Universität Rostock

Traditio et Innovatio

# The detection of microplastics in beach sediments

Extraction methods, biases, and results from samples along the German Baltic coast


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von

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**Masterarbeit**

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*To my nieces, Liza and Leonie, and all children –*

*may the beaches you discover be full of joy, sand, and living things.....*

Börgerende beach in sunlight

# Thesis Abstract

This is the first study to investigate the spatial and temporal variations of microplastic concentrations in beach sediments at the German Baltic coast. Two extraction methods, centrifugation and air-venting in high-density saline solutions, are tested, and air-venting in calciumchloride solution is found to be most efficient and least biased for the extraction of microplastics from beach sediments. With the aim to study the sources of anthropogenic microplastic influx, a total of 11 locations were sampled to analyse spatial variations, including four beaches along the west-east current in the wider Rostock area, four beaches around the island of Rügen, and two beaches in the Oder/Peene estuary. One location at the North Sea Jade Bay known to be contaminated with microplastics was chosen for comparison. The four Rostock locations were sampled over a period of 5 months from March to July 2014 to investigate temporal fluctuations. Visual inspection under dissecting microscopes was employed to distinguish microplastics from residual natural sediment. With this method, coloured particles and fibres are shown to provide the safest identification of microplastics. Between zero and 9 coloured particles/kg dry sediment are found, with typical numbers of 1-3 particles/kg observed in most samples. The highest anthropogenic contamination in both microplastic particles and glass fragments is detected near the Oder/Peene outlet into the Baltic Sea, suggesting that industrial and urban river discharge as well as the nearby fishing harbour contribute substantially to microplastic contamination. Comparable concentrations of 1-11 coloured fibres/kg dry sediment are found, and high concentrations of several tens to hundreds of transparent fibres are detected in all samples. The highest total fibre concentration is observed in July at Warnemünde beach, indicating that touristic activity increases the fibre load by up to one order of magnitude. The microplastic concentrations observed in Baltic coast sediments are consistent with the concentrations of coloured particles and fibres reported in earlier studies on the North Sea island of Norderney and on beaches at the Belgian coast using similar methods.

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# 1 Introduction

## 1.1 Microplastics in the marine environment

The existence of microplastics in the marine environment has been known for more than four decades (Buchanan 1971, Carpenter et al. 1972, Carpenter & Smith 1972, Colton et al. 1974) and is confirmed ubiquitously in seawater and sediment samples today (see e.g. the complete review of all microplastics studies until mid 2013 by Ivar do Sul et al. 2014). Carpenter & Smith (1972) were also the first scientists to recognise the ingestion of resin pellets in a variety of pelagic fish species. While pictures of macroplastic debris in the Pacific and Atlantic gyres and of the excessive accumulation of litter on beaches in the most remote locations worldwide have fostered the awareness of plastic pollution over the past 13 years since the pioneering studies by Moore at al. (2001), microplastics have emerged as a an imminent source of plastic contamination in the marine envrionment only recently as a consequence of their eluding presence in sediments and seawater. Over the past decade, microplastics detections have become a growing concern in the scientific community, with a wide range of concentrations between one and thousands of potential plastic particles per kg of dry sediment or per liter of seawater reported (see Tables 4.1 and 4.3 in Leslie et al. 2011 for an overview). These numbers clearly raise the concern for contamination levels that will inadvertantly affect the marine food chain from the smallest planktivours to the largest fish and marine mammal species. Today, the chemical fingerprints of microplastics are detected in the muscle and blubber tissue of the largest filter feeders such as basking sharks and fin whales (Fossi et al. 2012, 2014). As microplastics cannot easily be removed from the marine environment, their presence not only causes health-adverse effects to marine organisms on all scales but are already shown to loop back and infiltrate the human food web (Van Cauwenberghe & Janssen 2014), such that health-adverse effects to humans must also be expected with the long-term presence and exposure to microplastics. With research on microplastics just emerging today and given its high migration potential, it is crucial to quantify the contamination levels and the distribution of microplastics in the world's oceans and seas to assess the ecological risks to sea-dwelling species on all scales from invertebrates to seabirds as well as humans.

Despite increasing standardisation attempts over the past decade, the comparison between studies is still limited by the methodology and the inspection methods employed for microplastics identificaton. The most common procedures include the extraction of microplastic particles and fibres from sediment via floatation and air-venting in high-density saline

solutions, followed by filtration and visual inspection under dissecting microscopes. Spectroscopy is known to be the unique secure way to identify polymers and distinguish especially transparent microplastics from natural minerals (e.g., Hidalgo-Ruz et al. 2012). However, microscopic FTIR or Raman spectroscopy necessary to analyse microplastic samples are rarely available in standard biological or chemical laboratories, and the analysis of large samples of sediment is not feasible even with micro-imaging spectrometry. The evaluation of apparent microplastic particles with micro-spectroscopy after sample extraction was recently shown to be dominated by natural sediment particles rather than synthetic polymers (Lorenz 2014). In addition, synthetic particle losses from artificially enriched samples were shown to increase with the number of refilling and handling steps during the extraction process (Imhof et al. 2012), such that a minimised number of processing steps increases the chances to obtain unbiased microplastics number counts. From these results, it has to be deduced that previous studies were biased in two different directions. First of all, only a small fraction of microplastic particles might have been recovered as a consequence of numerous refilling steps during extraction. Secondly, and more concerningly, large amounts of sediment might have been contaminating microplastics source counts extracted in high-density saline solutions. In this thesis, the biases during sediment extraction are quantified using a simple air-venting method to extract microplastics from sediments as might be used for monitoring purposes. The pitfalls of visual inspection and the consequences of natural mineral suspension in high-density solutions are revealed with the aim to raise awareness of these quantification biases, such that an increasing number of quantitatively comparable studies can be obtained in the near future.

Microplastics are now known to be omnipresent in the marine environment. To date, more than one hundred studies were conducted to measure the concentration of microplastic particles and fibres in surface waters (neustonic net samples), occasionally in the water column, and in sediments along coastlines. The locations and results of these studies were annotated into a world coverage map of microplastic detections as part of the extensive review by Ivar do Sul et al. (2014) reproduced in Fig. 1. This map shows the discovery of microplastics in coastal regions of all inhabited continents, but also illustrates how sparse our knowledge on microplastic contamination is at the present time. Note, in particular, that no measurements were obtained so far in the Baltic Sea. For sediments alone, Ivar do Sul et al. (2014) review 28 studies covering the Mediterranean, the Hawai'ian archipelago and North Pacific Central gyre, Southern Pacific beaches, the British coast including the English channel, the North Sea and Frisian islands, the South Atlantic Ocean, as far as the Japanese and Singa-

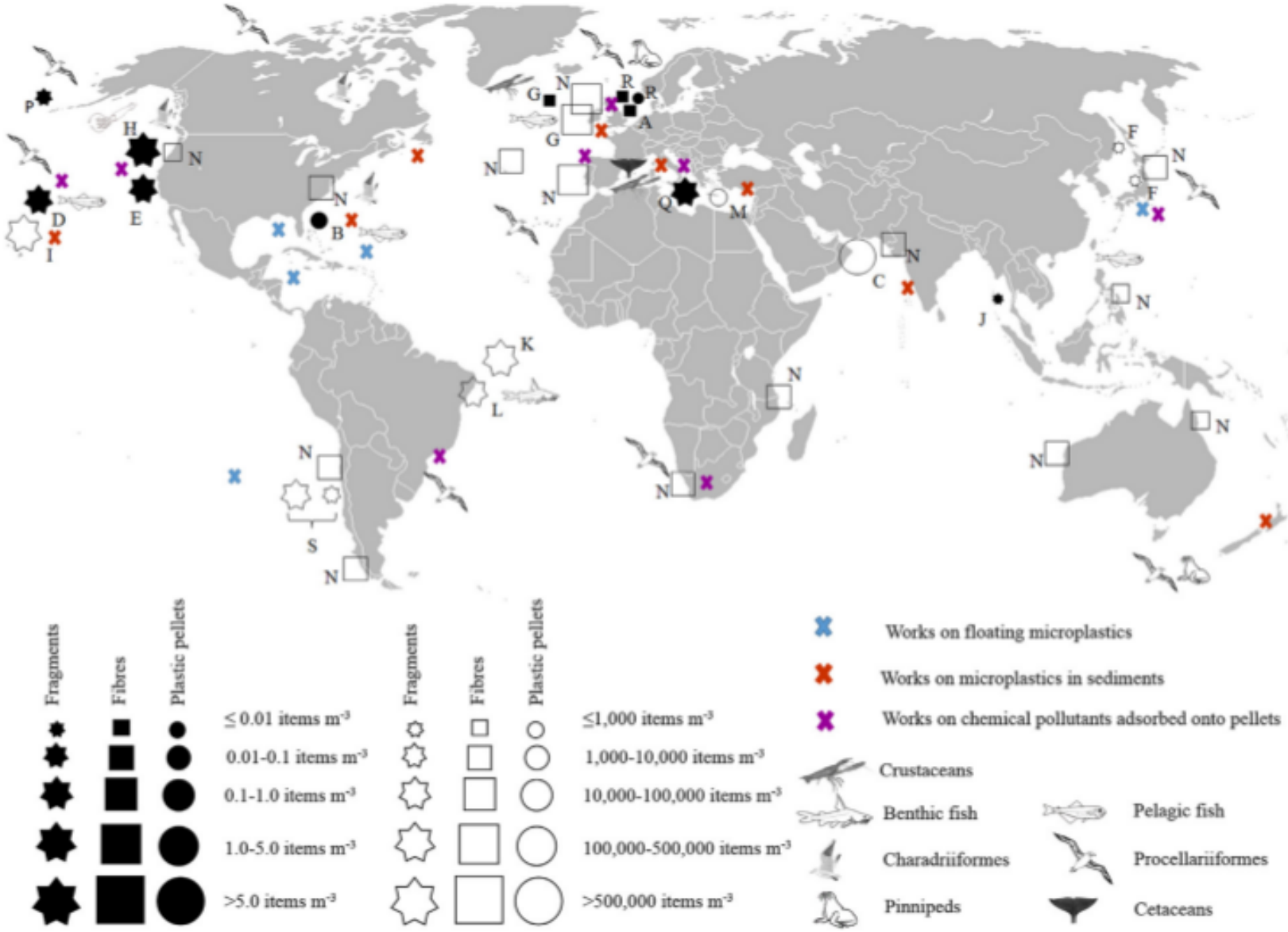


Figure 1: Worldmap of all microplastics measurements published until mid 2013, as compiled by Ivar do Sul et al. (2014). Symbol size represents the measured concentration of microplastic fragments (stars), fibres (squares), and pellets (circles), and is scaled with the mean number of pieces per cubic meter in seawater (filled symbols) and sediments (open symbols). Crosses represent detections outside the presented scales, and detections of microplastics in animals are displayed as representative animal groups. The extensive list of individual references can be found in Ivar do Sul et al. (2014, letters (A)-(S) in their caption to Fig. 1).

pore coasts (see Sec. 2.2 in Ivar do Sul et al. 2014 for individual references). Although the concentrations of microplastics display large variations among beach samples, all published surveys detected microplastic fragments and/or fibres and pellets/granules in their sediment samples. While early studies reported on large concentrations of pre-production pellets as primary microplastic contaminants, the majority of microplastics are composed of degraded fragments and synthetic fibres in varying relative amounts more recently, and hence of secondary sources. While the occurence of primary pellets could be traced to industrial plastic production sites, and contamination levels have declined since better precautions against spilling are in place (e.g., Moore et al. 2001, Ivar do Sul et al. 2014), the contamination by secondary fragments was shown to have increased in recent decades in the North Atlantic survey region (Morét-Ferguson et al. 2010). Secondary microplastics originate from a much larger number of more diffuse sources, with the implication that their influx into the marine environment cannot be as easily controlled and diminished. The degradation of macroplastics to meso- and microplastics over time renders microplastics increases almost impossible to counteract unless an ecologically sensitive way is found to remove macroplastics from the marine world.

The durability of plastics, rendering synthetic polymers [1] beneficial materials in the production of consumer and industrial goods, is also the cause for the long-term persistence of plastic contamination in the marine environment. Although macroplastics break down to microplastic and possibly nanoplastic sizes, mineralisation under marine conditions is slow compared to air exposure (Andrady 2011), and the polymer content is expected to survive over hundreds of years (Thompson et al. 2004). Especially in deeper ocean layers, either in the benthos or in sediments not exposed to mechanical wave action and UV radiation from sunlight, plastics degradation is expected to be very slow (e.g., Andrady et al. 1998). Until the polymeric structure of individual molecules is broken up into monomers and harmless carbon-hydrate compositions (mineralisation), plastics cannot be considered biodegraded. During the entire time of this process, plastics serve both as adsorbers for persistent organic compounds and as leachers of chemical and organic additives. With an increasing number of studies on microplastic contamination and the transfer through the food chain, the consequences for marine organisms are just beginning to emerge.

[1] Polymers such as polyhydroxyalkanoates are produced naturally by bacteria under certain conditions and can also be metabolised for energy consumption when conditions change, and the degradation processes of such natural polymers are reviewed in Shah et al. (2008). Throughout this thesis, the term “polymers” refers to synthetic, anthropogenic materials unless otherwise mentioned.

## 1.2 Definition & origin of marine microplastics

In the executive summary of the *International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris* (IRW), microplastics are defined as particles with sizes of less than 5mm (Arthur et al. 2009). The IRW sets this upper boundary to allow for ecological effects beyond the accumulation in gastrointestinal tracts to be considered (see page 10 in Arthur et al. 2009 for details). No lower boundary is determined, although seawater samples are frequently limited to 333$\mu$m by the mesh size of neuston nets. The minimum boundary of sediment samples is frequently lower when 50-100$\mu$m sieves or 1-5$\mu$m filters are used to collect particles (see also Dubaish & Liebezeit 2013).

Two kinds of marine microplastics are distinguished throughout the literature on the basis of their origin, and were also defined by the IRW (Arthur et al. 2009). *Primary microplastics* originate from spillage during plastic production or recycling, from sandblasting in shipyards and other abrasives, and from microcleansing particles in personal care products. All of these primary microplastics share the common property that they are designed to be small during their production process. *Secondary microplastics* comprise broken fragments of larger plastic pieces, including, but not limited to, marine litter, derelict fishing gear from industrial and recreational fishing, litter from landfills, painting flakes from ship hulls, synthetic fibres from laundry discharge, and foil fragments from packaging, industrial or agricultural sources.

In the European Union (EU), 57 million tonnes of plastics were produced in 2012 (PlasticsEurope 2013), and global plastic production increased by 2.8% from 2011 to a total of 288 million tonnes in 2012. Of the 25.2 million tonnes post-consumer plastics accrued in the EU in 2012, about 60% (15.6 mio t) are claimed to be recycled or burned for energy recovery, while almost 40% (9.6 mio t) needed to be disposed off in landfills (Figure 10 in PlasticsEurope 2013). While in Germany 98% of post-consumer plastic waste is quoted to be recycled or combusted, many other European countries predominantly use landfills to dispose of plastics (disposal rate in countries with landfills between 37% and 87%, Figure 13 in PlasticsEurope 2013). The fraction of plastic litter entering the seas from this reservoir is not known. From the large amount of macroplastics produced and discarded both in industry and in household items every year, and from the observation that a significant fraction of macroplastic litter at sea originates from fisheries and ship transport (OSPAR 2009), it can be expected that secondary plastics comprise by far the largest volume of microplastic debris found in the marine environment. Biodegradation is extremely slow, which creates the valueable effect of durability of plastic products, but causes a major problem in

the marine environment. As all rivers flow to the sea, the oceans provide the largest sink for undegraded synthetic polymers down to molecular sizes. With UV, oxidation, mechanical or bacterial degradation times of several hundred years (Thompson et al. 2004), the current rate of increasing plastic production and the expected enrichment of the environment and oceans with both macro- and microplastics imply that contamination of the food chain will proceed, even if particle input could be stopped instantaneously. The contribution of fishing line fibres and the degradation timescale of synthetic net material are presently unknown. Synthetic clothing likely comprises a major fibre source especially in coastal waters. A single polyester fibre shirt released 1900 fibres in a single washing (Browne et al. 2011). One particular problem for marine and riverine environments is that both fibres as secondary microplastics as well as (primary) microspheres from personal care products can pass sewage treatment plants (Magnussen & Norén 2014). While rivers serve as transport vectors for anthropogenic litter, sea and ocean sediments serve as the ultimate sink for both light-weight and heavy polymer fragments (see also Leslie et al. 2011). Yet, concentrations found in the seawater column also point towards a land-based origin. In a seawater survey along the North Canadian coast and into the Pacific, Desforges et al. (2014) found a 4-27 times increase of microplastics concentrations from the open ocean to near-shore locations (Fig. 2, left panel). In their seawater samples at a depth of 4.5m, average fibre concentrations are 75% of all microplastic pieces, yet fibre concentrations of $> 90\%$ are found near the shore (Fig. 2, right panel), which leads Desforges et al. to conclude that land-based sources are the most likely origin of the high microplastic concentrations. Desforges et al. also found that microplastic concentrations are unexpectedly high in the little inhabited Queen Charlotte Sound, suggesting that closed ocean basins are particularly sensitive to the capture and enrichment of seawater with microplastics. The same conclusion is anticipated for enclosed estuaries such as the Oder/Peene river outlet into the Baltic Sea, and trapping causes the Baltic Sea Basin to serve as a sink for microplastics.

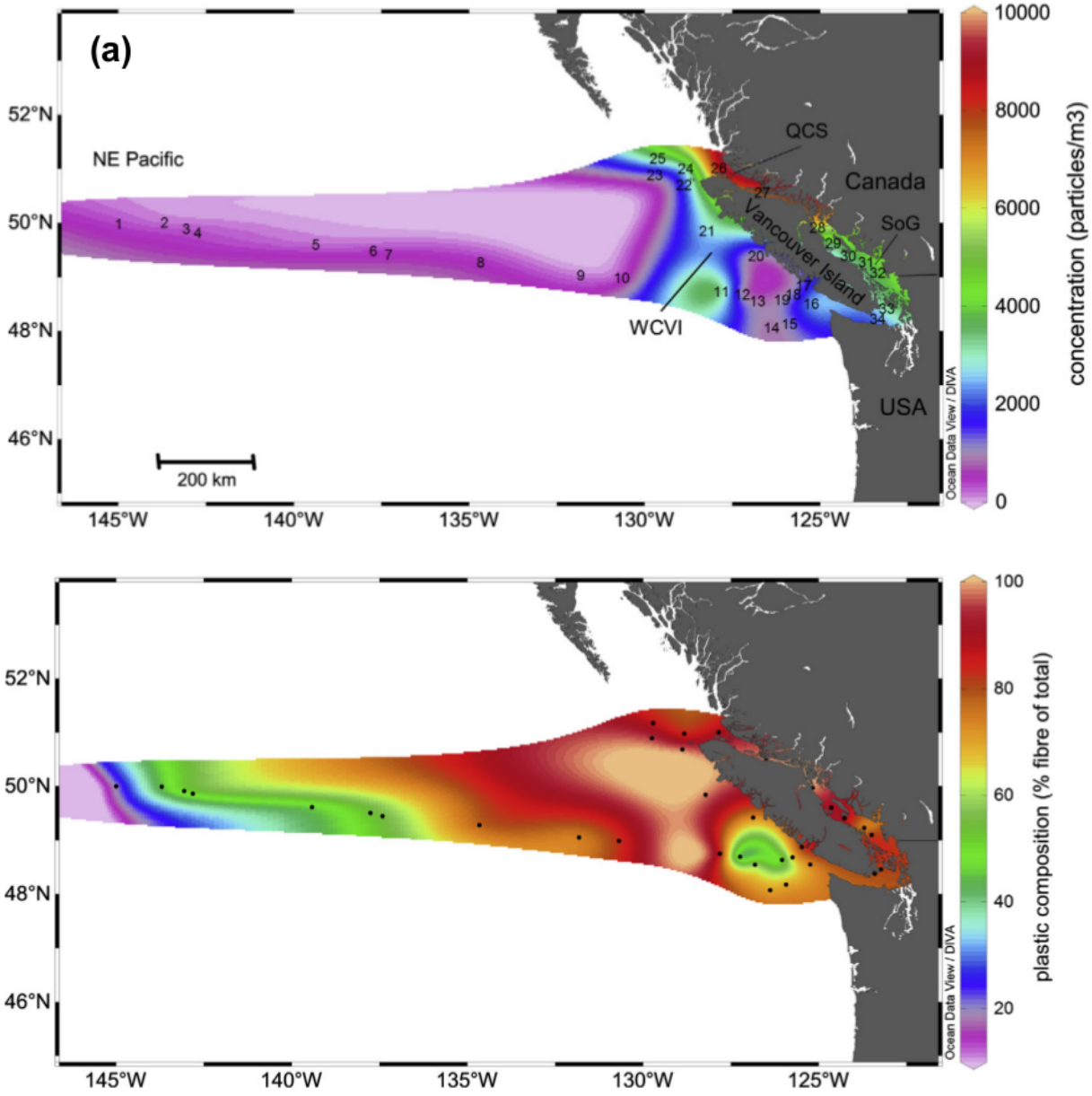


Figure 2: Map of the concentration of potential microplastic pieces (including fragments, filaments, thin foils, and fibres) in 0-10000 pieces/m$^3$ sampled at a seawater depth of 4.5m from the Canadian Coast into the open Pacific Ocean (*left panel*), and percentage of synthetic fibres among all microplastic pieces (*right panel*). Figures reproduced from Desforges et al. (2014), their Figs. 1 and 2.

## 1.3 Detections of microplastics in the marine environment

In Baltic Sea coastal waters, Magnusson & Norén (2011) found average concentrations of 4 fibres/liter and 32 anthropogenic debris particles/liter, as quoted in the WP3 GES-REG report (Ojaveer et al. 2013, p. 3, original study not in english). While further studies in the Baltic Sea area are not yet available, several groups have addressed microplastics contamination in the North Sea. In seawater samples obtained in a Skagerak transect at the outlet of the Baltic into the North Sea, Norén & Naustvoll (2011) found blue particles in 15 of 17 of their samples, with a predominant size range of 10-100$\mu$m. Alarmingly high concentrations of 102 microplastic spheres per liter of seawater are found by Norén (2008) in Stenungsund industrial harbour near a polyethylen production plant. The characteristic size range of 0.5-2mm of these spheres is large for marine microplastics and covers the size range of prey for juvenile fish. Increasing evidence indicates that the vicinity of urban areas increases the concentration of microplastics in surface waters and in beach sediments. In excess of 1200 particles/liter, by far the highest microplastics concentrations reported in the North Sea environment, are detected in seawater samples in the densely populated Jade Bay serving as a discharge site for industry and the Wilhelmshaven sewage treatment plant (Dubaish & Liebezeit 2013).

In submerged sediments in the UK, microplastics and fibres are found in 23 out of 30 samples (Thompson et al. 2004), indicating that microplastics were effectively transported from the water column to sediments over the past decades, and are omnipresent in benthal environments today. As in seawater samples, a wide variety of concentrations of potential microplastic particles is reported in sediments as well. In remote locations, microplastic contaminations between 1-2 particles/kg dry sediment are found at the island of Norderney (Dekiff et al. 2014), while a maximum of 50,000 particles/kg is reported for the island of Kachelotplate (Liebezeit & Dubaish 2012). However, Lorenz (2014) recently found between 34 and 74 particles/kg dry sediment in three off-shore locations on the wider Helgoland shelf and two beach sediment samples on the island of Sylt and showed that a significant fraction of particles after floatation are natural minerals using FTIR spectroscopy, rendering previous high number counts uncertain.

Globally, maximum meso- to microplastic loads are observed in sediment samples obtained close to the drift line at the highly littered Kamilo beach on Hawai'i, where a mean plastic load of 3.3% and a maximum of 30% by weight is observed (Carson et al. 2011). In the most recent ecological status report from the $\sim 50$ year timebase of the Continuous Plankton Recoder (CPR), the amount of microplastic fragments is mentioned to be increasing

in the Northeast Atlantic region, and an increasing number of captures of monofilament netting at the CPR unit are recorded in the southern North Sea (Edwards et al. 2007). In the North Atlantic region, the number concentration of microplastic particles increased by 18% between 1991 and 2007 (Morét-Ferguson et al. 2010, but see also Law et al. 2010), although the concentration of plastics per weight decreased in the same timeframe. During a period of $\sim 40$ years, an increase of marine microplastics is observed in the North Pacific central gyre (Goldstein et al. 2012). Comparably high concentrations (0.3 particles/m$^2$ of seawater) as found in the North Pacific gyre are also reported for the Mediterranean Sea (Collignon et al. 2012, Fossi et al. 2012), and can be expected to increase further with the increasing influx of litter and degradation over time. If fragmentation is the major source of secondary microplastics, this implies that increasingly smaller sizes are available to be mistaken for food and infuse the marine food web.

