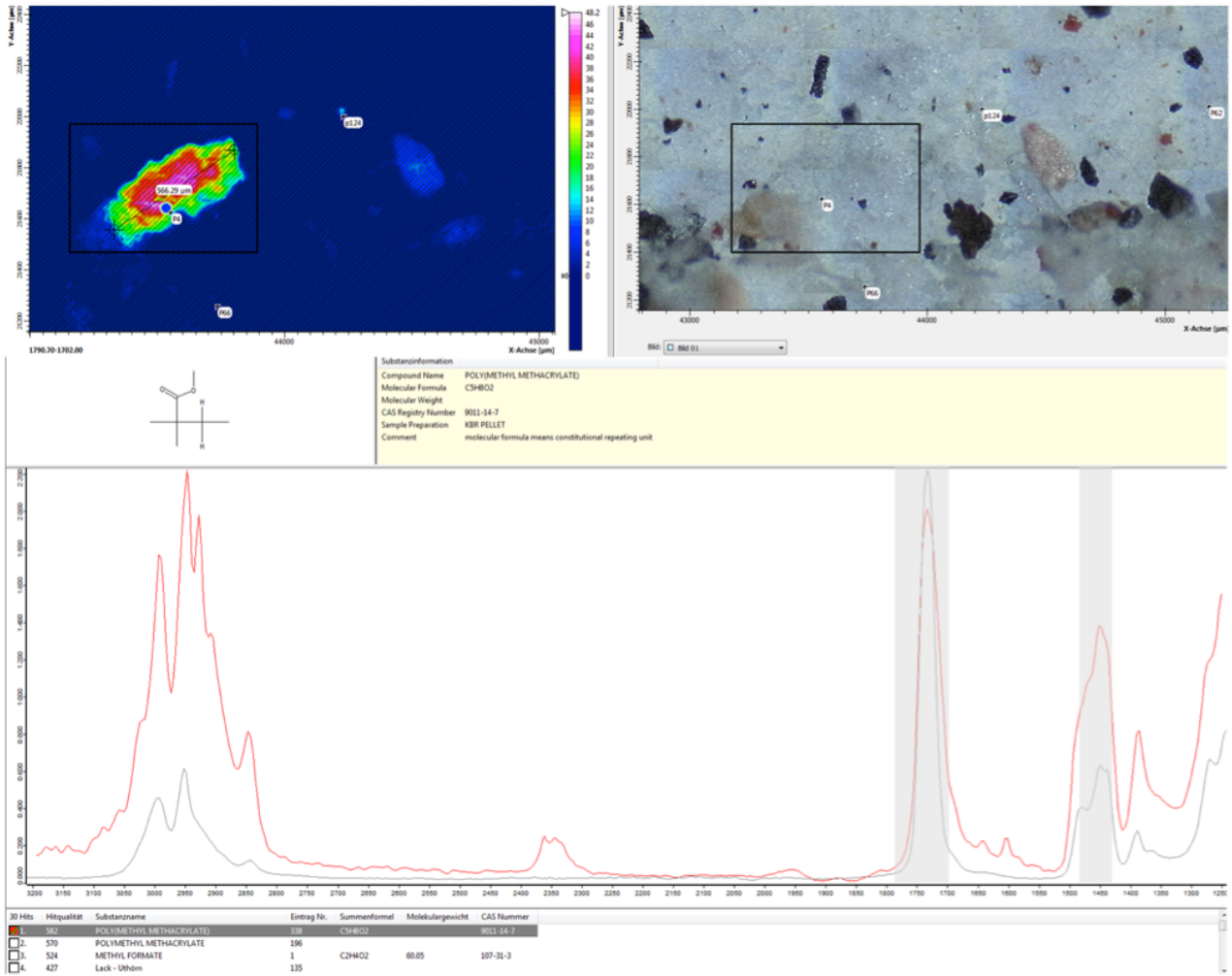

Figure S11: Spectrum ascribed to PMMA extracted from sample H18_21: The optical image on the right showing the surface of the Anodisc filter with a transparent fragment of about 566 µm in size (black frame) on it and the matching false color plot on the left. Below the extracted spectrum and the matching PMMA-spectrum obtained from the database

Table S2: Abundance and distribution of plastic types in the samples that were assigned to the corresponding plastic type with less certainty than the other particles and were therefore excluded from calculations

sample	H19_22	H18_21	H16_19	SW_456_16	SH_456_13	H30_8*	Σ
Polymer type							
EVA	2	1					3
PA			2		1	1	4
PC							0
PE	1	8	4	2	4		19
PEST						1	1
PLA					2		2
PP	11	2	4	5	14	2	38
PPSU							0
PS		1					1
PUR	1						1
PVA					2		2
PVC							0
PMMA		2		1			3
Σ	15	14	10	8	23	4	