Beaches with high macroplastic loads are reported to contain microplastics as well, e.g. on Hawai'ian beaches (Carson et al. 2011) and in the Greek Archipelago (Archipelagos institute 2014). Beach litter at the German Baltic Coast and the North Sea is dominated by plastics, with 59% of all beach macrodebris found to be plastics on North Sea Beaches (Umweltbundesamt 2010a). At German North Sea beaches, fishing gear (rope & net) and shipping litter constitute the majority of marine debris (OSPAR 2009), whereas plastic bags and bottles from land-based sources are the predominant litter items at the Baltic coast (Umweltbundesamt 2010a). While broken down fragments are expected to accumulate at severely littered beaches, microplastics and macroplastics are exposed to different mechanical forces over the course of time. A systematic investigation on the North Sea island of Norderney yielded no direct spatial correlation between beach microplastics and macrodebris (Dekiff et al. 2014). Such a correlation is also not expected on physical grounds, as microparticles and -fibres must have different wind and water (rain or surf) resistence and relocation properties than macrolitter pieces. Microplastics are therefore expected to accumulate in locations that cannot be deduced from the presence and amount of macrolitter alone. One of the major differences between macro- and microplastics is the expected infusion of sediments with microparticles and -fibres, which might lead to increasing levels of plastic enrichment over time. The large volume of microplastics increases the chances of chemical leaching, such that microplastics have a higher per weight capability to release toxic additives into the environment. At the same time, they resemble prey items for a substantially larger variety and number of zooplankton species, but possibly also for beach-feeding bird species such as sandpipers, thereby penetrating the marine food web from the bottom upwards at an unknown scale. For these reasons, microplastics have to be moni-

tored individually and the presence of microplastics and their potential for adverse effects in the marine environment cannot be deduced from the quantity of macroplastics alone.
With this study, we contribute to the detection of microplastic particles and fibres, and hope to contribute to the definition of standardised methods for the extraction, observation, and quantification of microplastic contents in sediments.

## 1.4 Hazards of microplastics

The numbers of potential synthetic particles are found to be increasing with decreasing sizes (Norén 2008). In a Skagerrak seawater survey, 95% of particles with sizes 10-500$\mu$m were found to be smaller than 100$\mu$m and hence in the same size range as phyto- and zooplankton (Norén & Naustvoll 2011), rendering microplastics probable prey targets for plankton feeders. As microplastic particles and fibres with their resemblance to phyto- and zooplankton occupy the bottom of the marine food chain, contained toxic compounds infiltrate the marine ecosystem from filter feeders to increasingly larger predators.
Both macro- and microplastics contain on average 4% of chemical additives, predominantly plasticizers such as phtalates, phenols, and bisphenol A now known for their adverse health effects in humans (Meeker et al. 2009, Umweltbundesamt 2010b), on animals with potential relevance for human health (Talsness et al. 2009), and on wildlife including marine species (Oehlmann et al. 2009).
Additives may consist of persistent organic compounds with high toxicity levels which enter the tissue of marine organisms upon consumption, e.g. as endocrine disruptors shown to interrupt the natural sexual development of fish (Oehlmann et al. 2009). Microplastics build up a growing surface area as they fragment, facilitating the adsorption of hydrophobic persistent organic pollutants (POP) and toxic molecules from the water column. POP concentrations were observed to be $10^5 - 10^6$ times higher in resin pellets collected on Japanese beaches than in surrounding seawater (Mato et al. 2001, Endo et al. 2005), and were found to be similar to concentrations in microplastic particles collected in the North Pacific Central Gyre (Rios et al. 2007, Teuten et al. 2009). Surveys find elevated POP levels in plastic debris collected both in the open ocean and in beach samples (Hirai et al. 2011), and pellets are used as tracers for global mapping of POP contamination from fertilisers and other anthropogenic sources (Ogata et al. 2009). In contrast to the spatial distribution of species, microplastics are not limited by the thermal and trophic food production boundaries of marine ecosystems. The large ocean circulations distribute both macro- and microplastics and their constituents across the worlds ocean bodies continuously (e.g., Moore et al. 2001,

Law et al. 2010). With the potential accumulation of microplastics in the marine food web, microplastics and their additives are prone to come back to the human plate. Knowledge of health effects in humans is still limited, but becomes a growing concern in the presence of the plastic mixing in the oceans.

A large number of studies concerned plastic intake of seabirds and the variation of plastic amounts and types over time. Microplastics are frequently mentioned, yet specific studies for microplastics are not reported, and macro- and microplastics are not analysed separately. Nevertheless, the intake of small plastic fragments is certainly concerning in seabirds as well as pelagic marine species. As ingestion in plankton species is more specific to the problem of microplastics in near-shore environments addressed in this project, we focus on pelagic species here. A concise summary of microplastic intake by seabirds is given in Ivar do Sul et al. (2014).

In laboratory experiments, ingestion of microplastic granules is evidenced in a growing variety of marine species (Fig. 3). Among them bivalves (Blue Mussel, von Moos et al. 2012), copepods (Cole et al. 2013), as well as amphipods, barnacles and lugworms (Thompson et al. 2004), representing some of the most omnipresent zooplankton species in the oceans. A concise overview of the increasing amount of references is given in the introduction of Van Cauwenberghe & Janssen (2014). Although the microspheres used in laboratory feeding and transport experiments are 1-2 orders of magnitude smaller than the microplastics investigated in this study, they illustrate the potential for ingestion of microplastics on all trophic levels of the food chain.

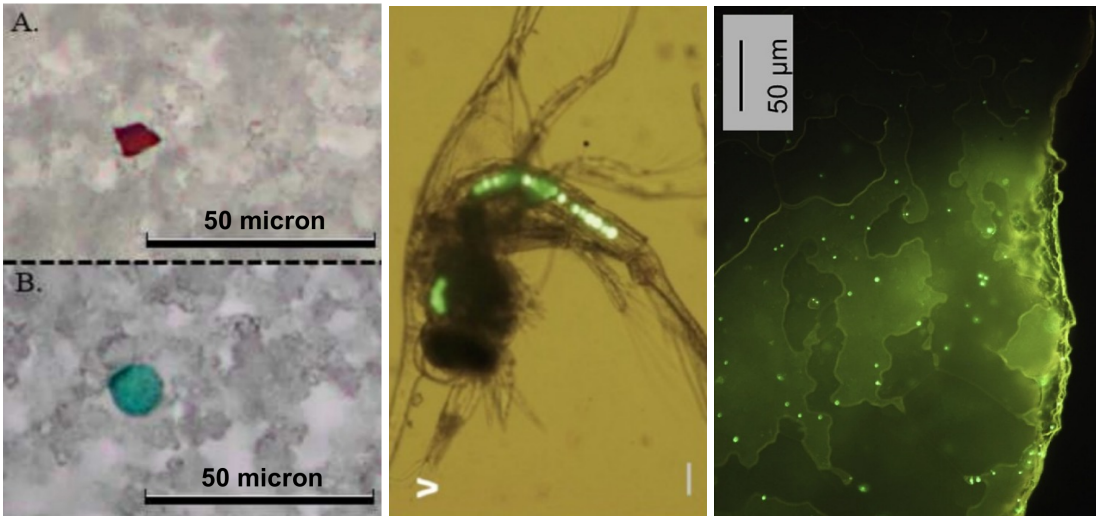


Figure 3: Ingested microplastic particles in mussel tissue produced for human consumption (*left panel*, Van Cauwenberghe & Janssen 2014, their Fig. 1), fluorescence marked microspheres ingested in copepods (*middle panel*, Cole et al. 2013, their Fig. 1), and transported into the gill lamella of crabs after feeding on microplastics-fed mussels (*right panel*, Farrell & Nelson 2013, their Fig. 2).

Laboratory exposure of different types of invertebrates resulted in microplastics ingestion (Graham & Thompson 2009). The ingestion and transfer of microplastic spheres of $10\mu$m size from mesozooplankton to the macrozooplanktonic level was shown by Setälä et al. (2014). All of the six varied mesozooplankton species exposed to microspheres ingested these plastics at various levels, and zooplankton prey as well as marked microspheres were identified in mysid shrimp intestines after just 3 hours of exposure to microsphere-fed mesozooplankton. In a similar study, feeding 0.5$\mu$m fluorescent microspheres to mussels which were then offered to crabs, the microspheres occupied vital organs including the gills and ovaries and had penetrated into the haemolymph of the crabs (Fig. 3 (right panel), Farrell & Nelson 2013). In addition, tissue inflammation was observed in mussels after microplastic particles were deposited in their intestinal tracts (von Moos et al. 2012). The presence of microplastics was recently confirmed in aquacultured bivalves produced for human consumption (Fig. 3, left panel). Van Cauwenberghe & Janssen (2014) found on average $0.4 \pm 0.1$ particles/g of wet tissue in mussels (*Mytilus edulis*) commercially cultured in the German North Sea and Atlantic oysters (*Crassostrea gigas*) from France. Estimating an average intake of 1800 microparticles/year for typical amounts of yearly European bivalve consumption per person, the study shows that microplastic particles do not only affect the marine ecosystem but perfuse the human food chain already today.

Although lab experiments work with high concentrations of microspheres and not under typical environmental conditions, the ingestion of microplastics is demonstrated in an increasing number of species in the wild. In the North Pacific Central Gyre, 33% of gooseneck barnacles comprising the rafting community on macroplastic debris contain ingested microplastics (Goldstein & Goowdin 2013). Similarly, Lusher et al. (2013) analysed the digestive tracts of five pelagic and five benthic fish species and found microplastic pieces in 36.5% of all animals, with a precedence for fibres (68%). The material of recovered plastic pieces was identified to be polyamide, polyester, and rayon by FTIR spectroscopy, suggesting anthropogenic fibres (fishing net, clothing, hygiene products) as the source for plastic intake. Comparable fractions of plastic intake are found in fish residing in the North Pacific Central Gyre (Boerger et al. 2010) and Brasilian estuaries (Possatto et al. 2011, see also the discussion in Lusher et al. 2013).

The IRW report identifies three physico-chemical effects on zooplankton species upon ingestion of microplastics (Arthur et al. 2009). The physical blockage of the digestive tract is reminiscent to the effects of macroplastics on species in higher trophic levels of the food chain. The large surface-to-volume ratio of microplastics and the adsorbing power for organic compounds raises the toxicity level with increasing microplastic intake. Leaching of

toxic and endocrine disruptive molecules in the intestinal tracts of zooplankton species might contaminate the blood stream and directly affect the neural system. Leaching of endocrine disruptive chemicals into the water was claimed responsible for changes in both the sex distribution of fish as well as for abnormal transsexual mutagenesis and limited reproduction capability (Oehlmann et al. 2009, Carlisle et al. 2009). These effects would be enhanced if leaching occurs inside the organism instead of into the ambient water at a much higher rate of dilution. The third effect of concern is bioaccumulation, which affects all species throughout the food web via direct or indirect intake of microplastics. Plastic additives (phtalates) were detected in the muscle tissue of basking sharks (Fossi et al. 2014) and in the blubber of stranded Mediterranean fin whales (Balaenoptera physalus) by Fossi et al. (2012), who correlated the phtalate rates to the measured abundance of microplastics in surface water samples and their phtalate content, concluding that microplastics are a likely origin, causing phtalates to be accumulated in the blubber through the large amounts of filtered water and small prey intake in these baleen whales (see also the discussion in Baulch et al. 2014). The map presented by Fossi et al. (2012) of the concentration distribution of microplastic particles in the Mediterranean shows the strongest concentrations in coastal waters, where the breeding grounds of fisheries are located. The same authors provide a summary of the detections of microplastic particles *in vito* in a diversity of planctivorous fish species in different benthic layers (see their Sec. 4, and references therein). Although not discussed in their study, their map is one of the first indications that microplastic contamination might be capable of influencing the juvenile stages of higher marine species dependent on phyto- and zooplankton in the sensitive coastal ecosystems.

Lithner et al. (2009) showed that leachates from 32 plastic materials caused toxic effects in freshwater fleas *Daphnia magna*. Toxic effects of the most common microplastic materials found in the marine environment were established in green algae, Baltic Sea amphipods, and freshwater fleas (Balode & Muzikante 2013). On the basis of these tests, negative effects were observed on all zooplankton species from 60% of the analysed plastic products. Polyurethan in the form of dishwashing sponges had the most adverse effects of all polymers tested. While this is not surprising for green algae, where adverse effects are warranted to avoid algae growth in wet sponges, the high mortality rate of 30-100% observed after 72h exposure in freshwater amphipods in the presence of dishwashing sponge leachates is particularly concerning, as comparable items and materials are used in most household kitchens. Of the six materials tested, polypropylen proved to have the least adverse effects on crustaceans.

In addition to toxicity effects, microplastics are capable of altering the physical properties

of beach sediments. Carson et al. (2011) analysed sediment cores designed to represent the mean sediment grain and microplastic size distribution as found in the drift line at Hawai'ian Kamilo beach. Carson et al. demonstrated that the permeability of beach sediments had increased due to the addition of 15% or more microplastics (by weight) with a larger mean particle size than the natural sediments. Increased water flow into and evaporation from the sediment might change the distribution of nutrients and organic matter as well as zooplankton species and hence the biological and chemical composition of the litoral and sublitoral zones. At the same time, thermal transport and maximum warming temperatures are decreased with only 1.5% microplastics as compared to uncontaminated beach sediments with the same natural properties. These physical changes might affect hatching of beach-nesting species and particularly might alter the sex determination in sea turtles in a systematic way. Carson et al. (2011) suggest that the decrease in temperature could lead to a lower fraction of female seaturtles, possibly increasing the high strain on the populations even further. This is particularly crucial as Hawai'ian beaches are one of the predominant nesting sites for various turtle species, but also in view of the fact that increasing numbers of Asian beaches are littered with plastics. Although the impairing effects of such a bias on other populations are not yet known, the evolutionary adaptations of nesting and sand-dwelling species on thermal properties of selective beaches are undermined in the presence of altered physical conditions imposed by microplastics.

In addition to transport of toxic compounds not naturally found in the marine environment, microplastics (and plastics in general) were suggested to facilitate the transport of pathogenic germs and plankton species from their native regions into uncontaminated zones. Microplastics serve as floatation devices, but might also serve as feeding grounds for organisms in the presence of biofouling (algae, bacterial growth, Ye & Andrady 1991).

In the most current census, 663 species of marine animals and birds are found to be affected by marine debris (Galgani et al. 2013). While most of the physical encounters between species and marine debris are linked to entanglement in derelict fishing gear (Galgani et al. 2013), the ingestion of both macro- and microplastics has become an increasing thread with the rising levels of debris deposited in the marine environment. With the aim to counteract the described hazards and ensure the good ecological status of the European marine environment, the European Union explicitely refers to marine litter in Descriptor 10 of the Marine Strategy Framework Directive (EU, MSFD, Annex III), and requests the characterisation of *"trends in the amount, distribution and, where possible, composition of microparticles (in particular microplastics)"* (Criterion 10.1.3), including microplastics ingested by marine animals (Criterion 10.2.1). Even though international initiatives exist on

the alleviation of marine litter (e.g., UNEP regional seas, Jeftik et al. 2009, MARPOL, HELCOM), microplastics are not included in the monitoring guidelines for marine litter due to the technical challenges involved (UNEP/IOC Guidelines on Survey and Monitoring of Marine Litter, Cheshiere & Adler 2009, see especially page 16). The fact that no uniform monitoring strategy is presently available, and that the true extent and influence of microplastics in the marine ecosystem are only beginning to emerge, underlines the necessecity for systematic microplastics analyses.

## 1.5 Technical challenges in the detection of microplastics

Naively, the assumption might be made that microplastics are light-weight particles that always float on the water surface. With specific densities of up to 1.5 g/cm$^3$ for polyvinylchloride (PVC), one of the most frequently employed material for hardshell plastics (DVDs, drinking bottles, cell phones, and many more standard household items), this assumption fails for a majority of secondary plastic fragments. In addtion, biofouling causes sinking of buoyant plastics (Ye & Andrady 1991), such that sediments from the deep sea to the litoral regime are expected to contain increasing levels of microplastics (Leslie et al. 2011). Among these, beach sediments are most easily accessible, and reflect the amount of microplastics washed towards the coastlines with the tidal flows and storm events as well as local influx pathes from the shore.

The methods for collecting microplastics from water and sediment samples were recently reviewed by Hidalgo-Ruz et al. (2012). For the extraction of microplastics from sediments, two methods are identified, which are both based on density separation between microplastic particles and fibres from natural minerals with a higher specific density: i) air-venting or shaking in high-density saline solutions (zincchloride $ZnCl_2$, sodium tungstate $Na_2WO_4$, and sodium iodide NaI) and decanting of the supernatant onto membrane filters, and ii) centrifugation of small amounts of sediment, possibly with a previous floatation stage. These density extraction methods are sensitive to two types of biases. Depending on the decantation of the supernatant from the saline solution and on the chosen solution density, the smallest size fraction of natural sediment particles (minerals) is likely to contaminate the light-weight floating particles. Indeed, the spectroscopic examination of presumable microplastics extracted via air-venting in $ZnCl_2$ solution confirmed only a few percent as synthetic polymers, while in excess of 90% of the extracted particles were natural minerals (G. Gerdts, private communication). In this case, density separation without further inspection methods has led to a severe overestimation of the microplastics contamination in sediments. The second

bias relates to the high level of stickiness of microplastics even to smooth glass and metal walls. Imhof et al. (2012) systematically analysed sediment samples artifially enriched with the eight microplastic particle types predominantly retrieved from ecological systems and found loss rates of up to 70% due to refilling and handling steps. Designing a cylindric metal extraction system optimised for sediment-plastic separation in zincchloride solution, the *Munich Plastic Sediment Separator* (MPSS), Imhof et al. (2012) were able to recover 95+-2% of small plastic particles $< 1$mm, while they found that on average only 40% of the same type of particles were extracted with the standard density separation procedures employed in previous studies. These authors added raman microspectroscopy as a final step for particle characterisation, and found neither residual minerals nor organic material in their floating fraction after density separation. While this system provides by far the most unbiased extraction system available at the present time, it is expensive and build individually (see Imhof et al. 2012 for details), and hence will not be available for monitoring purposes in standard biological/chemical laboratories across Europe or worldwide. Furthermore, the system is currently operated with toxic $ZnCl_2$ solution, and only the topmost few 100 ml of the supernatant are extracted. The use of cheaper and non-toxic salts such as NaCl or calciumchloride ($CaCl_2$) with no health-impairing potential imply a lower specific density of the solution, and their extraction efficiency with the MPSS system still has to be examined. Extracting only the top layer of the supernatant especially in lower-density solutions might again lead to a significant loss of higher-density plastics such as PVC, but might also hamper the detection of particles and fibres exposed to biofouling from natural environment samples.

With these options in mind, one of the major aims of this thesis was to quantify the potential biases imposed by extraction methods with standard laboratory equipment likely to be used for monitoring purposes, and to reveal the losses of low-density particles as well as the positive biases of residual sediments in the decanted solutions. For this purpose, a series of technical methodology tests were performed with standard laboratory equipment as described in Sec. 2, with results presented in Sec. 3.1 to Sec. 3.3. The most commonly employed method of visual inspection of the extracted samples is applied to Baltic coast sediment samples with results presented in (Sec. 3.4). As the identification of microplastics among sediment introduces the largest uncertainty in the measurements, the applied methods are scrutinised throughout the thesis, with a conclusive discussion provided in Sec. 4. The possible origins of microplastics in individual locations are discussed in Sections 4.4 to 4.5, and a detailed comparison of detected microplastics concentrations with literature values is given in Sec. 4.6. The major findings are summarised in Sec. 5.

## 1.6 Hypothesis

At the start of this project, we phrased the hypothesis to be tested as follows: *Microplastics reach the sea from a diversity of anthropogenic sources. If the concentration of microplastics is not influenced by tides and weather events on a daily or weekly basis, the spatial and temporal distribution of microplastics is expected to indicate the sources (entry pathways) at each location and in each season.*
With the aim to shed light on the origins of microplastics, the spatial and temporal concentration fluctuation of microplastics in Baltic Sea beaches and river outlets were sampled from March 2014 to September 2014.

During the course of the project, the distinction between microplastics and natural minerals revealed itself as the major problem when employing visual inspection after density separation to detect microplastics among sediment samples. Similarly, the distinction between synthetic and organic fibres, especially fibres originating in or near the marine habitat such as crustacean or insect antennae, proved difficult to discern. In a recent study of seawater samples, Norén & Naustvoll warned that

*"... one conclusion is that contamination of the samples is a serious threat for overestimation of particle concentrations. Due to [the] contamination problem, previous reported concentrations should be handled with care and are not reliable."*

*Norén & Naustvoll 2011, p. 5*

Sediment samples are even more susceptible to misidentifications than seawater. The spectroscopic identification of minerals and polymers in North Sea sediment samples previously extracted via density separation revealed a residual contamination rate of more than 90% natural minerals instead of 100% polymer material (G. Gerdts, private communication). With this high failure rate in mind, we set out to characterise the extraction of microplastics from sediment samples with various methods and chemical solution compositions in the first part of the thesis. In the second part, the spatial and temporal concentrations of identified microplastics from the sediment and water samples in four survey locations at the Baltic and North Sea coasts are analysed. These results are discussed in the context of previous findings with similar methods, and the biases and pitfalls of the current most widely used techniques are exposed.

# 2 Methods & Materials

## 2.1 Methodological background

In this chapter, the sampling of beach sediments and water samples is introduced, and the methodology applied for density separation of natural sediment and synthetic materials is described. With the general steps of sieving, density separation, filtration, and visual inspection we follow the suggestions of Hidalgo-Ruz et al. (2012) for microplastic extraction from sediments and sea surface water samples. The first to describe saturated saline (NaCl) solution for the extraction of microplastics from sediment was Thompson et al. 2004. The method was later modified by Claessens et al. (2011) to allow for larger sample sizes of up to 1 kg sediment to be analysed. A combination of these previous procedures was used to optimise the extraction of microplastics from beach sediments, as described below.

## 2.2 Materials

The materials used in the laboratory were restricted to glass whereever possible. Only glass flasks were used, including in particular the 2 liter Erlenmeyer flasks employed for air-venting. The surface solution was extracted with a 30 ml graded glass pipette after air-venting. Nevertheless, the use of synthetic materials was unavoidable at several stages. The suction bulb attached to the glass pipette was made of red rubber, and the lint-free cleaning cloth consisted of light-blue polyamide. In the initial experiments, glass fibre filters were adopted to filtrate the heavy saline solution to the clean level required prior to sample contact. Over the course of the experiment, fibre *"nests"* were routinely found in a large number of samples. These were initially not thought to originate from breakup of glass fibre filters, yet approximately in the middle of the experiment blind reference samples were also found to contain fibre nests. After this point, glass fibre filters were replaced with polyacetat membrane filters with a pore size of $5\mu$m for pre-filtering to avoid glass fibre filters as an entry path for fibres.

Even though no clean room was available to analyse the samples, all clothes worn by the author in the lab were made of cotton. During the initial tests light-blue rubber gloves were worn to handle the toxic zincchloride samples. These gloves showed signs of flaking after contact with the aggressive zincchloride solution. After switching to non-toxic calciumchloride solutions, no gloves were used for sample handling to avoid flaking and synthetic rubber as an entry path for microplastic particles.

**Materials in direct contact with samples:**

- Stainless steel sieves with pore sizes 0.063, 0.5, 1.0, and 2.0 mm
- 2l Erlenmeyer flask
- 25cm glass tube for air-venting (approximate opening diameter 2mm)
- 30 ml glass pipette
- 250 ml filtration glass flask
- $55\mu$m mesh size zooplankton net, cut to 7cm filter size
- plastic & wire-mesh filter holder

Glass fibre pre-filters and $5\mu$m polyacetat membrane filters were used to clean the saline solution after every experiment. For the methodical experiments, samples were extracted onto glass fibre or membrane filters for analysis under the dissecting microscope, while all science samples were filtered through zooplankton nets to allow sediments to be rinsed into deionised water for particle and fibre counting.

## 2.3 Sampling locations

Four areas were sampled: 5 locations along the Rostock coast, 4 locations on the island of Rügen, 2 sites at the Oder/Peene outlet into the Baltic Sea, and 2 sites at the Jade outlet towards the North Sea (Jade Bay). An overview of sampling locations with geodesic coordinates and sampling conditions is given in Table 7 in Appendix C. With the aim to probe the expected anthropogenic sources, sediments and seawater were sampled in the following scheme:

**Rostock gradient**

With $\sim 700,000$ visitors per year (Statistisches Amt der Stadt Rostock 2014), Rostock is one of the most frequented cities at the German Baltic coast. The seaside resorts of Warnemünde, Markgrafenheide, Hohe Düne, and Diedrichshagen account for half of the overnight stays. Adding day tourists, Warnemünde beach faces a visitor density comparable to the heavily frequented seaside resort of Binz on the island of Rügen. The Warnemünde quaye is host to the international cruise ship terminal and the ferry terminal to the Nordic states, and Rostock city hosts several warfts as well as the commercial overseas harbour.

Table 1: Sampling locations and strategies.

| Area | Location | Sampling strategy |
|---|---|---|
| **Rostock gradient** | Nienhagen/Börgerende<br>Wilhelmshöhe<br>Warnemünde<br>Markgrafenheide | West to East sampling<br>along westward coastal current &<br>seasonal March to July sampling (tourist activity)<br>Warnow & overseas harbour outlet |
| **Rügen gradient** | Dranske<br>Heidehof<br>Breege<br>Binz/Seaside resort | Westbeach, moderate activity & fishing<br>Northbeach, low tourist activity<br>Eastbeach, moderate tourist activity<br>Eastbeach, high tourist activity |
| **Oder/Peene estuary** | Kamminke<br>Freest | inner Oder estuary *"Stettiner Haff"*<br>outer Peene estuary, Oder effluent into Baltic Sea |
| **Jade Bay** | Varel/Nordender Leke<br>Dangast beach | Freshwater sampling at paper recycling plant<br>Seawater & sediment methodology testing |

Samples were obtained both at expected low and high anthropogenic impact sites. Five sampling sites were chosen to monitor the gradient of microplastic contamination in beach sediments along the coast in the wider Rostock region. From West to East, the sites as shown in Fig. 4 cover Nienhagen/Börgerende assumed to be a low touristic/anthropogenic plastic contamination site [2], Wilhelmshöhe halfway towards Warnemünde as an intermediate station along the westerly current, Warnemünde main beach as a major tourist impact site, and Markgrafenheide to the East of the Warnow outlet. The latter location was chosen to monitor the influence of monthly harbour activity as well as beach contamination carried in the Warnow outflow from the Rostock municipal water treatment plant.

## Rügen

The island of Rügen served as a comparison site to Warnemünde as a major tourist area without the urban influence of Rostock and the overseas harbour. Four locations were sampled on Rügen, as shown in Fig. 5. With the main beach in Binz, a seaside resort hosting 1.8 million overnight stays in 2011 (Statistisches Amt Mecklenburg-Vorpommern 2011), one of the predominant tourist destinations of Rügen was captured, while Breege beach faces with a length of almost 7km less dense activity, yet lies close to several touristic villages.

[2]The sampling location was moved from Nienhagen beach to the east end of Börgerende Bay below the sand cliff from May 2014 onwards, as Nienhagen beach proved to be more crowded in the summer season than originally expected.


Figure 4: Sampling locations along the Rostock sea coast. The sites Nienhagen and Börgerende are analysed as one location. Green points mark the sampling sites.

Dranske and Heidehof, on the other hand, contain one to a few holiday camps, and hence are less influenced by touristic activities than Binz and Breege. These sites on the west and north-west coast of Rügen also receive fresh seawater directly from the open Baltic Sea transported on the westerly current.

**Oderfahne**

As a nutrient-rich and chemically loaded comparison location to the Warnow river outlet, two samples were obtained in the region of the river Oder estuary. The first sample was obtained at the freshwater inland *"Bodden"* side of the *Stettiner Haff*. A fine-sanded beach near Kamminke was chosen to collect a drift-line sample similar to the sea-side samples. In addition to a smaller outlet near Świnoujście (Poland), the bulk of the Oder waters flow into the Peenestrom and enter the Baltic Sea near Peenemünde. The second sampling location was chosen at a beach West of the Freest harbour at the West side of the Peene outlet into the Baltic Sea, where the bulk of the combined Oder/Peene flow discharges. Both sampling sites are shown in Fig. 5.

**Jade Bay**

As no microplastic sediment or water sample analysis was available in the Baltic Sea ecosystem in the literature at the time of writing, one location at the Jade Bay was measured with the same method to allow the direct comparison with earlier studies (Dubaish & Liebezeit 2013). Locations claimed to be heavily loaded with microplastics in the immediate


Figure 5: *Left panel:* Rügen island sampling locations. Green points mark the sampling sites. Binz and Breege beach are major tourist destinations, while Dranske and Heidehof are less frequented holiday camps.
*Right panel:* River Oder/Peene estuary sampling locations. Green points mark the sampling sites. Kamminke is located at the inner *"Bodden"* waters of the *Stettiner Haff*, while Freest captures the outer Peene flow into the Baltic Sea.

vicinity of a paper recycling plant in the city of Varel were chosen to ensure good number statistics (Fig. 6). Here, Dubaish & Liebezeit (2013) found more than 1200 particles/liter in seawater samples obtained 20 cm below the water surface. As previous studies analysed surface water samples, both water and sediment were sampled at this location. One freshwater sample was obtained from the surface of the *Nordender Leke*, a small canal passing directly in front of the factory grounds, with the paper recycling stacks in sight at a distance of about 50 meters. The second sampling site at Dangast beach was chosen such that both sea water and sediment could be sampled at the same location. The Varel coast is a protected mud flat area and does not provide direct access to a sediment bank where samples could have been obtained. Dangast is the nearest beach to the North of Varel, at a distance of $\sim$5 km from both the Varel Jade estuary as well as from the discharge pipeline extending into the central Jade Bay.

In Dangast, where clay and silt dominate the top-layer sediment, the surface 1-2mm of fine sand was collected with a flat spoon to obtain a comparable grain size distribution as at the Baltic Coast. The seawater sample was drawn several meters into the water at the same beach point where the sediment was obtained. Surface water was allowed to flow freely into two 5l canisters at a total water depth of 50-70cm. The comparison between seawater contamination and sediment contamination was expected to allow quantification of the input and trapping of microplastic particles in sediment from the water column.

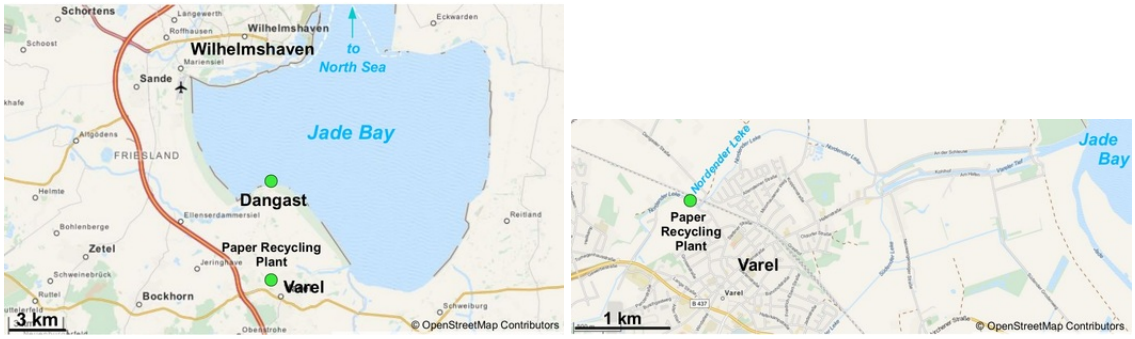


Figure 6: Sampling locations at the Jade Bay. Sediment and water were sampled at Dangast, while one freshwater sample was drawn from the *Nordender Leke* in the city of Varel opposite the paper recycling plant (distance less than 50m). Green points mark the sampling locations.

## 2.4 Beach sediment sampling

Samples of wet sand were obtained predominantly at the drift line above sea water level. All samples were obtained during calm conditions with low wave activity. Fine sediment was sampled as a larger number of microplastics were expected to be bound in the fine-grain layer than among coarse grains regularly rinsed with sea water. The majority of samples was collected at the drift line where small shell fragments were found to concentrate, under the assumption that microplastics would also accumulate there. Shallow-water samples were retrieved below the characteristic ridge of coarse gravel found a few meters below the drift line. Beach sediments at the Baltic Coast cover a wide variety of grain sizes from fine sediment $< 0.5$mm to large rocks. As a consequence, layers with grain sizes larger than 2mm (coarse gravel) are found at varying height levels less than 1 cm below the sand surface inside and outside the water near the surf zone. These conditions prohibit single-height sediment cores to be extracted. With the aim to avoid the coarse gravel zone, samples were scraped off the surface layer with a stainless steel table spoon either at the drift line or were carefully spooned off the surface of sand ripples under water with the same flat table spoon. Samples were limited to the top 1-2cm at most and frequently did not exceed 1cm depth. Studying the stratification of sediment cores to a depth of 25cm, Carson et al. (2011) found that 50% of mircoplastic fragments were contained in the topmost 5cm of each core, and that the top 15cm hosted 95% of all detected plastic particles. We therefore expect to capture the largest concentrations of microplastics when sampling the sediment surface. Samples were collected 500 ml each into screw cap glasses.

## 2.5 Water sampling

In addition to sediment samples, three water samples were obtained for comparison with the sediment content. Seawater was sampled at Warnemünde beach, the location used for all methodical experiments. In addition, one North Sea water sample was drawn at Dangast at the Jade Bay and one freshwater sample was drawn in the *Nordender Leke* in Varel near a paper recycling plant for comparison with earlier microplastic measurements extracted from water samples near these locations. For all water samples, surface water at the top layer at a depth of 2-4 cm was allowed to flow freely into 5-10l canisters previously rinsed several times with the ambient water. These samples were filtered over 55$\mu$m zooplankton net and treated with 30% $H_2O_2$ solution for 24 hours to dissolve organic matter. In the case of the *Nordender Leke* freshwater sample, organic content was so high that net filters were treated for a second 24 hour period after rinsing with deionised water.

### Special treatment of Dangast seawater sample

Seawater was poured into a cleaned glass flask and over zooplankton net filters without any previous treatment or handling. Because of the extreme zoo- and phytoplankton load of these samples, 500$\mu$m nets were used to retain the majority of plankton species. The residual solution was poured through 55$\mu$m net filters. The first filtering step was necessary to detect any particles and fibres among the dense layer of plankton on each net. At the same time, this step implied that only small particles and fibres could be analysed in these samples. The 55$\mu$m nets were soaked in 30% $H_2O_2$ for 24 hours to dissolve organic material, as in all other science samples.

All water sample zooplankton nets were then counted under the dissecting microscope, rinsed into deionised water, and recounted following the same procedures as for counting sediment extracted samples (see Sec. 3.1.4.2 below).

## 2.6 Preparation of Warnemünde test samples

Prior to analysis, all test samples were dried in a standard hot-air drying oven at 55$^o$C for 8 hours. The resulting clumpiness was smoothed with a spoon during sieving.
Each sample was first manually sieved through a 3-stage sieve. Stainless steel sieves with mesh sizes 0.5mm, 1mm, and 2mm were used, such that three fractions with grain sizes $< 2$mm were obtained. Given that samples were selected from the fine grained sand

fractions at the beach, there were no particles larger than 2mm in the top sieve. The largest fraction with particle sizes of 1-2mm was by far the smallest fraction by weight ($\sim 0.2$ %, or 1.4-1.7g in 500ml sediment). This fraction was investigated under the microscope, and not processed in a density separation bath. Several methods of density separation were tested to separate possible microplastic fragments from the sand in the medium fraction (0.5-1mm), as described below. This fraction contained 2-10% by weight or 13g to 61g in 500ml. The large variation is surprising in view of the homogeneously taken test samples, and might result from the drying and sieving procedure. Especially the larger fragments frequently consisted of glued finer particles, and were pushed through the sieve gently with a spoon. A slightly different clumpiness or stickiness after drying might have resulted in a larger fraction of medium-sized "grains". After sieving, the fractions were kept in glass containers and analysed separately.

The medium-size fraction (0.5-1mm) was analysed first using two separation methods suggested in the literature, centrifugation and air-venting in saline solutions for plastic extraction, as described in Sec. 2.8. Both procedures were then repeated with the small-sized fraction ($< 0.5$mm).

## 2.7 Preparation of science samples

After clumpiness was detected as a potential source of size bias in the Warnemünde test samples, all Rostock science samples were wet-sieved with 1l of deionised water. Wet-sieving provided the additional advantage that the drying procedure in the hot air oven, which likely introduced fibres from the sucked lab air into the samples, was avoided. The final science samples obtained at beaches in the Rostock area were sieved through 0.5mm, 1.0mm, and 2.0mm stainless steel mesh sieves. A separation of the large microplastic fraction $> 0.5$mm and the small fraction $< 0.5$mm is recommended in the review of Hidalgo-Ruz et al. (2012) for comparability with previous studies. Wet-sieved science samples were then transferred directly to the Erlenmeyer flask used for density separation. As in the case of the test samples, density separation was only applied to the $0.5-1.0$mm and the $< 0.5$mm fractions, while the small amounts of even larger grains $> 1$mm were visually scrutinised under the dissecting microscope without further processing.

During the investigation presented here, it was found that the selective collection of fine-grained sediment contained only small amounts of coarse sediment $> 0.5$mm, varying between a few and a few 10 grams, with the exception of the Markgrafenheide samples containing a maximum of 360g of coarse sediment (0.5-1mm, see also Table 7 in Appendix

C). Because of this large variation, all Rostock samples were consistently sieved, and the coarse and fine fractions underwent density separation and microplastic extraction individually. Given the time-consuming nature of this process, and as only small amounts of coarse material were found in particularly fine-grained samples comparable to all other survey locations, scientific samples beyond the four Rostock locations were not sieved. This implies that the four Rügen locations, the two Oder/Peene locations, and the Jade location were treated to only one density separation in which each complete 500ml sample was processed.

## 2.8 Density separation methods

Several density separation methods were tested with the aim to find a simple, efficient technique to extract light-weight plastic particles and fibres with mean densities of $< 1.2\mathrm{g/cm^3}$ from natural sediment with a specific dry density of solid quartz, $2.65\,\mathrm{g/cm^3}$ (Nuelle et al. 2014). Separation methods included centrifugation and air venting with high-density zincchloride and calciumchloride solutions. A compilation of plastic materials and corresponding densities observed in the marine environment is provided in Table 2 together with the relative frequency of their occurence in North Sea sediment samples (Lorenz 2014). The low-density materials polypropylene (PP) and polyethylene (PE) together account for more than 80% of all microplastic particles classified with infrared microspectroscopy, with PP contributing with 77.9% by far the largest fraction of microplastics. The solubilities of NaCl and the high-density salts used here for plastic extraction are shown for comparison in Table 3. Especially the predominant light-weight materials PP and PE have specific densities significantly below the densities of $ZnCl_2$ and $CaCl_2$ solutions.

## 2.9 Centrifugal density separation

Most studies use a time-intensive density separation method to extract synthetic polymers with characteristic densities $< 1.4\mathrm{g/cm^3}$ from sand grains with densities $> 2\mathrm{g/cm^3}$. As a first step, the sediment is air-vented in a high-density salt solution, typically a zincchloride solution at 1.4-1.6 g/ml densities. Air-venting is applied for several hours, before the floating light-weight particles are extracted from the surface. The currently most ideal method of analysis was described by Imhof et al. (2012), where the surface of the solution is contained in the filtering device, such that no decanting is necessary. In order to extract the plastic particles, the filtering tube is turned around, and the solution previously on the surface is immediately filtered and the zincchloride washed off with distilled water. The advantage of

Table 2: Characteristic specific densities of the most frequent plastic polymers, and the frequency of their occurence in percent of all eight spectroscopically identified synthetic polymers in North Sea sediment samples according to Lorenz (2014).

| Material | $\rho$ [g/cm$^3$] | frequency [%] | reference for $\rho$ |
|---|---|---|---|
| **Synthetic Polymers & Fibres** | | | |
| Ethylene-Vinyl Acetat (EVA) | 0.93 | 1.3 | (1) |
| Polyethylene (PE) | 0.92-0.96 | 6.1 | (1,2) |
| Polypropylene (PP) | 0.9-1.0 | 77.9 | (1,2) |
| Polystyrene (PS) | 1.05 | 3.9 | (1,2) |
| Acrylamide (Acrylic) | 1.13 | – | (3) |
| Polyamide (PA, Nylon) | 1.01-1.14 | 0.9 | (1,2,3) |
| Polymethyl methacrylat (PMMA) (acryl glass/plexiglass) | 1.16-2.0 | 6.1 | (1,2) |
| Polyvinyl chloride (PVC) | 1.2-1.4 | 1.7 | (1,2) |
| Polyester (Polycarbonate, PC) | 1.2-1.4 | 2.2 | (1,3) |
| Polyethylene terephthalate (PET) (Thermoplastic Polyester) | 1.37-1.40 | – | (1) |
| **Narutal fibres & materials** | | | |
| Cotton | 1.40-1.55 | – | (3) |
| Flax, Jute, Hemp | 1.50 | – | (3) |
| Silk | 1.33-1.60 | – | (3) |
| Wool | 1.31 | – | (3) |
| Viscose | 1.5 | – | (3) |
| Glass (Silicate) | 2.45-2.55 | – | (3) |
| Sand, quartz | 2.65 | – | (4) |

References: (1) - http://www.kern-gmbh.de: EVA fact sheets; (2) http://wiki.polymerservice-merseburg.de/index.php/Dichte, Table: *Comparison of polymer densities with other raw materials*; (3) Australian International fibre centre (IFC), 4.1.04 – Table of Fibre Densities (natural and synthetic), www.ifc.net.au; Polyester (PC, also denoted as PES in other references) – density of fibres, note that PES can also represent the entire group of ester polymers, and is used for sulfonic polymers in other contexts; (4) Nuelle et al. (2014).

this system is that no particles are lost on the container walls during decanting or pipetting of the surface solution.

As especially our medium-size fraction consists of very small samples, we attempted to simplify and shorten this procedure. Here, we followed suggestions in Claessens et al. (2013), where a combination of floatation in a high-volume stream of tap water and centrifugation is used to extract polymer particles from sediment. As our samples consisted of at most 32g of material, we did not apply the water-intensive floatation step. Instead, we split each sample into two to four portions of 6-10g each, which were filled with high-density saline solution into centrifugation tubes. Following the procedure in Claessens et al. (2013), the tubes were shaked vigorously before centrifuging at $3500 \times g$ for $3 \times 5$min. After each 5min

Table 3: Specific densities $\rho_{sp}$ and solubilities of salts in water at room temperature (20$^o$C). The solubility corresponds to the maximum density of the saturated saline solution that can be achieved at room temperature in the lab.

| Salt | $\rho_{sp}$ [g/cm$^3$] | solubility [g/ml] |
|---|---|---|
| NaCl | 2.17 | 1.20 |
| $CaCl_2$ | 2.15 | 1.47 |
| $ZnCl_2$ | 2.91 | 2.14 |

spin, 7ml solution were pipetted off the surface of each tube, and vaccuum-sucked through a 5$\mu$m polyacetate membrane filter. The filtered solution was used to refill the tubes to the same level of $\sim$40ml, shaked and centrifuged again. After 3 centrifugations, each filter was washed with 250ml of deionised water in the case of the acidic zincchloride solution, and with at least 100ml of deionised water to remove residual calciumchloride. All filters were then air-dried under a slanted glass cover for protection against further fibre input.

## 2.10 Air-venting density separation

In order to test density separation with the methods used predominantly in the literature, the sediment samples were air-vented inside a 2l Erlenmeyer flask with 0.5-1.1l of high-density saline solution. Pressured air was pushed through a glass pipe with an opening diameter of 2 mm inserted in the Erlenmeyer flask such that the pipe nearly touched the ground. The flask was tilted at an angle of $\sim 10$ degrees to allow for sediment to flow towards the bottom part of the flask (Fig. 7), where the air was inserted, and rotated at semi-regular intervals of 15-30 minutes to expose the complete sediment volume to the air flow. The air flow was adjusted such that the sediment was easily lifted from the ground, yet keeping a safe marging to avoid splashing through the neck of the flask. A constant air flow was kept for 3 to 4 hours in accordance with the amount of sediment to be stirred, and sedimentation was allowed thereafter for 12 hours (typically over night). Between 200 and 400 ml of the surface of the solution were pipetted off with a 30ml pipette, which was moved over the surface to capture the area of the dense solution as much as possible. Moving the pipette over the surface was applied to compensate the slow flow of the high-density solution towards the pipette. The pipetted solution was then filtered onto glass fibre or membrane filters (test samples) or zooplankton net (science samples, see Sec. 2.13), and the solution remaining above the sediment was decanted and filtered separately. Procedural details for extractions with the $ZnCl_2$ and $CaCl_2$ solutions are given in Sections 2.11 and 2.12 below.

Figure 7: Laboratory equipment used for density separation.
*Left:* Erlenmeyer flasks (2l) were used for air-venting and settling of sediments in high-density saline solution. The glass filtering equipment used to extract samples onto filters is shown on the left. *Right:* Erlenmeyer flask during air-venting.

## 2.11 Density separation in zincchloride solution

In the first test (sample P1 in Table 5 in Appendix A), a zincchloride solution with a density of 1.43 g/ml was used as a density separator. The solution was available in the lab, and was filtered through paper filters to remove particles. In test 1, the 32g of medium-sized 0.5-1mm sediment were split into 4 portions of 8g each and filled into 4 centrifugation tubes. The tubes were filled up to a total volume of 40ml with 37ml of $ZnCl_2$ solution. In addition to the sediment probes, 4 reference tubes were filled with 37ml $ZnCl_2$ solution only. All tubes were centrifuged three times. The surface of the sediment tubes was pipetted as described in Sec. 2.9, and washed with deionised water to remove residual zincchloride. In this test exclusively, each centrifugation run was pipetted onto a separate filter. As reported by Claessens et al. (2013), practically no fibers and particles were found after the third centrifugation. Very few, very short fibers were still present, which could be explained by contamination from laboratory air and clothing. After the second centrifugation, however, a significant number of particles was observed on the filter. Three centrifugation runs were therefore used for all tests hereafter. In addition to the 3 sediment centrifugation runs, the top 7ml of the solution in the reference tubes were also pipetted onto one filter to probe the level of fibre contamination during processing. Finally, the remaining zincchloride solution above the sediment in the samples and the solution in the reference tubes were decanted and filtered separately to probe any remaining particles in the water column below the surface.

Because the initial goal was to find particles with sizes larger than 0.5mm, and in an attempt to avoid polymer material used in membrane filters as a source of contamination, glass fibre filters were used in test 1. It turned out that most detected objects were fibres, which were difficult to separate from the glass fibre structure, even if they were clearly seen under the dissecting microscope. Therefore, membrane filters were employed for all following tests.
In order to probe the small size fraction ($< 0.5$mm) with the centrifugation method, small portions needed to be selected to allow for the efficient separation of high- and low-density sediment. For comparison with the medium-sized fraction, 4 tubes were again filled with 32g of small-grained sediment with 8g in each tube. Tubes were filled up to 40ml with $ZnCl_2$ solution and processed in the same way as described for the medium-size fraction.

In the second test (P2 in Table 5), the sediment samples were infused with 1.1l of zincchloride solution in a 2l Erlenmeyer flask vented with a glass pipe from the bottom of the flask. The air flow was adjusted such that bubbles readily lifted the sediment particles from the ground without overshooting the neck of the flask. To allow for the exchange of particles from the sides into the bubble stream, the flask was tilted slightly and rotated regularly (see Sec. 2.10). Air-venting was applied for 4 hours as described above, and the sediment was allowed to settle over night thereafter. The top 750ml of the $ZnCl_2$ solution was pipetted off and vacuum-sucked over a 5$\mu$m membrane filter. Care was taken to pipette off the surface of the solution covering as much area as accessible. The pipetting method was used to avoid decanting the solution, as Imhof et al. (2012) had shown that up to 60% of the floating plastic particles are lost during decanting alone. The pipette was rinsed with deionised water to capture all remaining small particles possibly stuck to the pipette walls. As in the case of the centrifugation experiment, the remaining solution was decanted over a separate filter to check for residual synthetic material in the water column above the sediment. The same procedure was applied to both the 0.5-1mm and the $< 0.5$mm grain size fractions.