N

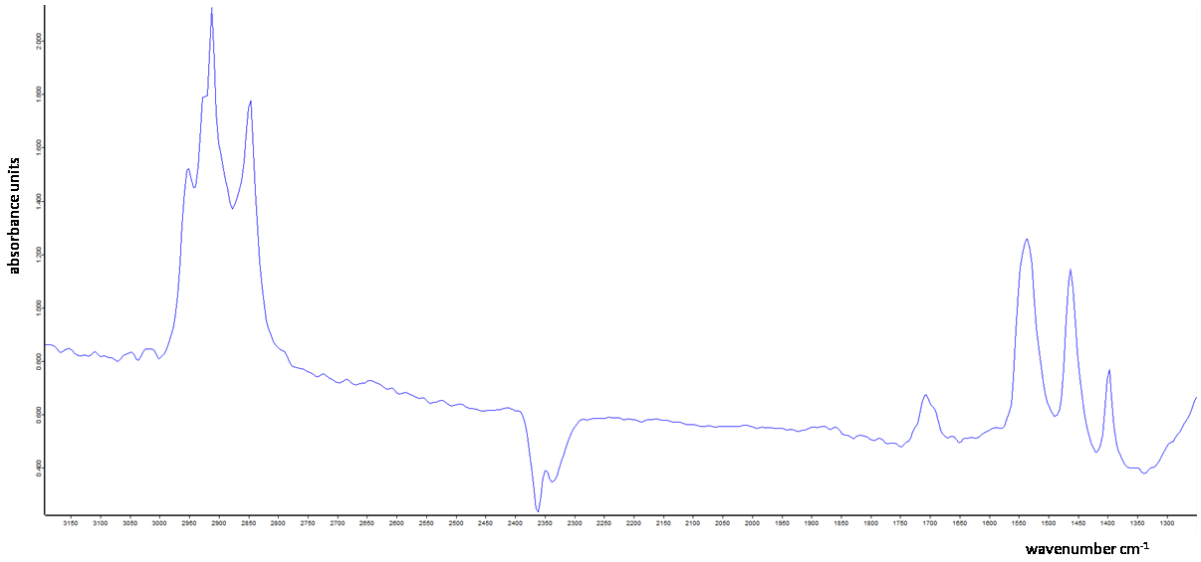

Figure S12: Not assignable spectrum found 35 times in sample H16_19 and 3 times in sample SH_456_13

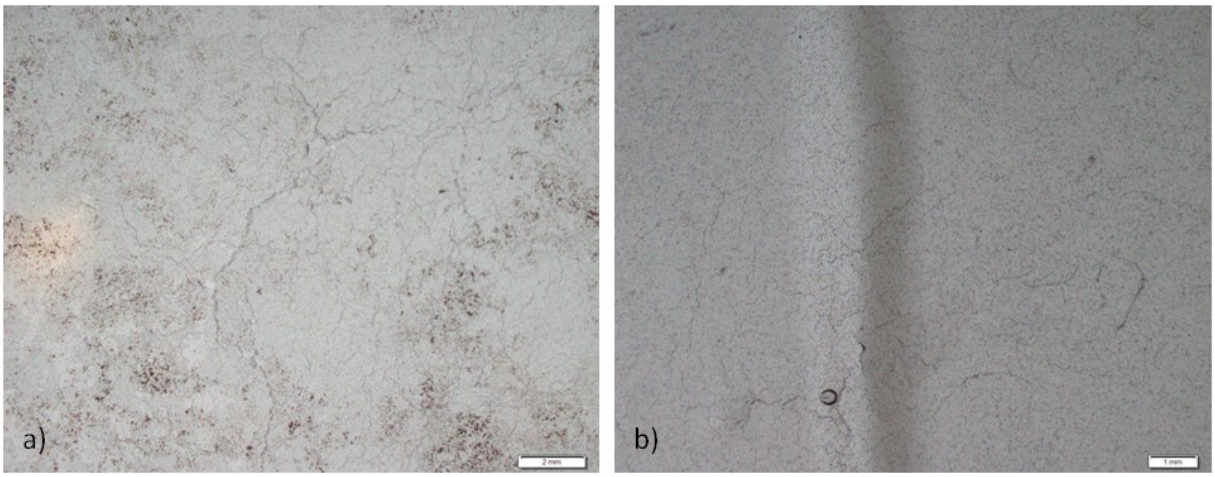

Figure S13: Polycarbonate filter of sample H32_10 (a) and LW_12_1 (b) after a second treatment with H_2O_2 at 50 °C

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Declaration of academic honesty / Eidesstattliche Erklärung

Declaration of academic honesty

I hereby declare that I have written this master`s thesis “Detection of microplastic in marine sediments of the German Coast via FT-IR spectroscopy” on my own having used only the auxiliaries and literature listed. All citations that are taken directly or indirectly out of other sources are indicated as such. Furthermore this thesis has not been submitted to any other university.

Location, Date Signature of the author

Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Masterarbeit „Erfassung von Mikroplastik in marinen Sedimenten der Deutschen Küste mittels FTIR-Spektroskopie“ selbständig und ohne Benutzung fremder Hilfe oder Hilfsmittel angefertigt und nur die angegebenen Literaturquellen verwendet habe. Die aus fremden Quellen direkt oder indirekt übernommenen Textstellen sind zitiert und als solche kenntlich gemacht. Diese Arbeit wurde bisher keiner anderen Prüfungsbehörde vorgelegt, weder in gleicher noch in ähnlicher Form.

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