## 2.12 Density separation in calciumchloride solution

The experiments were repeated with a second 500ml sample of Warnemünde beach sediment, which contained only 13.2g of medium-sized 0.5-1mm particles (P3 in Table 5). The sample was therefore split into 2 portions of 6.3g and 6.9g of sediment in 2 tubes filled up with 37ml of calciumchloride ($CaCl_2$) solution. These tubes, along with 2 reference tubes filled only with $CaCl_2$ solution, were then centrifuged 3 times and filtered over a membrane filter as described above. Given the results from test 1, all 3 centrifugation runs were filtered

over the same filter. The reference tubes were decanted over a separate filter, as in test 1. To probe for residual material in the sediment after the $3\times$ centrifugation process, the sediment was retrieved from the tubes and filled into the clean Erlenmeyer flask. The flask was filled with 500ml of $CaCl_2$ solution because of the low sediment amount of only 13g, and air-vented for 3 hours. The flask was manually rotated approximately every 15 minutes during this time. After air-venting was turned off, the solution was allowed to sediment and cleared entirely within several minutes. The 300ml solution of the surface area was pipetted off and vaccuum sucked over a membrane filter, and calciumchloride was rinsed off with 100ml of deionised water. The remaining solution was decanted over a separate filter, as in the case of the $ZnCl_2$ experiment. The same procedure was conducted with 40g of small grained sediment ($< 0.5$mm) after centrifugation. Here, the finer sediment was allowed to settle over night prior to filtration.

## 2.13 Filtration & digestion

After air-venting and settling samples over night, the surface supernatant of each sample was extracted by moving a 30ml pipette across the solution surface, and expelling the pipette onto a membrane or glass fibre filter with a pore size of $5 - 10\mu$m. The remaining supernatant was decanted over a separate filter to analyse particles and fibres lower in the water column individually. Filters were air-dried in small petri dishes with lids almost closed to minimise laboratory air contamination. After the test samples were conducted, it was found that visual inspection on filters was hampered in the presence of large residual sediment loads. The pipetted and decanted fractions of scientific samples were therefore extracted onto zooplankton net filters precut to a diameter of $\sim 7$cm with a mesh size of $55\mu$m. This mesh size provides the lower detection limit in all scientific samples.

Depending on the nutrient content and the grain properties of the sediment, a varying amount of organic material was observed. Organic matter can be efficiently disintegrated to distinguish potential microplastic particles and natural minerals from organic protein and carbohydrate structures by digestion with hydrogen peroxide ($H_2O_2$), as shown in Lorenz (2014). The remains after digestion will contain natural sediments, plastic particles with the exception of polyamides (dissolved in $H_2O_2$), and chitin-based crustacean or insect shell and exoscelleton fragments. The latter could, in principle, be dissolved with chitinase (Lorenz 2014), but the long treatment times rendered this extra digestion step impractical for the large volume of samples analysed here. All membrane and zooplankton net filters were soaked in 30% $H_2O_2$ solution for 24 hours and rinsed with deionised water afterwards

in the clean filtering equipment.

## 2.14 Visual inspection

All filters were inspected under a dissecting stereo microscope (Olympus SZ51 or similar) with 3-4$\times$ magnification. Suspicious particles and fibres were analysed under the Olympus SZX16 stereo microscope equipped with the DP21 digital camera with a magnification of up to 11 to facilitate the distinction between microplastics and natural sediment or organic matter, as well as between synthetic/anthropogenic fibres and organic fibres. Because spectroscopy was not availabe, no distinction is made between synthetic and non-synthetic anthropogenic fibres in the remainder of the thesis. Potential microplastic particles and synthetic fibres were photo-catalogued with the Olympus SZX16 stereo microscope or the Zeiss BH2 stereo polarisation microscope. Microplastics and natural materials were distinguished on the basis of colour, surface structure, and morphology (shape). As transparent particles are most susceptible to misclassification by visual inspection, transparent particles are only included as potential microplastics if their surface structure was clearly distinct from natural sediment. All particles and fibres investigated by visual inspection alone are considered *potential* microplastics (e.g., Dekiff et al. 2014). As material proof via spectroscopic identification was not available, we implicitely assume all pieces to be *potential* microplastics when the terms microplastic particles and fibres are used throughout this thesis. Particles and fibres with colours different from natural sediment, such as intense blue, green, pink, and violet, are visually identified as the most certain microplastic contaminants.

## 2.15 Artificial samples

Two artificially enriched samples were created by adding 200 polyethylen particles (PE) with a density of 0.9 g/cm$^3$ to $\sim$500 ml of sediment with grain sizes $< 0.5$mm, corresponding to a sediment dry weight of 802.6g and 743.3g, respectively. Before enriching sediment with PE particles, microplastic particles were extracted from Nienhagen beach March and April sediment samples as described in Sec. 3.1.4.1. The PE particles were cleaned, post-processed recycling fragments covering the approximate size range 100$\mu$m-1mm.

The original PE mix contains predominantly transparent and white-transparent particles, which are difficult to distinguish from natural sediment. While the freshly produced recycling fragments can be distinguished on the basis of their surface structure and shape, it is likely that aged plastic particles in the natural Baltic environment are not easily discerned on the basis of their surface structure. With the aim to test our method to separate light-weight

particles from natural sediment, and the efficiency of air-venting and lifting of plastics in the high-density saline solution, a large number of coloured particles was selected to enhance statistical recovery in these artificial samples. The artificially enriched samples were air-vented for 4 hours in $\sim 1$l calciumchloride solution with a density of 1.24 g/ml. Each sample was allowed to settle overnight for at least 12 hours after air-venting. 400-500ml of the surface solution were extracted by moving the pipette across the surface systematically as in the case of the science samples. The remaining saline solution above the bottom sediment was decanted onto a separate zooplankton net filter, and the pipetted and decanted fractions were counted individually. The results of these tests are evaluated in Sec. 3.2.

# 3 Results

## 3.1 Results of Warnemünde test samples

### 3.1.1 General results

Density separation of the Warnemünde test samples was carried out in $ZnCl_2$ as well as $CaCl_2$ solution, and centrifugation was also used as a means to separate light-weight particles from sediment in small samples of up to 40g. Although zincchloride can theoretically be saturated to densities of 2.1 g/ml, the $ZnCl_2$ solution proved difficult to saturate, and densities above 1.45 g/ml were not achieved. A likely cause for the low densities are impurities in the available $ZnCl_2$ salt. In contrast, the $CaCl_2$ solution was readily saturated to densities of 1.35 g/ml, close to the saturation density of 1.43 g/ml at 20°C. While $ZnCl_2$ is a highly toxic, oxidising, and chemically aggressive medium, $CaCl_2$ is non-toxic and suitable for use in food as a coagulation agent. Given the minimal difference between the achieved solution densities, and the substantial difference in ecological impact and handling in the lab, all scientific samples were air-vented with $CaCl_2$ solution at densities between 1.3 and 1.35 g/ml.

One of the aims of this thesis was to develop a method that allows comparability between spatially and temporally separated measurements. When counting particles on illuminated filters, the dominating uncertainty originated in the fact that particle counts suffered from insufficient size limits. On membrane or glass fibre filters, the detected number of particles and fibres varied according to the provided contrast on the filter material available with surface light or transmitted light at the microscope. While membrane filters displayed a higher contrast compared to glass fibre filters, it was still difficult to discern fibres among larger amounts of sediment and sediment particles from organic matter. The most subjective decision process originated from the smallest particle to count. With large numbers of several hundred to thousand particles, it is not practically feasible to measure the size of each object near the counting lower limit. While the upper size limit is set by the sieve to 1mm, the smallest fraction with grain sizes $< 0.5$mm includes numerous tiny pieces of sediment and organic material as well as microplastics.

The use of zooplankton nets with a pore size of 55$\mu$m allowed to set a fixed lower limit, below which particles were excluded from the science samples. Despite their synthetic material, plankton nets displayed several advantages when used in the final test sample. First of all, zooplankton nets are not a potential source of plastic contamination. Even when the hand-cutted filter edges disintegrated, the mesh was so characteristically woven that

zooplankton net pieces were easily discerned from all other synthetic matter in the samples (see Fig. 8).

In sample 4 (P4 in table 5 in Appendix A), the pipetted and decanted surface solutions were poured over zooplankton nets and counted under the microscope in two different stages. First, the material on the net was counted, including all particles and fibres visible unless particle numbers were too high to count. In a second step, zooplankton nets were rinsed with deionised water into glass petri dishes, and high-density material located on the ground as well as material floating on the surface was counted individually. The total of the hence-forth called *"ground"* and *"float"* fractions was then compared to the total number of particles and fibres counted on the plankton net prior to rinsing. In general, very small particles might stick to the net pores and might be lost in the count rate. On the other hand, clear fibres comprising the dominant amount of all fibres detected are substantially easier to recognise after rinsing, such that fibre numbers increased. Therefore, this two-stage procedure was applied to all latter science samples. Except for very small particles and dissolved organic matter after treatment with 30% hydrogen peroxide solution, the rinsed zooplankton nets were very clean. Because of the more objective counting method before and after rinsing, this net material was cut to filter-size circles and used as filters in all science samples.

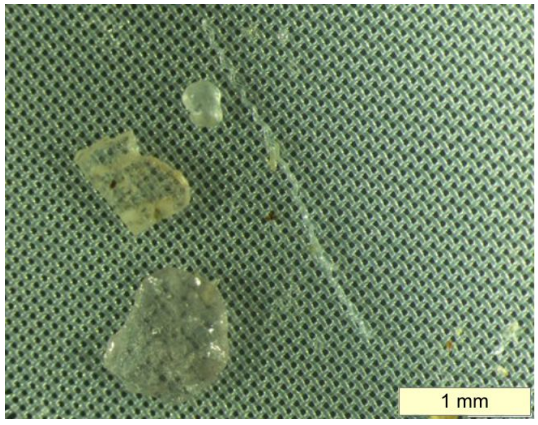


Figure 8: Image showing sediment grains and extracted net fibre on zooplankton net with a mesh size of 55$\mu$m. Note the characteristic curly shape and thickness of the zooplankton net fibre in the centre of the image.

### 3.1.2 Number counts of particles and fibres

#### 3.1.2.1 Centrifugation

A small amount of sediment, 30-40g, distributed into 4 plastic centrifugation tubes, could be analysed in each centrifugation experiment. Naively, one would expect that most light-weight particles (plastics) and fibres flow on the surface after the first of the three centrifugation runs (see Sec. 2.9), and that the lowest number of low-density material remains in the decanted solution. The number counts of fibres and particles on the filter after centrifugation in $ZnCl_2$ solution are shown in Fig. 9. The three centrifugation runs are denoted *c1* to *c3*, and number counts of the decanted extraction are denoted *dec*. The coarse and fine

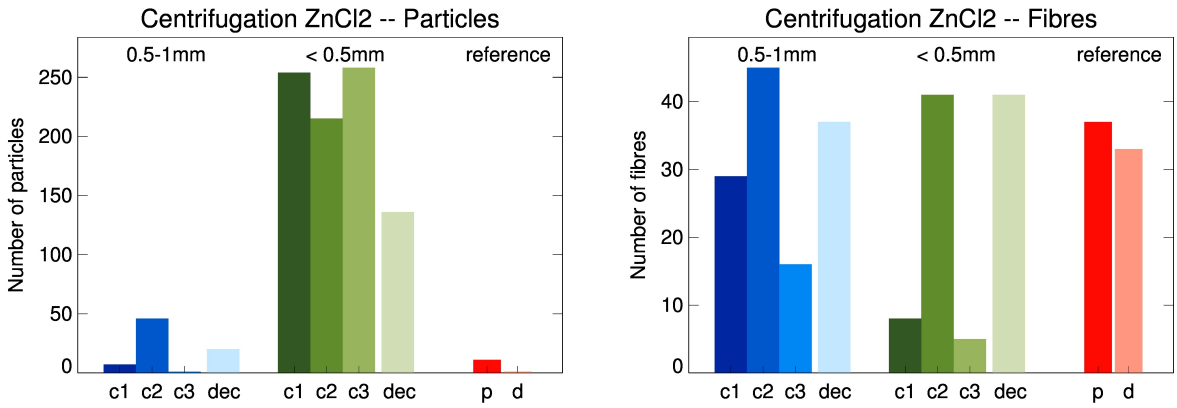


Figure 9: Results of particle (*left*) and fibre (*right*) extraction via centrifugation in $ZnCl_2$ solution. The three centrifual runs (*c1*-*c3*) and the decanted supernatant (*dec*) are counted on glassfibre filters individually for the coarse $0.5 - 1$mm (blue bars) and fine-grained $< 0.5$mm (green bars) sediment fractions. Note the particularly high particle counts after all three centrifugation runs in the fine-grained sediment fraction $< 0.5$mm. Number counts of the reference solution without sediment sample are derived for the pipetted surface solution ("p") and the decanted solution ("d") individually (red bars).

sediment fractions are displayed in blue and green, respectively, and the reference $ZnCl_2$ solution containing no sediment is shown in red. The extraction of fibres and coarse particles is most efficient in the second centrifugation run *c2*, and declines, as expected, rapidly after the third centrifugation. However, in the small size fraction, particles are comparably frequent in the 7ml pipetted surface soluation after all three centrifugation runs, suggesting that sediment is stirred up shortly after centrifugation in the heavy $ZnCl_2$ solution and floats above the bottom sediment layer. The fact that unexpectedly large numbers of particles are located near or at the surface implies that centrifugation in a heavy $ZnCl_2$ solution does not provide a clean means to extract synthetic particles from sediment samples. This result is strengthened by the fact that even after the third centrifugation *c3*, more than 200 particles reside near the surface of the saline solution. Even in the coarse size fraction (0.5-1mm), particle numbers are larger after the second centrifugation run than after the first. In both size fractions, the fibre detection rate also does not follow a systematic decrease from the first to the third centrifugation run. Furthermore, the decanted solution remaining after pipetting off 7ml from the surface after the third centrifugation run contains a significant number of fibres and particles.

The reference sample with $ZnCl_2$ solution and no sediment displays a concerningly large number of 70 fibres (pipetted plus decanted, red histograms in the right panel of Fig. 9), which might be introduced in the process of extensive handling during the centrifugation experiment (opening of tubes, pipetting, re-filling with residual solution, decanting). As $ZnCl_2$

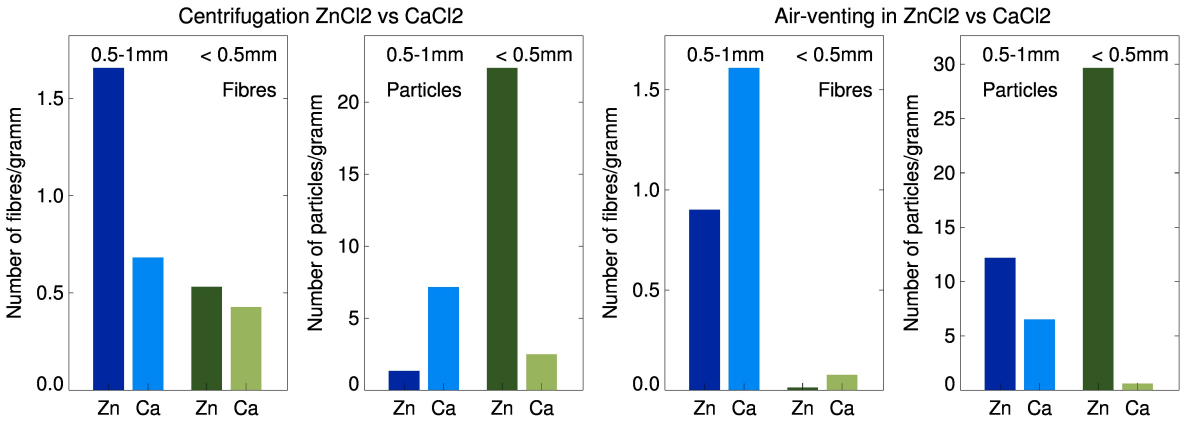


Figure 10: Comparison of density separation methods using $ZnCl_2$ and $CaCl_2$ solutions. Particle and fibre number counts are shown as concentrations per gramm of dry weight sediment for comparison (blue: coarse sediment $0.5 - 1$mm, green: fine-grained sediment $< 0.5$mm). The left two panels display concentrations of fibres (*left*) and particles (*middle left*) after centrifugation and the right two panels display concentrations of fibres (*middle right*) and particles (*right*) after air-venting.

is extremely sticky, this large reference fibre load might also be the cause for the differences between centrifugation in the $ZnCl_2$ and $CaCl_2$ solutions, with only 15 fibres found in the reference sample of the $CaCl_2$ solution. The differences between the centrifugation samples P1 with $ZnCl_2$ and P3 with $CaCl_2$ are displayed in Fig. 10, where the numbers of fibres and particles are shown relative to the total weight of each sample. Only fibres and particles extracted with the pipette are displayed, as the decanted number counts were influenced by sand stirred up during extraction. Particle concentrations are higher after both centrifugation and air-venting in three of the four test samples with $ZnCl_2$ solution, and fibre densities are higher with $ZnCl_2$ in the case of centrifugation (*left panels* in Fig. 10). Despite subtraction of the reference sample number counts prior to weight scaling, the 0.5-1mm fraction shows more than twice the fibre load after centrifugation in $ZnCl_2$. The large particle load in the fine-grained sediment fraction discussed above stands out prominently even after subtracting the reference sample number counts and scaling with the total weight of each sample. As a consequence of these effects, the particle and fibre load per weight of sediment is inconsistent within and between the test samples.

Two additional problems occured during centrifugation. After three centrifugal runs, the remaining sediment is expected to contain no more fibres and light-weight particles according to Claessens et al. (2013). This expectation seemed justified, as the first centrifugation tests showed low number counts particularly after the third centrifugal run. However, as discussed above, this trend could not be confirmed in the later tests. In addition, a large number of fibres is detected in the four blanks used as reference, where tubes were filled

with $ZnCl_2$ solution only. The 37 and 33 fibres detected in the pipetted and decanted part of these solutions, respectively, suggest that without clean-room conditions, the tubes or the handling might introduce fibres from the lab into the samples. In order to test this result further, the second centrifugation sample, P3, was air-vented in $CaCl_2$ solution after the 3 centrifugation runs were completed. If the extraction were near complete, only small amounts of fibres and light-weight particles are expected to remain after the bubble bath. However for the coarse as well as the small size fraction, large amounts of fibres are found both in the pipetted surface solution as well as in the decanted solution despite the previous centrifugal extraction. This lends additional evidence that either centrifugation introduces fibres or that the extraction is highly incomplete. These results supported our conclusion that the centrifugal method, while interesting, is not sufficiently robust for a spatio-temporal comparison study.

In summary, both particles and fibres do not show the expected number decrease during the three centrifugal extractions. A significant number of potential synthetic particles and fibres remains above the bottom sediment, and can only be extracted when decanting the remaining solution. It is questionable that the majority of these particles can be claimed plastics, as their optical appearance is not distinguishable from bottom sediment. In view of the small weight fractions of $\sim 40$g that can be processed within the one hour centrifugation procedure, centrifugation does not seem an efficient method to extract microplastics from larger sediment samples. Nevertheless, centrifugation in a lower-density solution might be a valueable method to extract microplastics from small residual sediment samples after another density separation method, such as air-venting, was already applied.

#### 3.1.2.2 Air-venting in high-density saline solution

Both air-venting in $ZnCl_2$ and $CaCl_2$ solutions enabled the extraction of lighter particles and fibres from substantial amounts of up to 800g sediment samples. The fact that calcites dissolve in the aggressive $ZnCl_2$ environment, while $CaCl_2$ preserves mussel and other calciferous material, led to substantial differences in the optical analysis of both samples. The $ZnCl_2$ sample (P2 in Table 5 in Appendix A) produced thick layers of calcites on the filter (Fig. 11), biasing the detection and count rate of both fibres and particles. Fibres were particularly affected, as the detection of thin threads is practically impossible in a dense layer of calciferous material. This aspect adds to the arguments that calciumchloride substantially facilitates the density separation method, material handling, and optical analysis.

In the right panels of Fig. 10, number counts from both air-venting experiments are com-

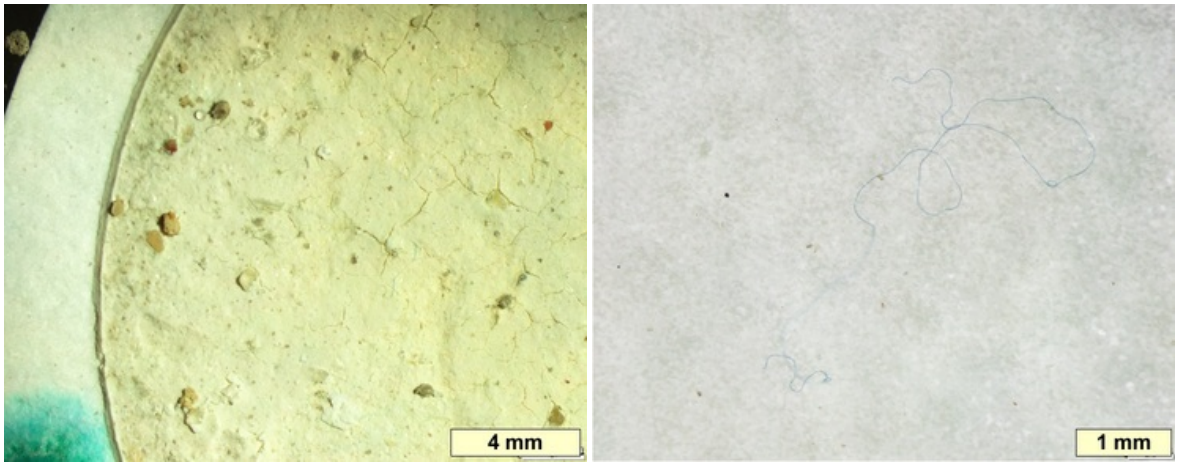


Figure 11: Comparison between a glass fibre filter with the supernatant of sediment air-vented in zincchloride (left) and calciumchloride (right) solution. The $ZnCl_2$ filter is densely covered in calcites originating from dissolved mussel shell. The glass fibre structure is exposed on the $CaCl_2$ filter, and a long, blue fibre is visible across the center.

pared. Fibres could not be counted on the $ZnCl_2$ filter, because the large number of more than 6000 particles pipetted off the $ZnCl_2$ solution from just 217g of fine sediment ($< 0.5$mm) prohibited the detection of fibres. Even in the coarse sediment fraction (0.5-1mm), 744 sediment grains were counted in the pipetted solution extracted near the surface even after settling for at least 12 hours. As these particles are visually indistinguishable from sediment grains, small grains appear to be easily suspended in the $ZnCl_2$ solution, which implies that a clean sediment-plastic separation is difficult in such a medium. The more viscous zincchloride solution lifts a larger number of small sediment particles than the less viscous calciumchloride solution. As a consequence, synthetic particles will be more easily picked out after air-venting with calciumchloride, where the residual contamination with sediment is not as extreme. Fibres are more easily extracted in the case of air-venting with $CaCl_2$ (*middle right panel* in Fig. 10), suggesting that fibres are extracted efficiently in the calciumchloride solution when air-venting is applied. Fibres were also more readily counted when lower numbers in the range of several hundred particles were present on the zooplankton net adopted in experiment P4 for the first time. Rinsing of the net had the additional advantage that a large percentage of more than 50% of the sediment particles were sinking to the ground in deionised water, additionally facilitating the counting of both particles and fibres (Fig. 12). While particles are easily detected on the plankton net, especially clear fibres can be lost among the sediment heaps and are more readily detected after rinsing into a petri dish with deionised water. Particle counts on the zooplankton nets in these test samples are overestimated as compared to the scientific samples presented below, as very small particles and remains of organic matter were counted in these comparative tests.

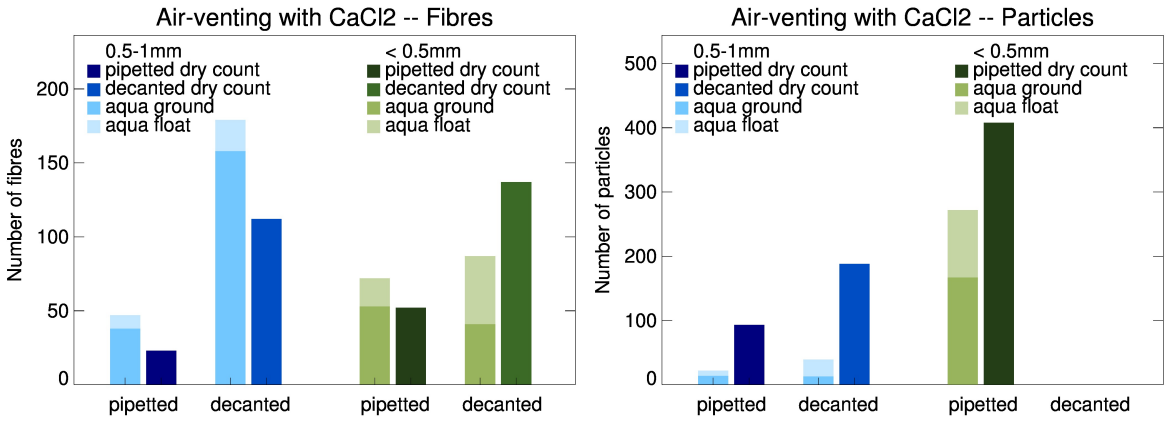


Figure 12: Fibre and particle counts in the Warnemünde test sample (P4) air-vented with calciumchloride solution. The coarse-grained sample with sizes 0.5-1mm is shown as blue bars, the fine-grained sample $< 0.5$mm is shown in green. Number counts of the pipetted and decanted solutions are displayed individually, as indicated on the x-axis. Dry counts on zooplankton net are shown as dark bars, and counts retrieved after rinsing into aqueous solution are shown as light bars separated into the ground and the floating fraction. Particles in the decanted solution of fine-grained sediment were too numerous to be counted, hence the outermost bars are missing in the right panel.

Identifiable organic matter and particles $< 70\mu$m were not counted in the scientific samples. Note that the large numbers of fibres observed in all of these test samples can be caused by contamination from the dry oven. This source of contamination is excluded in the final wet-sieving procedure applied to all science samples. Hence, absolute fibre number counts and concentrations are meaningless in these test experiments.

### 3.1.3 Polarisation microscopy

With the aim to distinguish synthetic from natural fibres, including anthropogenic natural cotton fibres, Zubris & Richards (2005) have employed high-resolution polarisation microscopy. Examples of fibres in polarised light with a magnification of 430 are shown in Fig. 13 (Zubris & Richards 2005).

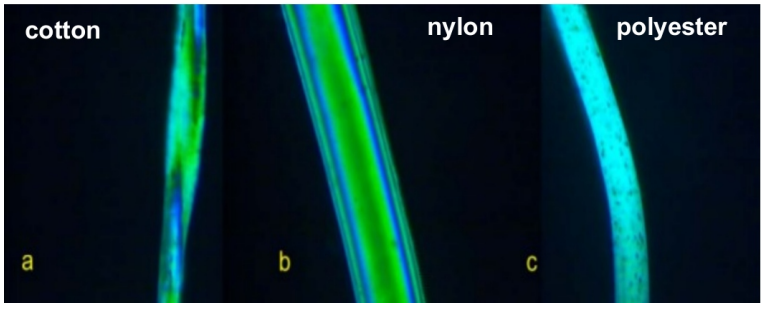


Figure 13: High-resolution fibre selection imaged with a magnification of 430 in polarised light (dark-field polarisation) as shown by Zubris & Richards (2005).

Fibres were collected from the filters of the test samples onto a microscope sled, and were

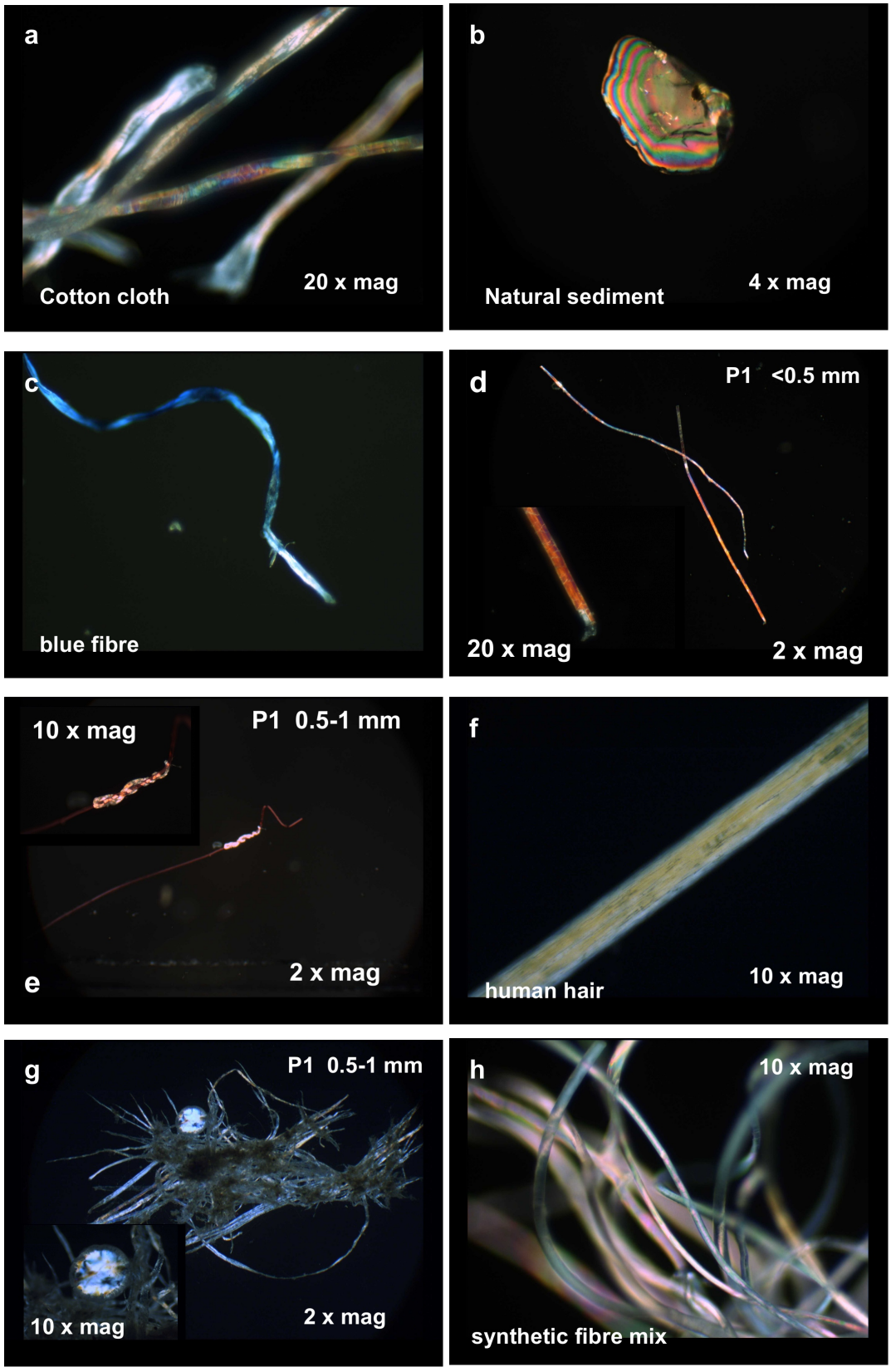


Figure 14: Examples of fibres and particles in polarised transmitted light. a) Cotton wipe at 20×magnification, b) natural sediment (4×mag), c) blue fibre (likely cotton, 10×mag), d) red synthetic fibre with disintegration marks, fibre kernel-husk structure is clearly seen at the fibre end, e) red fibre overgrown with algae, f) human hair, g) microsphere embedded in organic fibrous matter, h) synthetic fibre mix.

compared with likely contaminants from the lab. These samples included clothing worn during the experiments and polymer cloth fibres used for surface wiping. Several of the fibres detected in the test samples showed a uniform structure and diameter, suggesting a synthetic origin (Fig. 14 d,e). Nevertheless, fibres could not be distinguished uniquely under polarised light, as cotton and wool fibres also exhibited polarisation. In addition, the dark-field polarised light microscopy employed here required light transmission through the sample, such that fibres and particles analysed under the polariser had to be picked off the filter or the plankton net samples. Especially fibres and small particles were frequently lost in the test process when sticking to the collecting equipment. As polarised light microscopy is also used to highlight plastic particles in thin-layer organic material such as mussel tissue, the polarising properties of potential microplastic particles were compared to sediment polarision. However, the crystalline structure of the natural sediment caused strong polarisation signals as well, which were indistinguishable from possible transparent synthetic polymer signals (Fig. 14b). Given the limited possibility to collect large numbers of particles and fibres without loss from each sample, and the restricted distinction of synthetic and natural materials found in these experiments, polarised light microscopy was only used in occasions where the synthetic nature of particularly suspicious fibres should be confirmed.

### 3.1.4 Analysis procedure of scientific samples

#### 3.1.4.1 Extraction of light particles and fibres from sediments

After the experiments with Warnemünde test samples, and following as far as possible the recommendations in Imhof et al. (2012) and Hidalgo-Ruz et al. (2012) using inexpensive laboratory equipment available in standard chemical or biological laboratories, the procedure was refined to encompass the steps displayed in Fig. 15.

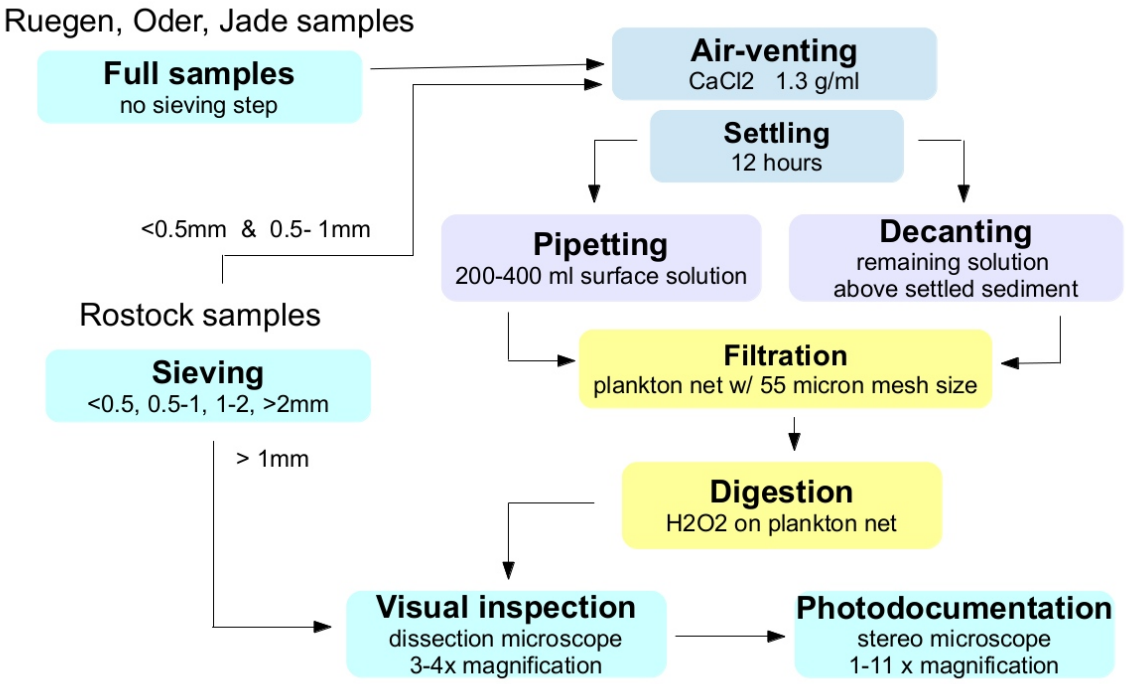


Figure 15: Procedure employed for all scientific samples.

**Sieving:**

Separating size fractions with $< 500\mu$m sieve, 0.5-1 mm sieve, 1-2 mm, and $> 2$ mm sieves (Rostock gradient samples).

**Air-venting:**

Stirring sediments in calciumchloride solution with densities of 1.30-1.35 g/ml.

**Extraction:**

Pipetting 200-400 ml off the surface onto zooplankton net filters, decanting the remaining $CaCl_2$ solution above the settled sediment to maximise extraction of higher-density particles and fibres, including particles affected by biofouling.

**Digestion:**

Treatment of zooplankton net samples with hydrogen peroxide ($H_2O_2$), rinsing with deionised water after 24 hours.

**Visual inspection:**

Visual inspection with 3-4 x magnification with a standard laboratory dissecting microscope, photographic documentation of suspicious items with up to 11 x magnification under Olympus SZX16 stereo microscope.

This procedure is designed to maximise the extraction rates of potential microplastic particles and fibres while minimising the exposure of samples to laboratory air and minimising the number of handling steps to reduce the risk of contamination with fibres. Following the recommendations in Imhof et al. (2012), the number of refilling stages is also kept to a minimum to avoid the sticking of microplastic particles and fibres to flask walls and the corresponding biases.

#### 3.1.4.2 Counting procedure

The counting procedure established in test sample P4 was used for all scientific samples. Particles and fibres were first counted after filtration on the zooplankton net filters under a dissecting microscope at 3-4x magnification (*dry count*). Zooplankton nets were then rinsed with deionised water into petri dishes, and particles and fibres settled to the bottom of the petri dish were counted separately from fragments floating on the surface of the aqueous solution (*ground* and *float* number counts). Although particles and fibres floating on the surface are expected to have a higher likelyhood to be composed of synthetic polymers, intensely coloured particles and fibres were routinely discovered in the ground fraction as well (as expected for nylon or polyamide with a higher specific density than deionised water). Hence, both *ground* and *float* fractions were counted in all scientific samples. After counting, every zooplankton filter sample was rinsed off the petri dish into a small glass flask for preservation.

## 3.2 Artificial samples

In the test samples artificially enriched with 200 polyethylen recycling particles (Sec. 2.15), coloured particles are used as tracers for the potential to recover plastics among large amounts of sediment. Examples of inserted particles and sediment samples containing recovered coloured particles are displayed in Fig. 16.

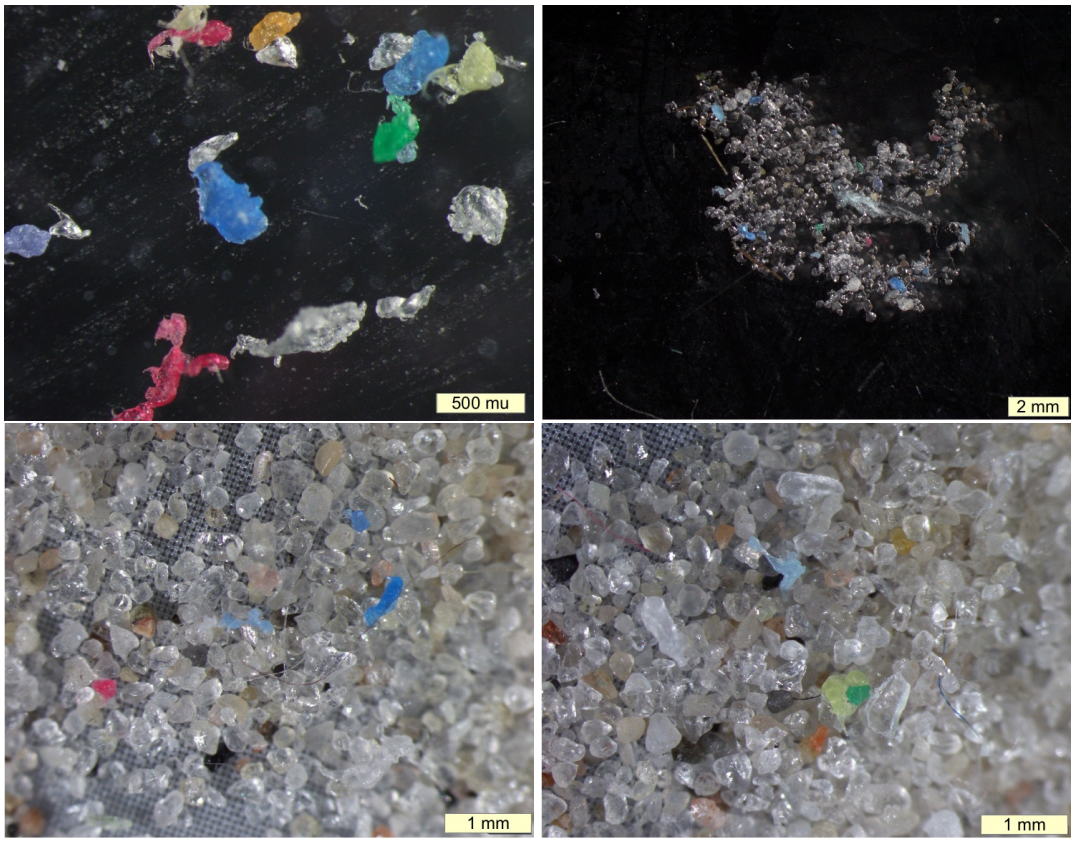


Figure 16: *Top left:* Polyethylen recycling fragments inserted in sediments to create artificial samples. The scale bar is $500\mu$m, and particle sizes are typically less than 1mm. When all inserted particles are considered, transparent particles are more ambundant than shown here. *Top right and bottom panels:* Examples of recovered microplastic particles on zooplankton net (*bottom panels*) and floating on the surface after rinsing with deionised water (*top right*). Note that blue, green, and pink particles are easily detected by eye, while the yellow particle in the bottom right panel could be mistaken for sediment.

As in the real samples, the obtained count statistics are dominated by coloured particles, which easily stand out from the natural sediment (Fig. 16). This is particularly true for blue, green, and violet particles and fibrous structures (employed to mimick the discovery of coloured fibres). The redetection of yellow, orange and pink particles proved more difficult because natural sediment is interleaved with red-orange granite and light-rose and yellow transparent quartz grains.

Recovery rates assorted by colour are shown in Fig. 17 and number counts are provided

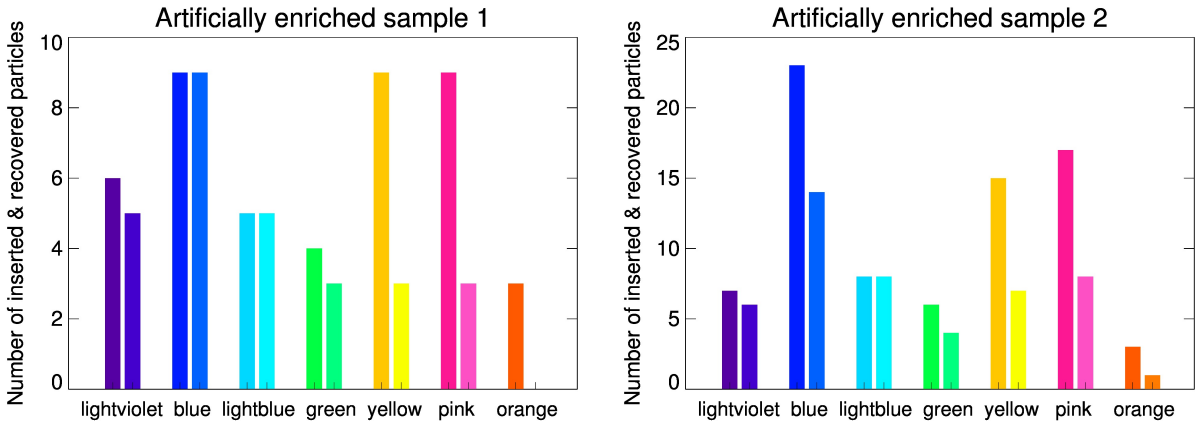


Figure 17: Numbers of coloured inserted and recovered polyethylen particles in the two artificially enriched sediment samples. Inserted particle numbers are shown as the left bar of each colour, and recovered particle numbers are reported in each right bar. Colours indicate the colours of inserted particles, although PE pieces were more lightly coloured than shown.

in Table 4. Recovery rates of intensely coloured particles range from 60-100%, while sediment-coloured particles are not always recovered, with rates ranging from 47% to 0% for yellow, pink, and light orange particles. Particles with colours blue, lightblue, light violet, and green stand out particularly clearly: recovery rates can be as high as 92-100%, especially after rinsing zooplankton filters into petri dishes and counting particles in the floating fraction. Particles of these colours are also easily detected even through thin layers of natural sediment when mixed into sediment heaps on the dry zooplankton nets, such that high detection rates of blue, green, and violet microplastics are also expected in the science samples. Yellow particles, on the other hand, are difficult to discern from both sediment and organic material in real samples, and orange particles are barely recovered.

The combination of dry number counts on the zooplankton filters and recounts after rinsing with deionised water into petri dishes proved very efficient for the recovery of both coloured and transparent plastic particles. Especially clear particles are not easily distinguished in the natural sediment heaps on the net filter, while their surface structure and charateristic shapes stand out more prominently when floating on the surface above the majority of the sediment in aqueous solution. The very small number of just 200 particles in a large volume of sediment achieves total recovery rates of 49% and 62% in both samples when transparent particles are included. These high recovery rates affirm air-venting in saline solutions as a valueable method to extract light-weight plastics from natural sediment. In addition to particles, five fibrous structures located in the PE recycling mix were also introduced to each sample. As in the case of particles, coloured fibres were easily recovered, while white

fibres could not be retrieved among residual sediment.

Table 4 also illustrates that both pipetting and decanting are important to obtain high recovery rates of synthetic particles and fibres. The majority of coloured plastic particles and fibrous structures are only recovered in the decanted solution. This suggests that pipetting or extracting the surface solution, despite containing a much lower residual sediment load, is not sufficient for a maximum microplastics detection rate. Even light-weight particles and fibres with a lower density than water, such as the PE particles with a density of 0.9 g/cm$^3$ employed in this experiment, are frequently attached to natural sediment and hence kept in the water column or at the wall of the glass flask as a consequence of adhesive forces. In the pipetted solution, the floating islands after rinsing of the plankton net are dominated by plastics. In the decanted solution, up to 1200 particles were floating on the water surface. Nevertheless, a few plastic particles and fibres were located at the ground among the sediment. These fragments were likely bonded to the sediment by adhesion. Adhesion is also prominent among the floating islands, as both floating sediment and plastic pieces come together rapidly after being rinsed into the petri dish. In addition, several plastic particles were sticking to the edge of the petri dish immediately after rinsing. This illustrates how readily microplastics are captured by the surfaces of the laboratory equipment, which was identified by Imhof et al. 2012 as one of the major sources of plastic particle losses during extraction experiments, confirming our attempt to avoid extra refilling steps whereever possible prior to the number count.

Table 4: Results of artificially enriched samples: Inserted particle numbers (*column 2*) sorted by colour (*column 1*). Particles recovered in the pipetted ("pip", *column 3*) and decanted ("dec", *column 4*) solutions are shown separately, and the sum of all recovered particles ("pip+dec") is given in *column 5*. The recovery rate is calculated as the fraction of recovered to inserted particles in *column 6*. *Columns 7 & 8* show the number of recovered particles in deionised water after rinsing the plankton net filter (total of plastic particles attached to ground sediment plus floating particles) and the corresponding recovery rate, respectively.

| **PN3 + 200 PE particles** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Colour** | **insert** | **pip** | **dec** | **pip+dec** | **rate** | **aqua** | **rate** |
| blue (bl) | 9 | 1 | 7 | 8 | 0.88 | 9 | 1.00 |
| lightblue (lb) | 5 | 3 | 1 | 4 | 0.80 | 5 | 1.00 |
| green (gr) | 4 | 1 | 2 | 3 | 0.75 | 3 | 0.75 |
| violet (vi) | 6 | 2 | 3 | 5 | 0.83 | 5 | 0.83 |
| yellow | 9 | 2 | 0 | 2 | 0.22 | 3 | 0.33 |
| pink | 9 | 0 | 0 | 0 | – | 3 | 0.33 |
| orange | 3 | 0 | 0 | 0 | – | 0 | – |
| **coloured** | 45 | 9 | 13 | 22 | 0.49 | 28 | 0.62 |
| **bl+lb+gr+vi** | 24 | 7 | 13 | 20 | 0.83 | 22 | 0.92 |
| transparent | 155 | | | | | 70 | 0.45 |
| **all** | 200 | | | | | 98 | 0.49 |

| **PN4 + 200 PE particles** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Colour** | **insert** | **pip** | **dec** | **pip+dec** | **rate** | **aqua** | **rate** |
| blue (bl) | 23 | 7 | 7 | 14 | 0.61 | 12 | 0.52 |
| lightblue (lb) | 8 | 4 | 4 | 8 | 1.00 | 8 | 1.00 |
| green (gr) | 6 | 2 | 2 | 4 | 0.67 | 4 | 0.50 |
| violet (vi) | 7 | 1 | 5 | 6 | 0.86 | 2 | 0.29 |
| yellow | 15 | 2 | 5 | 7 | 0.47 | 5 | 0.33 |
| pink | 17 | 2 | 6 | 8 | 0.47 | 7 | 0.41 |
| orange | 3 | 0 | 0 | 0 | – | 1 | 0.33 |
| **coloured** | 79 | 18 | 29 | 47 | 0.59 | 39 | 0.49 |
| **bl+lb+gr+vi** | 44 | 14 | 18 | 32 | 0.73 | 26 | 0.59 |
| transparent | 121 | | | | | 76 | 0.63 |
| **all** | 200 | | | | | 123 | 0.62 |

Two results are most striking when interpreting the numbers of microplastic particles recovered. i) In both samples, the largest number of microplastic pieces is recovered in the decanted solution. Two reasons are identified for this behaviour. First, the mentioned adhesive forces cause microplastic particles to stick to the edge of the Erlenmeyer flask, such that they are missed by the pipette, but are recovered when the remaining solution is carefully decanted while turning the flask. Secondly, synthetic particles as well as fibres are routinely found in the water column rather than at the surface in all of our scientific and test samples. This suggests that synthetic particles sink more easily than expected from their pure material density alone. Additives might additionally increase the density of particles, as shown in Nuelle et al. (2014, see their Table 5). The fact that this also occurs in lightweight PE particles (0.9 g/cm$^3$) not exposed to biofouling indicates that adhesion cannot be ignored. ii) The number counts are optimised when both dry and wet counts are used. In the first artificial sample, the wet count caused more plastic particles to be exposed and a larger recovery rate was obtained after the wet count. In the second sample, the sediment content both on the ground and in the floating fraction was very high after rinsing the plankton net, impeding redetection of several coloured particles. In the dry count, however, searching systematically through the sediment allowed a redetection rate of $\sim 60$% among coloured particles despite the very high sediment load of several thousand sediment particles in the decanted fraction. In both samples, transpartent and white microplastics were only recovered in the aqueous solution. Especially in residual sediment, the structure of transparent particles does not stand out, and only a few isolated particles were identified on the plankton net of the pipetted fraction. On the water surface, however, the structure of PE particles is clearly discerned from the smoothed surfaces of natural sediment. While this structural difference might change after exposure to wave forces in the natural sea environment, this observation stresses that several means of visual inspection enhance the chances of microplastics discovery.

**Summary of artificial experiments**

Coloured particles, especially in shades of blue, green, and violet, are most easily discovered among natural sediment, even if their average size is smaller than the size of the immersing sediment layers. Particles as small as $\sim 70\mu$m are easily spotted by eye through the dissecting microscope with a magnification of 3. We therefore conclude that the air-venting, pipetting plus decanting method employed here to retrieve plastics from natural sediment works most efficiently on blue-tinged fragments, and that the detection of coloured

particles in these colour regimes should be complete to at least 60% (Table 4).

For transparent and yellow, orange, or pink particles, spectroscopy would be particularly beneficial in order to unambiguously identify all synthetic polymers among the mix of natural sediment. Despite larger losses, the overall recovery rates range from 50% to 60% when both coloured and transparent particles are taken into account. The fact that about half of the 200 particles with sizes less than 1mm could be extracted from 800g of sediment renders the developped method highly efficient.

It has to be noted here, however, that in natural sediment, we expect the detection rate to be lower. Bleaching and the presence of large amounts of white and transparent fragments will decrease the detection rate. From the artificial experiments, it becomes immediately clear that the detection of transpartent particles, especially after biofouling or mechanical smoothing, faces severe limitations when visual inspection has to be used to distinguish microplastics from natural sediment. This point will be stressed further during the analysis of the scientific samples, yet special emphasis will be placed on both coloured particles and coloured fibres because of this finding.

## 3.3 Blind & reference samples

Blind samples were processed in the same way as science samples as much as feasible. Cleaning procedures of the Erlenmeyer flasks, the filtering equipment, and the zooplankton net filters were identical to the procedures applied between sediment samples. Air-venting for 4 hours with pre-filtered calciumchloride solution in the same Erlenmeyer flasks was conducted. The solution was then decanted, and in two blind samples pipetted as well as decanted, over cleaned zooplankton net filters as in the case of the real samples.

The results of the blind sample number counts are shown in Table 6 in Appendix B. From five blind samples, the laboratory contamination of particles and fibres is expected to be low. For particles, the blinds contain between 1 and 8 particles as counted on the zooplankton net filters (dry count), and after rinsing with deionised water, between 0 and 3 to 5 particles are found in the floating fraction and on the ground, respectively. The average particle contamination in the dry count is 3.4 fragments, while it is 2-3 particles in the ground and floating fractions in aqueous solution. This low particle contamination is expected, as sediment is not easily entering clean sample volumes in the lab. The counted particles are likely residual contamination in the Erlenmeyer flasks or in the filtering equipment, or were stuck to the plankton nets after cleansing due to sticky protein residuals. Given the large volume of 2l of each flask, as well as the several handling steps, and the fact that the net

filters cannot be cleaned to an entirely pristine state, the contamination of 1-8 transparent particles in all blind samples is very low, and no coloured particles are found.

For fibres, the situation is not as clear. In two of the blind samples, small fibre nests were observed. This suggests that glass fibre material was disintergrated from the glass fibre filters during pre-filtering of the $CaCl_2$ solution. As the first blind sample (18 Aug 2014) did not contain any glass fibre residuals, this finding came as a surprise. After fibre nests were detected in the blind samples, the calciumchloride solutions for all scientific samples were pre-filtered through 5$\mu$m polyacetate membrane filters instead. The fibre contamination in the first blind plus the membrane-filtered blind samples was as low as 1-8 fibres in the dry count. As fibres are harder to see on the plankton net material, fibre loads in the wet count are slightly higher, with an average of 9 fibres on the ground and 7 fibres floating on the surface, implying a total fibre contamination of 16 fibres on average. This fibre load increases to 21 when the two samples with obvious fibre nests are included in the mean. Most of the detected fibres are thin and transparent, and at the thin edge of being counted in scientific samples. However, each blind contained on the order of 1-2 long, thick fibres, several of which are also intensely coloured. As a consequence, we expect up to 2 coloured fibres to be introduced from laboratory air and/or handling procedures into each sample. This is confirmed by the laboratory air sample also shown in Table 6. After drawing laboratory air through a membrane filter for 2 hours, 2 coloured, long fibres are detected on the filter. Note that the numerous very small particles and fibres also counted on this air filter are very small (particles) and thin (fibres) and would not be included in the real samples, as they would be removed by the 55$\mu$m zooplankton net filter. As actively drawing lab air for 2 hours through a membrane with a vacuum pump is longer than all of the scientific sample handling, we can consider the 2 coloured fibres detected on the air filter again as an upper limit of contamination from laboratory air alone.

In the first blind sample, 2 microspheres were detected on the plankton net. One of these spheres was recovered in the aqueous solution, while the second sphere was lost in the wet count. This is the only blind sample that contained any microspheres. Both spheres, despite displaying different sizes and different colouring, had the appearance of potential cosmetic polymer spheres also found in several of the scientific samples. One of them, with a yellowish hue, was particularly similar to the spheres detected in the Nienhagen May samples, where microspheres featured prominently among the raps pollen. No such microspheres were found in either of the laboratory water samples also included for reference in Table 6. In 10l of cold tap water, only 1 particle and 1 fibre were found on the plankton net. In the 10l deionised water sample, on the other hand, in addition to the 1 particle, 13 fibres were de-

tected, and one of these fibres was intensely coloured in red. The higher fibre load is likely a consequence of the longer processing time and handling in the lab, as deionised water is first pulled through the deionisation tank, then filled into a rinsed canister, and refilled into either glass flasks or the laboratory spray bottle. The two spheres found in the first blind sample are therefore likely remnants from the previous science sample (the Warnemünde May sample) on the Erlenmeyer flask walls. The high stickiness of microplastic fragments and spheres renders the cleaning of flask walls to a zero contamination level practically impossible. As all 4 later blinds do not show any microsphere contamination, and as no spheres are found in either tap or deionised water, the contamination with microspheres is expected to be less than 1 microsphere on average in each science sample.

In summary, the most important source of contamination are coloured and transparent fibres. On the order of 16 contaminating fibres can be expected in the aqueous solution, and up to two coloured fibres are found in blind samples. This contamination level is surprisingly low in view of the fact that clean room conditions were not available for these experiments. The contamination with particles and microspheres is found to be negligible.

## 3.4 Analysis of Baltic Sea and North Sea coastal samples

All scientific samples were processed as described in Sec. 3.1.4.1, and particle and fibre numbers were counted on zooplankton net filters (dry count) and in aqueous solution with individually counted ground and floating fractions. The detailed results of all sample counts, along with comments about the appearance of each sample and special particles and fibres standing out among natural sediment, are provided in Appendix D (Tables 8 to 14). Examples of detected microplastic particles and fibres are shown for illustration in Figs. 32 to 37 in Appendix E. The results are summarised in histograms presented in Sections 3.4.2 to 3.4.5 for each corresponding location. Before analysing source counts in detail quantitatively, a brief overview of the general results observed in all samples is presented in the next section.

### 3.4.1 General observations

Most particles ($> 99\%$) extracted after air-venting have the same optical appearance as the sediment particles in all samples. In particular, even most particles floating on the surface of the aqueous solution after rinsing of the filters are visually indinstinct from natural sediment (see Fig. 33 in Appendix E for examples). Only a small number of uniquely identifiable plastic particles are found in all sediment samples. These particles stand out mostly on the basis of their intense blue, turquoise, green, or bright red colours, in agreement with the finding in the artificial samples above. Orange particles with smooth surfaces are frequently detected, yet those particles are visually indistinct from natural orange-red quartz fragments. Several microplastic pieces are discovered on the basis of their shape and their surface structure together with their floatation properties. Another source of anthropogenic contamination in the sediment samples that might enter the food chain are glass pieces, although the absence of toxic additives leaching into the tissue of absorbing organisms suggest less adverse health effects than feeding on microplastics. Green glass pieces down to very small size scales ($\sim 70\mu$m) are regularly detected in almost all sediment samples. Most green glass bits are tiny and smoothed by erosion and must have been exposed in sediment and water for a prolonged time. Despite their similarity to sediment in shape, the characteristic green colours stand out among natural grains prominently, consistent with the high recovery rates of coloured fragments regardless of shape and size in the artificially enriched samples.

### 3.4.2 Results of Rostock gradient

#### 3.4.2.1 Analysis of particle & fibre number counts

**Particles:**

With the finding in mind that particles floating on the surface of extracted samples after air-venting are visually indistinguishable from natural sediment, the particle number counts in Fig. 18 are not discussed under the presumption that all particles floating near the surface in heavy saline solution are mircoplastics. The fluctuation is large both in the coarse 0.5-1mm size fraction as well as in the small $<$0.5mm size fraction. In the pipetted solution, particle numbers can range from zero to more than 150, and are most frequently between 10 and 50 in the plankton net counts as well as in the combined wet (ground + float) counts. The instances where particle numbers are particularly high in the May Warnemünde and March Markgrafenheide samples had a large fraction of fine-grained sediment floating above the settled bottom sediment in the Erlenmeyer flask. Under the microsope at a magnification of 3-4, these grains were visually indistinguishable from natural sediment despite being more numerous than in the other samples. The fluctuations in particle numbers are observed to be even larger in the decanted solution, where the layers above the settled bottom sediment are sifted onto the zooplankton net filter. In the coarse sediment fraction, number counts in excess of 100 particles are observed in the April Nienhagen, Wilhelmshöhe, and Markgrafenheide samples. Especially in the small size fraction, most sediment counts are lower limits because of the very high sediment loads of several hundreds to thousands of particles. The maximum number count in excess of 2000 particles is again observed in the Nienhagen April sample. Systematic seasonal variations are not observed from March to July in either of the locations, and systematic spatial trends between each of the four locations are also not detected.

**Fibres:**

Substantial statistical differences are observed in the fibre loads both in sediment and seawater samples. Between a few and several hundred fibres are observed. Nevertheless, the distinction of natural and anthropogenic fibres is not as ambiguous as for uncoloured particles. Although natural fibres might also be included in the presented number counts in Fig. 19, several fibres displayed a long, thick and very regular structure unlikely to be found in natural organisms. It would be interesting to study the appearance of natural fibres

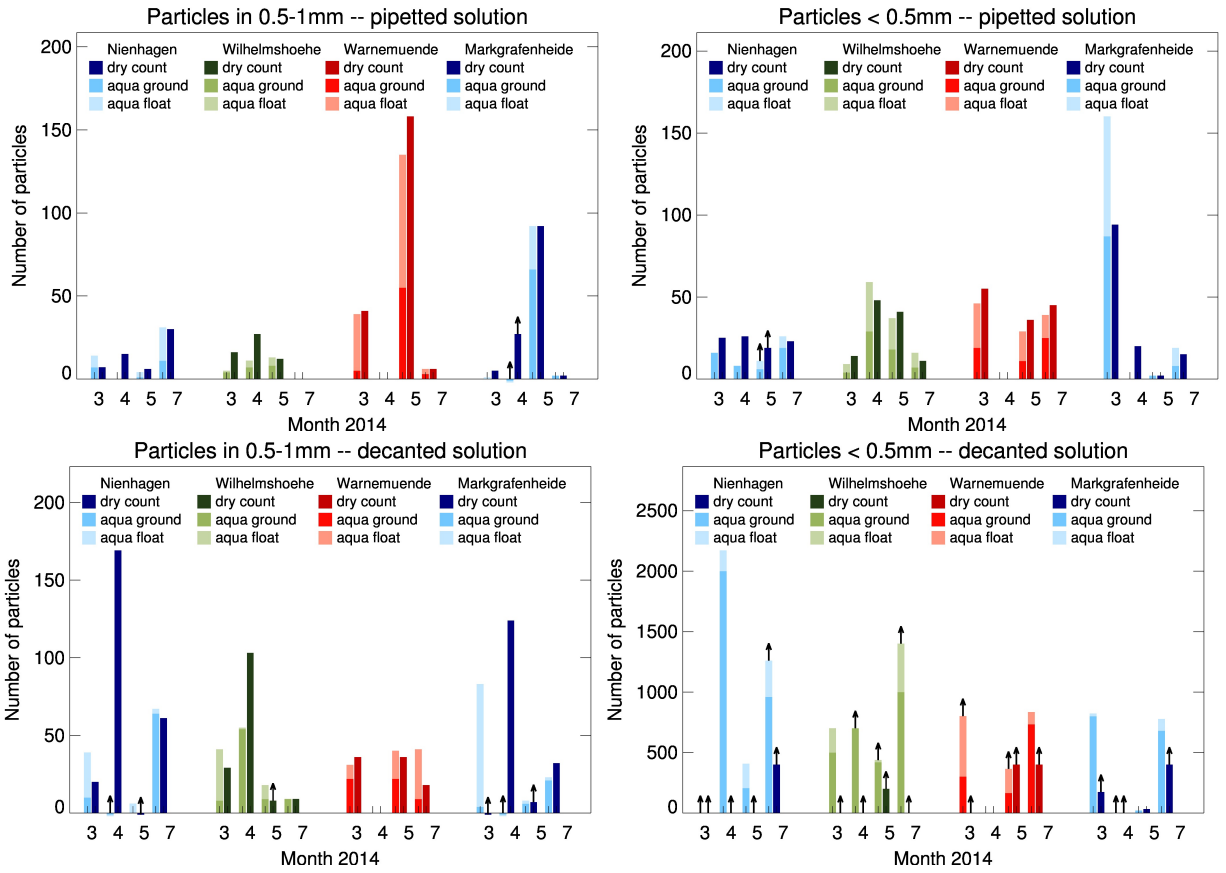


Figure 18: Spatial and temporal variation of particle number counts observed along the Rostock west-east gradient. Sampling locations are displayed from left to right: Nienhagen (blue), Wilhelmshöhe (green), Warnemünde (red), Markgrafenheide (blue). The x-axis labels denote monthly measurements for March (3), April (4), May (5), and July (7). Particle numbers are counted on zooplankton net filters (dry count, dark bars) and in aqueous solution after rinsing of the filters (lighter bars). Arrows indicate lower limits, or cases where the dry count was prohibited by dense sediment on the filter. Particles sunken to the ground (bottom part of lighter bars) and particles floating on the surface (light part of lighter bars) are displayed separately. The fine fraction with grain sizes $< 0.5$mm (*right panels*) and the coarse fraction with grain sizes 0.5-1mm (*left panels*) were analysed individually. Note the different scale in the bottom right panel.

in detail and create a comparison database, but this is beyond the scope of this thesis. The distinction between natural and synthetic or at least anthropogenic fibres is more easily obtained for coloured fibres than for coloured particles, as natural fibres do not display intense colouring. With the aim to increase the likelyhood to count synthetic fibres, the number counts in Fig. 19 were derived from either long, thick transparent fibres or intensely coloured fibres. The comparison between the darker and the lighter parts of the bars corresponding to number counts in aqueous solution indicates that fibres are comparably likely to be found on the ground along with the settled sediment fraction as well as in the floating “islands” on the surface. In particular, fibres are by no means predominantly floating on the water surface. In both the large and small size fractions, fibre numbers in the pipetted sur-

face solution range from 10-40 in most samples and stay below 30 in all pipetted $<$0.5mm surface samples. Fluctuations from a few fibres (consistent with lab background levels, see Sec. 3.3) to 25 fibres are detected for grain sizes $<$0.5mm, and do not show systematic patterns for specific locations or months, such that no seasonal trend is observed for fibres among small grains. In the pipetted solution of the coarse grain 0.5-1mm fraction, two peaks with fibre counts above 40 stand out among all other samples. The highest fibre load in coarse sediments is observed in Nienhagen in April, reaching levels of 80 fibres. The large fibre load is confirmed in the decanted solution of the fine-grained $<$0.5mm fraction of the same sample (110 fibres). The second peak that stands out prominently concerns the Warnemünde July sample. This sample displays a high fibre load in the 0.5-1mm pipetted solution, but additionally exceeds all fibre loads in the coarse and fine-grained decanted fractions with count rates of 220 and $>$120 fibres, respectively. The fibre number counts in Markgrafenheide, on the other hand, do not stand out significantly. With numbers between 20 and 40 in most Markgrafenheide samples, with only the April sample reaching a maximum of $\sim 70$ fibres, the numbers are comparable to Nienhagen and Wilhelmshöhe.

**Microspheres:**

A total of 18 microspheres is observed in 6 of 18 Rostock sediment samples.[3] Microspheres display a perfectly spherical shape with a diameter of 70-100$\mu$m. Most microspheres are highly transparent with a light-yellow hue, and are identical in size and colour. Exerting mechanical tension with the lanzette on one of the spheres, the shell of the sphere yielded to pressure and a thick gel emerged. The shell proved to be extremely stable and the exerted pressure had to be high to break the surface. Up to a magnification of 11, no substructure was observed inside the shells or in the escaping gel.

#### 3.4.2.2 Occurence of coloured particles and fibres

The least ambiguous anthropogenic contaminants in beach sediment are intensely coloured particles and fibres. Although the numbers of coloured particles and fibres are generally low (Fig. 20), almost all samples contain coloured fibres and about half of the samples contain coloured particles. Qualitatively, coloured microplastics serve as tracers for anthropogenic influx of synthetic material into natural beach sediment and seawater (see also Dekiff et

[3]There is no fixed term in the literature for spherical microplastic structures. Encountered terms included spheres, microsperes, globules, and round, spherical particles. We use the term microsphere here for simplicity when implying potential microplastics with a perfectly spherical shape.

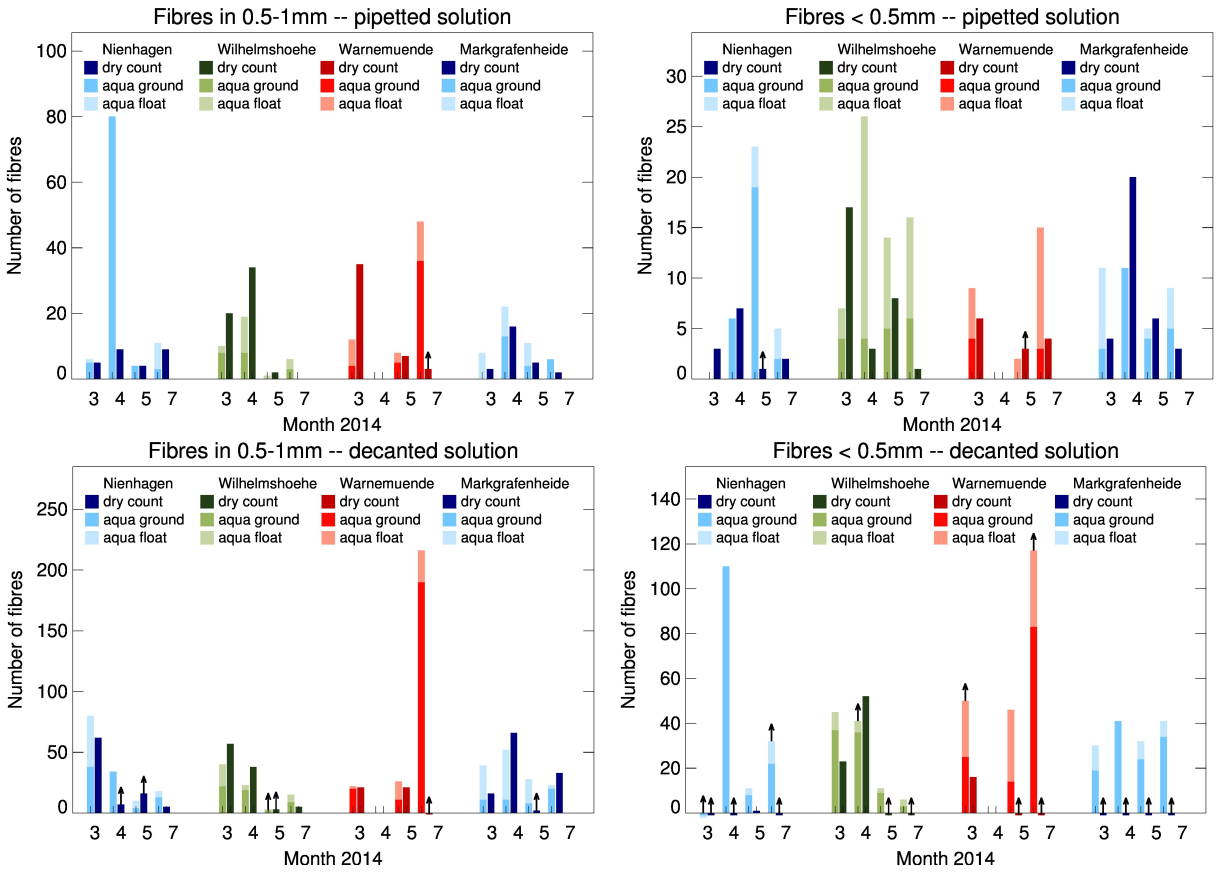


Figure 19: Spatial and temporal variation of fibre number counts observed along the Rostock west-east gradient. Locations and colour coding are as indicated in the legends (see also Fig. 18). The x-axis labels denote monthly measurements for March (3), April (4), May (5), and July (7). Fibre numbers as counted on zooplankton net filters (dry count) are shown as dark bars and in aqueous solution after rinsing of the filters as light bars. Arrows indicate lower limits or cases where dense sediment on the filters prohibited the dry count. Coarse (*left panels*) and fine-grained (*right panels*) samples are displayed separately. Note the different scales.

al. 2014). Number counts of intensely coloured fibres and particles are therefore compiled in Fig. 20, where the displayed colours in each sample reflect the colour range of the discovered particles and fibres. As expected from the artificially enriched samples discussed in Sec. 3.2, coloured fibres and particles are particularly well separated from sediment in hues of blue, turquoise, intense green, and intense red by visual inspection.

**Coloured Particles:**

The spatial and temporal variation in the number counts of coloured particles is shown in the left panel of Fig. 20. Between zero and five intensely coloured particles are found in all samples, with the maximum load of coloured particles detected in the March Warnemünde sample. As no coloured particles are observed in laboratory reference samples (Sec. 3.3),

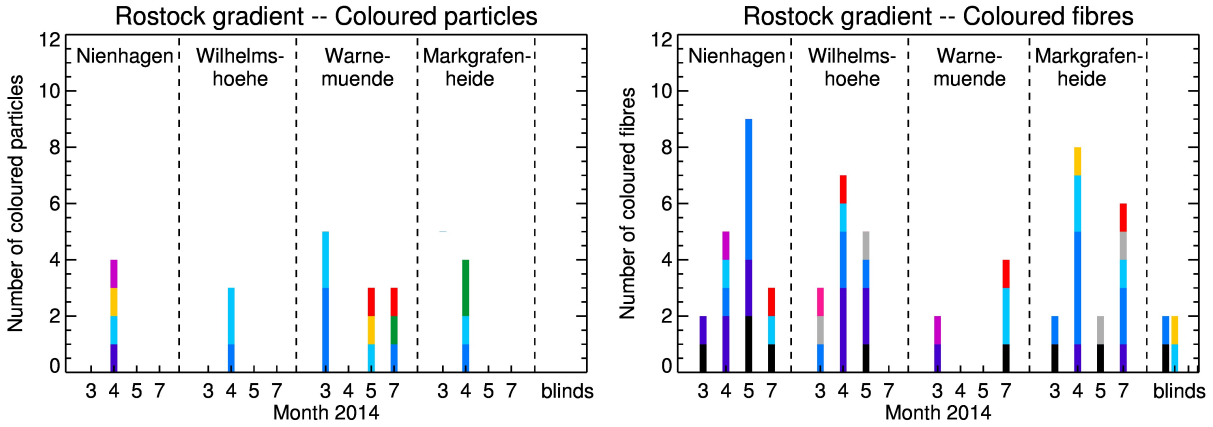


Figure 20: Number counts of intensely coloured particles (*left panel*) and fibres (*right panel*) observed from March (3) to July (7) at each Rostock sampling location. Colours are approximately coded to represent the real detected colour range. No bar indicates no coloured pieces were found, except for the April (4) Warnemünde position, where no sample was obtained. Coloured particle and fibre counts of laboratory blind samples are shown in the last bins to the right in each panel. No coloured particles were observed in the reference samples.

and as transparent plastic particles are not readily discerned from natural sediments by visual inspection, these numbers are considered the minimum microplastic particle load in Rostock beach sediments. While coloured fibres are detected in almost all samples, coloured particles are found in only 6 out of 15 samples or 40% of the Rostock beach sediment samples. A significant variation in the presence of microplastic particles is observed among the four sampling locations. Nienhagen, Wilhelmshöhe, and Markgrafenheide contain coloured microplastics only in April, and no coloured particles are detected in the March, May, and July samples. In the April samples, fibre loads are also exceptionally high, as discussed below. In contrast to these three sampling locations, Warnemünde has coloured microplastics in every sample (note that no sample was taken in April at this location). Numbers of microplastic particles range from 3 to 5 in all samples where intensely coloured particles are detected, with the highest number of microplastic particles observed in Warnemünde sediments in March. Especially blue and turquoise plastic particles are usually very small with sizes 50-100$\mu$m. These tiny particles were also not observed in any of the blind samples, but are observed in almost all sediment samples. That these particles are not contamination during lab processing should be confirmed with larger sediment sample sizes to provide better number statistics. Red particles unambiguously identified as plastics are only detected in the Warnemünde samples. One of the red particles dissolved in $H_2O_2$, suggesting polyamide as the polymer compound.

**Coloured Fibres:**

Coloured fibre number counts of all Rostock locations are shown in the right panel of Fig. 20. Coloured fibres are found in almost all sediment samples. However, up to two coloured fibres are also observed in several blind samples, such that contamination from laboratory air is not at the zero level, as expected without cleanroom conditions. In the blind samples, blue and darkblue/black fibres dominate, and only one yellow fibre was found. Blind samples contain between 0 and 2 fibres typically, and only one out of five blinds contains 3 coloured fibres. Sediment samples with only 2 coloured fibres can therefore not be distinguished from laboratory background levels. With 5 to 9 coloured fibres significantly above background levels, the Nienhagen, Wilhelmshöhe, and Markgrafenheide samples show large coloured fibre contents in April and/or May. This is consistent with the overall higher fibre loads discussed in the previous section, and with the intensely coloured plastic particles observed in these April samples. The coloured fibre load is with 4 fibres in the July Warnemünde sample moderately high compared to the March and May observations, where fibre contents were consistent with background levels.

In general, coloured fibres are more numerous than coloured particles. Fibre numbers range from zero to 9 coloured fibres per sample, while the maximum particle number is 5. So far, the focus of sediment studies was rarely on synthetic fibres. The fact that coloured fibres are present in almost all samples indicates that synthetic fibres represent a significant fraction of the microplastics contamination and should be studied in more detail in the future.

#### 3.4.2.3 Warnemünde Seawater Sample

Despite drawing seawater from the surface layer at a position where the water was more than 50 cm deep, a total of 69 particles are detected in the 7l water sample. Most particles lifted into the water column and collected in the seawater sample are very small as compared to sediment particles collected from the ground. The comparably small size range of these particles suggests that smaller-sized grains are supported more easily and for longer periods of time on the water surface than larger-sized grains. The most conspicious floating particle displays the shape of a droplet with a length of 1.6mm (see Fig. 21) and is clearly identified as microplastics. In addition, thin “foil” fragments are observed in the seawater sample, and one blue particle was attached to such fragments (see Fig. 21, right panel). While the darkblue particle is likely of anthropogenic origin, whether the thin foil fragments

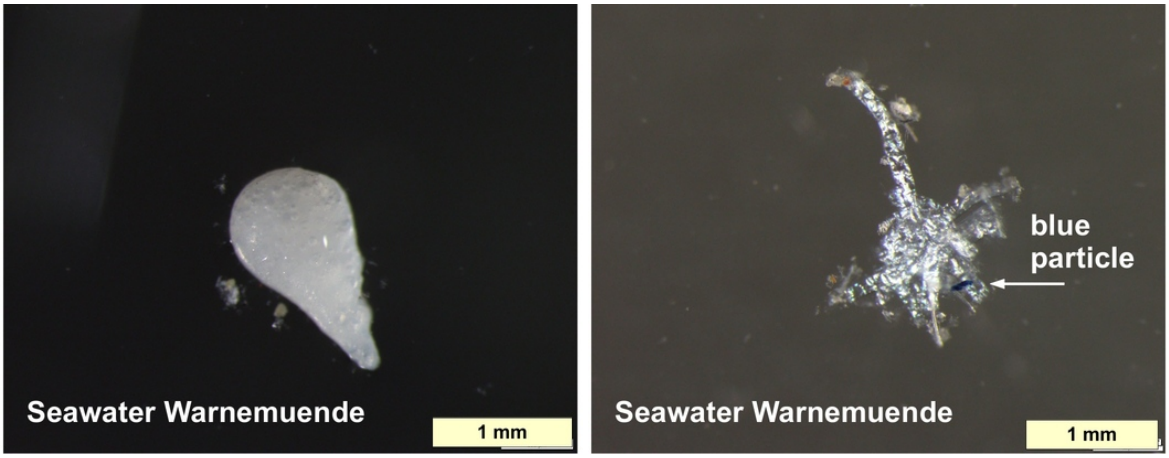


Figure 21: Microplastic particles discovered in the Warnemünde seawater sample. *Left:* Hard plastic droplet with a length of 1.6mm swimming on the surface after filtration of the Warnemünde seawater sample (scalebar 1mm). This is one of the largest and unambiguously identified microplastic particle found in all samples. *Right:* Thin foil fragments with darkblue particle attached (scalebar 1mm).

are organic matter or synthetic polymer sheets cannot be deduced without spectroscopic identification.

At the same time, there are surprisingly few fibres, and especially only 3 long, thick, coloured fibres (2 black, 1 blue) were suspended on the surface. This finding is consistent with the above observation that fibres, and especially thick, synthetic fibres, sink to the ground rapidly and would hence not be suspended at the water surface in large amounts.

### 3.4.3 Rügen gradient

#### 3.4.3.1 Particle and fibre number counts

The visual inspection of the Rostock samples showed that coloured particles and fibres provide the safest identification of microplastic contaminants in sediment samples. Hence, the coloured particles and fibres as potential microplastics are predominantly discussed below. As not intensely coloured particles are suspected to be natural sediment, only the total fibre number counts are shown in Fig. 22. The detections of coloured particles and fibres are displayed in Fig. 23. At the main beach in the seaside resort of Binz, both drift line and shallow water sediment samples were obtained. While the shallow water sample is not directly comparable to the drift line samples, the results are included in the discussion of microplastic contamination where appropriate.

**Particles:**

A total of seven tiny plastic particles are observed in the Rügen drift line samples when all coloured, potential microplastic pieces are counted (see Fig. 23). One additional blue particle was extracted from the shallow water sample. In each individual sample, between one and three coloured particles are detected. As in all other samples, coloured plastic pieces are predominantly smaller than 100$\mu$m and are dominated by blue and turquoise colours, as those stand out most prominently among natural sediment. Two intensely red coloured particles are identified, which is rare among coloured particle samples. Other than the dark red particles observed in the Rügen samples, brightly coloured red particles were only retrieved from Warnemünde beach sediments. These two beaches have the highest visitor density of all locations.

**Fibres:**

In the whole set of five Rügen samples obtained at four locations, only 5 coloured fibres are found (Fig. 23). With only 1-2 coloured fibres per sample, these numbers are consistent with laboratory background levels. Note that laboratory exposure is lower without sieving due to the decreased number of handling steps and exposure to lab air and equipment. The total load of 0-20 long fibres per sample (pipetted plus decanted solutions, Fig. 22) is also extremely low. This is even true for the touristic beach at Binz, where samples were obtained in the main beach area next to the sea bridge.

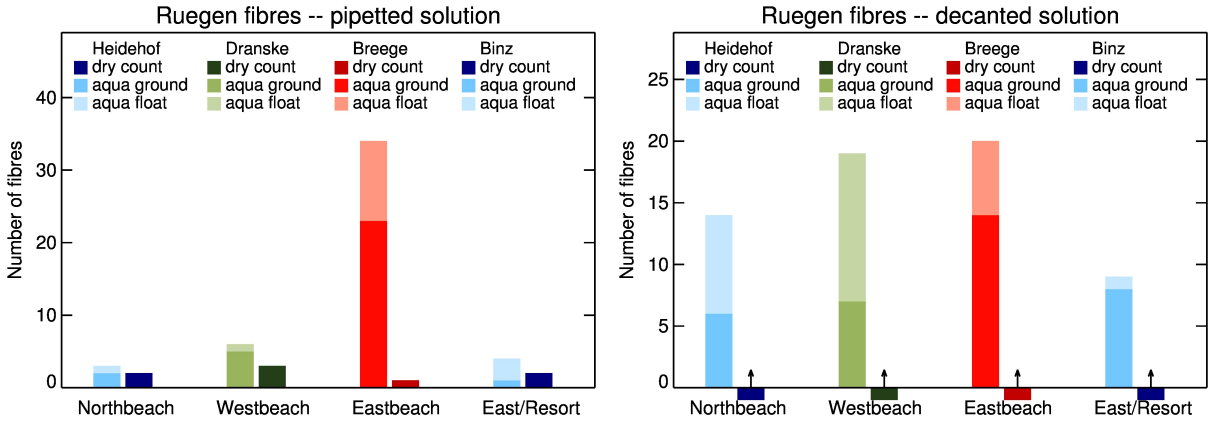


Figure 22: Fibre number counts at the four Rügen sampling locations. Locations are as indicated in the legends: Heidehof in the north (blue), Dranske in the west (green), Breege (red) and Binz (blue) in the east of the island. Dry counts are reported as dark bars, and counts in aqueous solution as lighter bars. Note the different scales and corresponding lower number counts as compared to Fig. 19 in both the pipetted solution (*left*) and the decanted solution (*right*). Fibres on the sediment-rich plankton net filters of the decanted solution could not be counted, as indicated by the arrows, such that fibre counts are only shown after rinsing into aqueous solution.

The decanted solution of the Breege and Dranske samples contains large amounts of fine, thin, and short fibres not counted in Fig. 22. While the sediment sample taken under the gravel line in shallow water in Binz shows a similarly rich frequency of very thin fibres, both the Binz and Heidehof drift line samples display no thin fibres at all. These are among the cleanest samples taken during the whole summer of 2014 in the entire Baltic coast sampling area. The fact that many fine fibres are observed in the Binz underwater sample, but not in the Binz drift line sample, suggests a possible natural marine origin of these thin fibres. Further samples and research are required to monitor the seasonal variation in fine fibre loads. A correlation between the growth rate of mussels and zooplankton species or other crustaceans and a detailed comparison with organic antennae would be beneficial to quantify the expected natural thin fibre load.

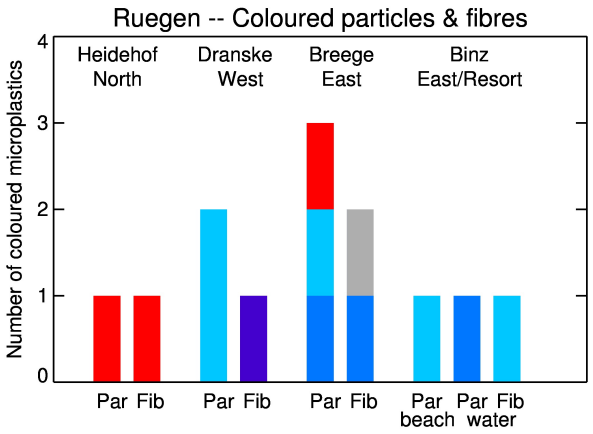


Figure 23: Coloured particle ("Par") and fibre ("Fib") number counts at all four Rügen sampling locations. In the case of Binz, both the drift line ("beach") and the shallow water ("water") sample are shown as both displayed coloured microplastic fragments. The Binz beach sample did not contain coloured fibres.

In summary, the five samples analysed from the island of Rügen show low numbers of tiny plastic fragments. At the same time, they display the lowest fibre content in all samples, and visually identifiable synthetic fibre numbers remain low even at the most frequented Rügen beaches.

### 3.4.4 Oder/Peene estuary

#### 3.4.4.1 Detection of anthropogenic particles, fibres, and glass pieces

Sediment samples of the outlet of the Oder into the Baltic Sea were taken landwards at the freshwater sand beach of Kamminke at the *Stettiner Haff*, near the Polish border, and seawards at the western Peene outlet near the Freest fishing harbour. As sieving did not yield distinctive results between the fine and coarse fractions in the Rostock samples, and as Oder samples are composed of fine-grained silt, the Freest and Kamminke samples were not sieved. Especially the Freest sample is dominated by very fine silt, such that numerous tiny sediment pieces (several 1000 to 10000 per sample) were found in the pipetted and decanted surface solutions above the bottom sediment layer, prohibiting sediment/particle

counts. Because large amounts of organic material (insect/crustacean shields, dragonfly shedded skins, waterplant blades, and dark-green algae) rapidly clogged the net, the remaining solution above the bottom sediment had to be decanted over 4 plankton nets instead of one. After rinsing the decanted solution from the plankton nets, large islands of mixed organic material and fine-grained sediment were floating on the surface of deionised water. Even tiny green glass bits were caught and suspended in these floating islands. To further test whether these floating particles are dominated by microplastics or natural sediment, the surface solution of two decanted nets was carefully poured from the petri dish into two centrifugation tubes, which were then filled to 37ml water level with deionised water. These tubes were centrifuged at low speed with 800 rotations/minute for 3 minutes. After centrifugation, all particles had sunk to the bottom of the tubes, indicating that the floating particles were dominated by fine-grained sediment with a higher density than water. Only a few insect shield and 5 skin/foil pieces as well as 4 fibres were still found at the surface of each sample (recovered fibre and foil number counts were identical in both samples), and no indication of transparent plastic particles was found in these samples after centrifugation.

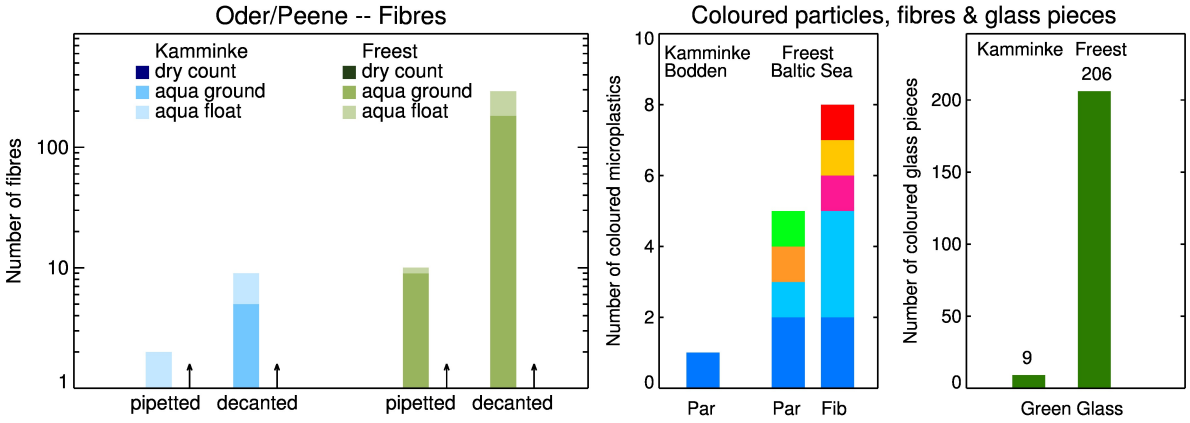


Figure 24: Oder/Peene fibre and coloured particle and fibre number counts.
*Left panel:* Fibre number counts in the Kamminke *Stettiner Haff* and Freest Oder/Peene estuary sediment samples. Plankton net counts (dry counts) were prohibited by the large number of fine-grained sediment on each filter, as indicated by the arrows. Note that a logarithmic scale is chosen in this plot, as fibre counts in the aqueous solution were very low in Kamminke and exceptionally high in Freest sediment.
*Right panel:* Coloured particles, fibres (*left*), and glass fragments (*right*) detected in the Kamminke (*left bars*) and Freest (*right bars*) samples, respectively. Bar colours indicate the colours of discovered particles and fibres.

Fibres were counted in the ground/float fractions after rinsing the zooplankton nets, as they were barely visible among the sediment heaps on the net. Results of the fibre counts, and remarks on the general appearance of the samples, are given in Table 13 in Appendix D and are shown in Fig. 24. The Kamminke *Bodden* sample contained one blueish plastic particle

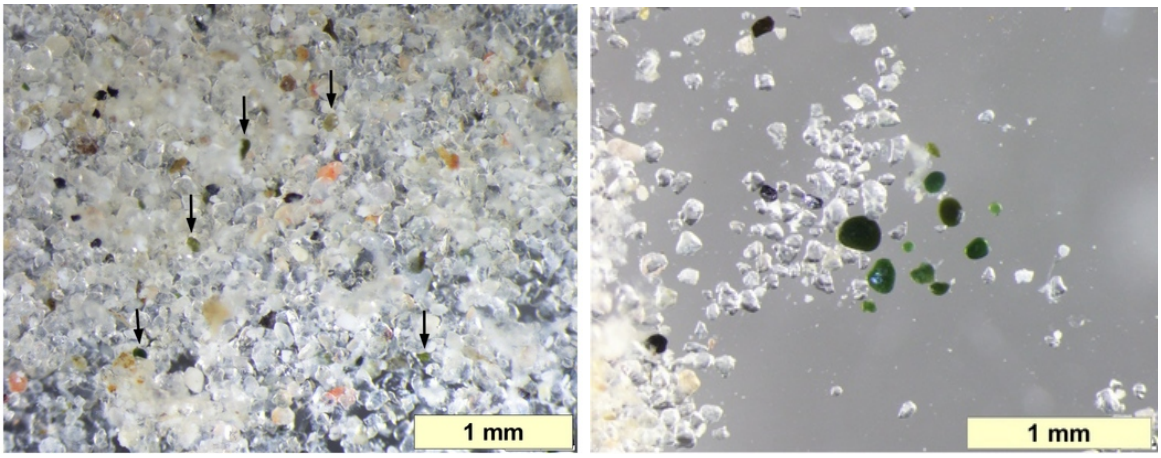


Figure 25: Selection of glass fragments detected in the Peene outlet sample obtained near Freest fishing harbour. In the left image, glass pieces identified in sediment heaps on the basis of their colour and shape are marked with arrows, and in the right image, a collection of glass pieces of various sizes and shades is shown.

and only 11 long fibres in total. Fine, thin fibres usually present in all Rostock samples were not observed. This unusually low fibre content might be a consequence of the lower tourist density and the fact that Kamminke beach is not a frequented bathing beach. In addition, no fibre influx is present from extensive fisheries or from sewage treatment plants discharging laundry effluent in the immediate vicinity of Kamminke. Nine green glass pieces with sizes on the order of 100$\mu$m are observed among the fine-grained sediment at the ground of the petri dish. Contamination is much larger in the Freest samples, where a total of 206 tiny green glass pieces were counted among the fine-grained sediment. Examples of the sizes and colours of glass pieces are shown in Fig. 25. Five small likely plastic pieces are found among the fine-grained sediment either at the surface or among the ground sediment (2 blue, 1 turquoise, 1 green, and 1 clear-apricot rod, Fig. 24). The fibre load in the 500ml Freest sample, identical to the sediment volume of the Kamminke sample, was 302 fibres instead of just 11. Among these were several very long (up to 2 cm length) and thick fibres both in the bottom as well as in the floating material. Although an organic origin for these fibres, e.g. chitin crustacean antennae, can not be excluded without chitinase digestion, the very regularly shaped structure of numerous fibres hints towards a synthetic origin. In addition to the large number of transparent fibres, 8 coloured fibres are found in the Freest sample (3 petrol, 2 blue, 1 rose, 1 red, 1 orange-ochre, Fig. 24). The low anthropogenic contamination in the *Stettiner Haff* at Kamminke beach starkly contrasts the very high microplastics and glass load at the Freest Peene outlet into the Baltic Sea.

### 3.4.5 Jade Bay comparison sample

The North Sea Jade Bay, known from earlier studies to be contaminated with microplastics, was chosen for method validation and comparison with Baltic coast sediment and seawater samples.

#### 3.4.5.1 Particle and fibre number counts

The particle and fibre counts, including coloured pieces, of the Jade Bay samples are shown in Fig. 26. As in the Baltic coast samples, the number fluctuations are particularly high for particles. Particle counts in seawater and the decanted solution of sediment samples range from a few hundred to a few thousand, while only few particles are observed in the freshwater sample. The fluctuation in the fibre contents is not as extreme. Between a few and a few tens of fibres are found in both sediment and water samples. A maximum fibre load of 77 fibres is observed in the canal *Nordender Leke* near Varel's paper recycling plant, where the load of 20 coloured fibres also exceeds detections in all previous samples. Remarks on the individual Jade Bay samples are provided in the following sections.

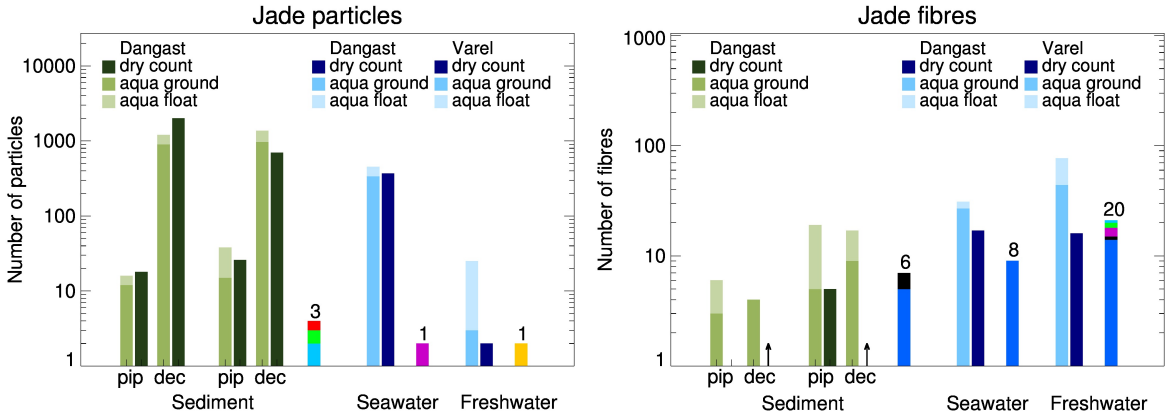


Figure 26: Particle (*left panel*) and fibre (*right panel*) number counts of all Jade Bay samples, including freshwater, seawater, and two sediment samples. Sediment samples are displayed in green, while water samples are shown in blue. For sediment samples, counts in the pipetted ("pip") and decanted ("dec") solutions are displayed individually. Dry counts and wet counts are separated in dark and light bars, as in previous figures. Arrows indicate prohibited dry counts due to dense organic material on the filters. Coloured fibres and particles are shown next to each sample, colour-coded to mimick the true colour variations found in potential microplastic particles and anthropogenic fibres, and annotated with the total number of coloured pieces above each bar. The majority of fibres displays various shades of blue. Note the differing logarithmic scales.

**Dangast seawater sample**

The high concentration of organic material in the Dangast seawater sample required that

particles and fibres were extracted onto three 55$\mu$m zooplankton net filters. After rinsing with deionised water, a total of 448 particles were counted with 3$\times$ magnification on all three 55$\mu$m nets (Fig. 26, left panel). The particles had a similar appearance to the sediment samples, and the seawater sample was overall very similar to the beach sample taken at the same location. Seven blue fibres of likely anthropogenic origin were detected, as well as one violett plastic particle (see Fig. 26). Among the 31 fibres counted in total, several fibres as long as 0.8-1.5cm were found. The fact that the longer fibres were folded into dense knots suggests that these fibres are composed of persistent synthetic material.

**Varel freshwater sample**

The freshwater sample of the *Nordender Leke* contained the largest load of coloured fibres. In a total of 20 coloured fibres, 13 were blue, 3 violet, 3 green, and one was black (Fig. 26). Two transparent particles showed a structured surface that indicated microplastics rather than sediment (in addition to the fact that no sediment was found, nor was expected, in the freshwater surface sample of the canal). A large number of 10 pieces with the appearance of (plastic/celluloid) foil were also observed.

**Dangast sediment samples**

In contrast to the freshwater sample, the two independent Dangast sediment samples contained a comparatively low overall fibre load of only 11 fibres in sample 1 and 36 fibres in sample 2 (right panel in Fig. 26). Except for one white fibre, all of the fibres in the first sample were transparent and might be of organic origin, given the large load of organic material in the Jade sediment. In the second sample, 7 coloured fibres with a potential anthropogenic origin were found. Despite the general appearance of most particles, especially in the decanted solution, being consistent with natural sediment, several plastic and likely plastic pieces were detected. In sample 1, one dark red, one blue-green, and one green particle share the appearence of plastic. An additional three clear particles were identified that display a surface structure consistent with synthetic material. Because of the large particle load in the decanted fraction, and the large number of particles floating on the surface of deionised water after rinsing off the plankton net, the surface solution was carefully poured into centrifugation tubes and centrifuged at 3000 rotations/minute. After centrifugation, no particles remained on the surface, yet several fibres were floating in the water column at various heights. This supports the conclusion obtained from the Rostock

samples that synthetic material is not predominantly floating on the surface.

In summary, the freshwater, seawater, and sediment samples taken at the Jade Bay display a large load of likely synthetic (colourful) fibres, a larger amount of small pieces with a plastic-like appearence, and a large amount of apparent foil fragments that might either originate from paper recycling or might be floating in the water column due to the high organic zooplankton load observed in each of these samples.

## 3.5 Summary of particle & fibre number counts

All samples display large numbers of hundreds to thousands of particles especially in the decanted solution extracted above the settled sediment in the Erlenmeyer flasks. Particles were in shape and visual appearance indinstinguishable from natural sediment. Although the pipetted surface solution allowed a cleaner extraction with much lower natural sediment levels, most coloured particles and fibres were detected in the decanted solution. Hence, a complete extraction of anthropogenic microplastics requires analysis of the decanted solution when densities of 1.3 g/ml are employed for plastic-sediment separation. Fibre number counts covered a large range from a few fibres to several hundred long, thick fibres as well. For transparent fibres, a unique distinction between organic fibrous material and fibres of anthropogenic origin (both natural and synthetic) was not possible by visual inspection under a dissecting microscope. Coloured fibres ranged from 1 to 9 in all sediment and seawater samples, and reached a maximum of 20 in the Varel freshwater sample. A high synthetic particle and fibre contamination is observed at the Peene outlet into the Baltic Sea, while a low coloured particle and fibre content is detected near the Warnow outlet at Markgrafenheide beach. No systematic trends are observed both spatially and temporarily in the Rostock and Rügen gradient samples.

# 4 Discussion

## 4.1 Method testing

One of the major findings of this study is that air-venting with high-density saline solutions (1.3-1.4 g/ml) does not provide a method to cleanly separate microplastics from natural sediment. Although small amounts of $\sim 200$ microplastic particles with low densities of 0.9 g/cm$^3$ are efficiently extracted from sediment samples with total weights of up to 800g (Sec. 3.2), all samples contained varying amounts of residual natural sediment in the floating pipetted or decanted solutions above the settled sediment as well. This implies that previous studies finding large particle loads with this method have to be treated with caution, and should be confirmed with more sophisticated methods where possible.

From the methods testing presented above, one of the major problems in microplastic detection is that natural mineral particles and transparent plastic fragments are visually indistinguishable. This is particularly severe for particles counted on filters, as the bright filter background renders the distinction even harder. Transparent fibres are difficult to detect on filter material as well. Using zooplankton net or stainless steel mesh filters allows the rinsing of particles and fibres into aqueous solution, which enabled a more reliable detection of both fibres and plastic particles on the basis of their shape and surface structure. A focus on intensely coloured particles and fibres increases the likelyhood that observed fragments are indeed synthetic polymers of anthropogenic origin. Especially after treatment with $H_2O_2$ only resistent coloured polymers should persist. This simple and cost-effective step allows the removal of organic residual matter from sediments and plastics, and causes synthetic fibres and particles to stand out more prominently among the sediment mix (see also Lorenz 2014). Polyamide/nylon particles and fibres are the only polymers that are dissolved by hydrogen peroxide, as was indeed observed for one red particle in Baltic coast samples. Coloured fibres were present in almost all sediment samples along the German Baltic coast, and coloured particles were present in 40% of the drift line samples. Coloured particles and fibres are therefore predominantly used as the basis for the discussion and literature comparison provided below.

## 4.2 General conclusions for particle extraction

The particle number counts in all samples display large fluctuations between several tens and thousands of particles. Among the particles extracted in calciumchloride solution, only a few particles can be identified as microplastics on the basis of their colour, shape or sur-

face structure. This implies that more than 99% of particles above the bottom sediment in calciumchloride solution are visually indistinguishable from natural sediment. Such particles would have to be claimed plastics under the assumption that high-density saline solutions provide a clear separation between microplastics and natural sediment. After centrifugation, sediment-like particles condensed at the bottom implying a higher density than water. Hence, caution has to be applied when the number counts of floating particles after air-venting in high-density saline solution are used unselectively to count microplastic particles. The systematically higher particle numbers extracted from the fine-grained sediment fractions indicate that smaller grains are more likely to contaminate microplastic samples. This finding is consistent with earlier observations by Imhof et al. (2012), who found that only the topmost volume in zincchloride solution at even higher densities of 1.6-1.8 g/ml was dominated by artificially added microplastic fragments. While their experiments were focused on the recovery rates of microplastics in artificially enriched sediments, the focus here is on the levels of contamination when real sediment samples are analysed. From the observations presented in Sec. 3, however, even in this topmost volume a large number of contaminating natural sediment particles is expected rather than pure microplastics.

Previous studies mostly used the top layer of the saline solution to search for microplastics. While this is advantageous in view of the results found by Imhof et al. (2012), in all samples presented in Sec. 3 many of the coloured microplastic particles were detected in the decanted rather than the pipetted solutions. In addition to high-density PVC and polyamide fibres and particles, aging and biofouling in the natural seawater environment might cause particles and fibres to sink, thus prohibiting their detection in the surface layer. In North Sea sediment samples, more than 80% of marine synthetic materials are composed of low-density polypropylen and polyethylen (Lorenz 2014, see also Table 2 in Sec. 2.8). If the Baltic coast samples are dominated by similar materials, the fact that numerous particles and fibres are found in the water column implies that biofouling or other effects must have altered the floatation properties of microplastics. Although evidence for biofouling is increasing with the increasing number of plastic degradation studies available (e.g., Ye & Andrady 1991), aging and sinking effects in microplastics have not been quantitatively studied so far. The visual inspection presented here leads to the conclusion that additional means are required after air-venting in high-density saline solution to ensure that a representative sample of light-weight and heavier microplastics are extracted.

## 4.3 Seawater samples and sediment floatation

Both the Warnemünde and the Dangast seawater samples contained substantial amounts of 69 and 450 particles. Both samples were drawn from the surface in shallow water near the beach. Finding sediment fragments on the water surface strengthens our conclusion that microplastics and sediment particles are not unambiguously separated from their floatation properties. The Dangast beach samples contained the finest-grained sediment among all locations. The large number of floating particles indicates that tiny, lighter-weight sediments are suspended more easily. As in the particles extracted from the sediment samples, surface tension must play a rôle in keeping particles on the surface. In this case, the water surface tension has to balance the gravitational pull causing high-density particles to sink. The gravitational force of a single particle, assumed to be spherical for simplicity, is given by

$$F_g = g\, V\rho = g\, \tfrac{4}{3}\pi r^3 \rho$$

where $g$ is the gravitational acceleration (9.81 m/s), $\rho$ the particle's density, and $V$ and $r$ the particle volume and radius, respectively. Gravity is counterbalanced by the force exerted by surface tension, which acts to minimise the energy $E$ and hence the area: $\Delta E = \sigma \Delta A$, where $\sigma$ is the surface tension and $\Delta A$ is the change in area (Gerthsen Physik, p. 100-101). For a spherical particle:

$$\Delta E = \sigma\, 2\pi r\, \Delta h$$

where $r$ is the radius of the sphere touching the surface, and $\Delta h$ is the height of the sphere segment with surface area $\Delta A = 2\pi r \Delta h$ causing the indentation. The surface tension force, $F_s = \Delta E / \Delta h$, acts as the force parallel to the surface of contact between the particle and the water. The vertical component of $F_s$ counterbalances the gravitational force and increases with the angle of indentation (as mesaured from the horizontal water surface):

$$F_s = \sigma\, 2\pi r \sin\theta$$

where $\theta$ is the indentation angle between the object and the surface, and $\sigma$, the surface tension of the liquid, is $72.7 \times 10^{-3}\,\mathrm{N/m}$ for water at 25°C. For natural sediment, a characteristic density of $2.5\,\mathrm{g/cm^3}$ and a particle radius of 100$\mu$m are assumed, consistent with observed grain sizes. With these values, the gravitational force of a single sediment particle is estimated to be $F_g = 1.0 \times 10^{-7}\,\mathrm{N}$. Assuming an indentation angle between the water surface and the surface of the sphere of 30°, the surface tension exerts a force of $F_s = 2.3 \times 10^{-5}\,\mathrm{N}$ on the particle. Thus, the suspending force due to water surface tension is two orders of

magnitude larger than the gravitational pull of particles with a radius of $100\mu$m and a density of $2.5\,\mathrm{g/cm^3}$. Note that the suspending force depends linearly on the size of the particle, while the gravitational force scales as $r^3$. With the same simplyfied assumptions, the gravitational force exceeds the surface suspension force for a particle with a radius of 1.5 mm ($r_{lim} = \sqrt{\frac{3\sigma \sin\theta}{2\rho g}}$) causing larger particles to sink. Given these considerations, the assumption that particles floating on the wave surface are of a lower density than water and natural sediment and hence have to be plastics needs to be revised. A very low surface tension appears to be sufficient to keep mineral grains floating for an extended period of time after being stirred into the water column from the bottom, e.g. by wave motion, touristic or boat activity. This conclusion implies that more sophisticated techniques are required to unambiguously distinguish microplastics from natural sediment even in seawater (or freshwater) samples. The major conclusion from this estimate is that natural sediment grains with diameters $< 1$mm are also expected to float on the surface of aqueous solution after rinsing of the zooplankton net filters.

## 4.4 Sediment samples along the Baltic coast

### 4.4.1 Rostock sediment samples

Almost all Rostock samples contained both coloured particle or fibre loads. Coloured microplastic particle contamination is found to be low in 500ml samples, ranging from 1 to 5 particles, with most samples displaying 1-3 coloured synthetic particles. Although number statistics are small, the fact that all Warnemünde samples contain coloured microplastics strengthens the strong anthropogenic influx of synthetic materials at this most touristic Rostock beach. While the fibre load is found to be exclusively large in the July seasonal sample (see below), microplastic particles appear to be persistent in all seasons, implying that particles have a longer resilience time than fibres in beach sediments. Particles might not be as affected by photo-induced UV bleaching as fibres, possibly retaining their synthetic colours for more extended periods of time.

The contamination with coloured, likely synthetic, fibres displays a much larger variety. Seasonal samples along Rostock beaches from March to July display a maximum of 9 coloured fibres in 500ml sediment (approximately 750-800g dry weight), with no evident seasonal pattern in the numbers of detected *coloured* fibres alone. Touristic activity and the proximity to the Warnow outlet carrying city and harbour discharge into the Baltic Sea are most likely responsible for the relatively high synthetic fibre loads. Transparent fibres are particularly

numerous in the July Warnemünde sample, while coloured fibres are only moderately enhanced, suggesting rapid bleaching under the influence of UV-intense solar radiation. The origin of the ecxeedingly high increase in the fibre load is likely seasonal touristic activity. Warnemünde is the most frequented beach in the Rostock area, and a major tourist destination as well as a major place for weekend recreation of the local communities. Visitor numbers in Warnemünde are on the order of one million beach guests in the vacation season, with July and August being the peak months (Statistisches Amt der Stadt Rostock 2013). Hence, the observed increase in fibre numbers is most likely caused by increased numbers of people on the beach and in the water. Here, the fact that swimsuits as well as UV protection clothes are made of synthetic fibres adds to the contamination load.

The seasonal variations in the fibre loads in the three other Rostock locations are not as extreme as in Warnemünde, as expected if touristic activity is one of the dominant sources of fibres. In particular, no systematic monthly trend in the fibre loads can be identified. The comparatively large pre-seasonal total and coloured fibre loads in the Nienhagen/Börgerende and Wilhelmshöhe April samples were particularly unexpected. Both locations were chosen as low anthropogenic influx sites as compared to Warnemünde and Markgrafenheide. Following the west-east drift as the major direction of wind and water flux, the heavy touristic activity westward of Nienhagen/Börgerende might influence the beach sediment contamination all the way to Warnemünde. A total of 410.000 guests with 2.4 Million overnight stays in Kühlungsborn (Kühlungsborn fact sheet 2013) might contribute to a high influx of synthetic fibres along the entire Rostock coastal area. A second possible origin of fibres is the artificial reef located 1.5km into the Baltic Sea from the Nienhagen coast. The reef is built from a combination of concrete structures and net material (http://www.riff-nienhagen.de), and the mesh netting could be a continuous source of disintegrating fibres. In these locations, the seasonal fibre load could be washed ashore and accumulate over the stormier fall and winter seasons, where wave activity during both the westwind drift as well as north-easterly winds drive the currents towards Nienhagen Bay (see, e.g., Staatliches Amt für Umwelt und Landwirtschaft Mecklenburg-Vorpommern 2014). After storm events, a larger amount of macrodebris was observed in the Nienhagen and Börgerende beach areas (Fig. 27). However, not all observed fibres need to be of anthropogenic origin. Increased natural activity in zooplankton/crustaceans and insect species enriching the samples with appendages, antennae, leg hair, and other fibrous material in the spring season cannot be excluded as the origin for the large fibre contents at the present time. In contrast to byssus fibres, any chitin fibres will not be dissolved in $H_2O_2$ and hence will be present on the zoo-

plankton nets and in the aqueous solution after sample processing. Without spectroscopy or a more complete digestion procedure with chitinase, it remains unclear whether these fibres are of organic or synthetic (anthropogenic) origin. Even if of anthropogenic origin, cotton or wool fibres are expected to disintegrate by natural processes more rapidly than synthetic fibres, and would not enter the marine food chain as a hazard. On the other hand, coloured anthropogenic fibres are observed inside dissolved organic matter (e.g. small crustaceans) as well. Whether these fibres already entered the food chain or were captured on the sticky dissolving proteins during sample processing needs to be studied further. In any event, a method to distinguish uncoloured synthetic fibres from organic material is urgently needed to quantify the potentially harmful microfibre load.

Figure 27: After a storm event in June 2014, insulation squares with a size of about 7cm had washed up in large numbers on Nienhagen beach. The squares were located underneath the sand cliff and coastal forest, a remote area where no construction activity is expected nearby.

A surprising result is that the fibre counts in Markgrafenheide do not stand out significantly. The effluent of the Warnow with its sewage treatment plant discharge as well as all discharges from the overseas harbour passes on the westerly current from the Warnow outlet directly in front of Markgrafenheide. However, the expectation of a particularly high fibre load at Markgrafenheide main beach is not confirmed. Further analysis of Warnow water samples and direct samples of the sewage treatment plant effluent would be valueable to compare these low fibre numbers with the discharge contamination. Such a study would provide a more detailed view on the amount of anthropogenic and synthetic fibres accumulating on nearby beaches and carried out to Sea. The comparison between Markgrafenheide and Warnemünde suggests that the influx of anthropogenic fibres accumulating in beach sediments is higher in beaches with the highest touristic activity than in beaches with passing river discharge near the Warnow outlet (but see Sec. 3.4.4 for a comparison with Oder discharges). From this finding, immediate and local anthropogenic fibre influx

seems to be a major source of synthetic fibres at Rostock beaches. The large fibre loads observed in Nienhagen and Wilhelmshöhe before the main summer season additionally indicate that in the wider urban area, other influx pathes add major contamination levels of plastic particles and of synthetic fibres to the total microplastics load.

### 4.4.2 Rügen sediment samples

The low fibre number counts observed in all Rügen locations are in stark contrast to the high fibre loads observed in Warnemünde in the tourist season (compare Fig. 22 to Fig. 19). Taken towards the end of June, on June 22 and 28, the Rügen samples are drawn during peak summer vacation. In the Binz drift line sample, total fibre counts amount to only 13 fibres (pipetted + decanted), and comparably low fibre counts are found both in beach sediment and in sediment retrieved in shallow water near the beach. In both samples together, only one petrol coloured fibre is found, and it cannot be excluded that this fibre was introduced in the lab. In the July drift line sample taken in Warnemünde, on the other hand, in excess of 300 fibres are counted (see also Fig. 19). Surprisingly, the total number of overnight visitors is comparable in both locations. Binz hosted 1.78 Millionen overnight stays in the summer season of 2010 (Touristic report, Statistisches Amt Mecklenburg-Vorpommern 2011, numbers for 2013/2014 were not available at the time of writing), while Rostock including Warnemünde hosted a total of 1.8 Million overnight stays in 2013 (Statistiches Amt der Stadt Rostock 2014). The very low fibre contamination found in Rügen beach sediments might therefore indicate that the waterflow around the island is generally stronger than along the Rostock coast, rapidly carrying off synthetic material.

### 4.4.3 Oder/Peene sediment samples

The fibre content in the Peene estuary sediment sample of 302 fibres is comparable to the excess fibre load in the July Warnemünde sample, and thus one of the largest fibre contents in all samples. With 5 coloured particles and 8 coloured fibres, the unambiguous microplastic contamination is also high in the Freest sample. Substantial fibre loads, coloured particles, and the large number of green glass fragments render the Freest sample the most evidently contaminated sample with anthropogenic particle and fibre influx. The high synthetic particle and fibre contamination observed at the Peene outlet into the Baltic Sea suggest that the Oder discharge carries substantial amounts of microplastics. The low coloured particle and fibre content detected near the Warnow outlet at Markgrafenheide

beach indicates a lower microplastic influx from the Rostock sewage treatment plant and harbour. The westward current along the Rostock coastline could additionally foster the fast dispersal of effluent into a wider area. Sampling along both extended Warnow and Oder river sections would be beneficial for quantifying the flux of microplastics towards the Baltic Sea.

**Origin of the Oder/Peene anthropogenic contamination**

Although a few green glass pieces are regularly encountered in most samples, the large number of 206 glass fragments found at the Freest beach location is not observed in any of the other samples. With its high fibre load, the Freest sample is also one of the most fibre-rich samples, comparable to the total fibre load in Warnemünde in the peak season. As the beach in Freest is not heavily frequented by tourists, two likely contamination sources are identified. The first source of fibre influx is, as expected, the Oder river with discharge waters from the city of Szczecin (Stettin), while a second origin of fibres might be the fishing industry. Freest itself has a fishing harbour with 54 small fishing vessels (Fischereigenossenschaft Peene/Freest 2010). Possible sources for the unusually large glass fragment content are therefore the glass spheres employed in the fishing industry for net floatation before plastic swimming spheres became available. A city discharge origin might seem less likely in the case of glass fragments as compared to plastic fragments, as glass sinks with sediment and is only transported into the Baltic Sea when sediment is washed out with river runoff during storm seasons. As glass behaves identical to natural sediment, those scenarios cannot be distinguished from the Freest samples alone.

In conclusion, the Freest sample obtained near the Peene/Oder outlet is the sample with the highest anthropogenic contamination in our survey. Although the proximity to the Freest fishing harbour and the military station at Peenemuende on the eastern side of the estuary likely contributes to the large anthropogenic particle, glass, and fibre load, the Peene runoff into the Baltic Sea is the major discharge location of the Oder river. Carrying effluent from the city of Szczecin and industrial areas along the river, a fraction of the anthropogenic contamination likely originates from urban and industrial activities along the river bank. Sediment sampling from the Freest outlet towards Szczecin at sediment deposit sites along the river would shed light on the spatial distribution of glass fragments and microplastics in the Oder/Peene estuary, and thus on the predominant entry pathes of anthropogenic microplastics (and glass pieces) into the Baltic Sea from the Oder river runoff.

### 4.4.4 Possible origin of microspheres

In 6 of all 18 Rostock sediment samples, including both beach and shallow water samples, a total of 18 round, glass-like spheres are detected. Most of these spheres have a yellowish tinge (Fig. 28, left panel), with a few being colourless. The sizes are identical within the visual inspection limitations and on the order of $80\mu$m for the yellowish microspheres. Colourless or milkywhite spheres were smaller, $\sim 60\mu$m in diameter, and just retained by the mesh size. One of the yellow spheres was destroyed with a lancette, and a thick liquid emerged from a sturdy shell with the soft, sticky consistency of a natural or synthetic polymer. Although we cannot definitely determine the origin of these microspheres, this observation is reminiscent of skin-care spheres containing oily substances in personal care products such as shower gels and lotions which unfold their skin-protecting virtue after the cleaning stage (Fig. 28, middle panel). Inside the microspheres, no substructure was observed. One potential natural origin of perfectly spherical shells without substructure could be unfertilised or undevelopped fish eggs (Fig. 28, right panel).


Figure 28: Microsphere (dark sphere near image center) found among raps pollen (white irregular particles) in the May Börgerende sample (*left panel*) with a size of approximately 80$\mu$m compared to silver-coated microspheres produced by Cospheric (*middle panel*). Unfertilised eggs of the marbled rockcod also display a perfectly spherical shape without substructure. Note the large size of these fish eggs (source & copyright: Aquatichyk 2012).

However, no more evolved or fertilised stages of fish eggs are present in any of the samples. Although fish spawning events can be highly synchronised, this would not explain the detection of identical spheres in later samples. The small overall numbers of spheres are also atypical for spawning events. Even if unlikely, a natural origin cannot be entirely excluded at this point. Just one sphere each is found in four of the samples, while the Nienhagen/Börgerende April sample featured 4 microspheres. The largest number of 10 spheres is detected in the May Börgerende sediment sample, where foil-like structures are also prominent (see Table 10 for reference). This sample is rich with more than 2000 raps pollen, suggesting that spheres and foil fragments were introduced with the pollen into the surface layer of beach sediment from the raps field directly above the sand cliff connect-

ing Börgerende to the Nienhagen coastal forest. The seawater was also rich with yellow raps pollen, confirming air-blown input from the nearby field. In a systematic study of field soils, Zubris & Richards (2005) found that synthetic fibres from sewage sludge fertilised fields are retained 15 years after the application. In the case of the Nienhagen sample, the application of sewage sludge could not be confirmed, yet a treatment with either sewage sludge or with other material containing sludge remnants would explain the high fragment and microsphere load found exclusively in the May sample. May samples from the other three Rostock locations do not show these anthropogenic contaminants, supporting the influx from raps pollen. An analysis of field soils at the Börgerende site would be valueable to confirm this tentative interpretation.

## 4.5 North Sea Jade Bay samples

### 4.5.1 Anthropogenic contamination in the Jade Bay

With 20 coloured fibres, the freshwater sample obtained in Varel's *Nordender Leke* near a paper recycling plant contains the largest coloured fibre load of all samples. As the plant does not discharge into the rivulet at a distance of just 50m to the paper stacking court, the origin of the large influx is most likely wind carriage. Fibres, including synthetic fibres from acrylic colour layers, are a natural byproduct of paper recycling due to the shredding process. Hydrogen peroxide is a potent bleaching agent employed in the paper industry that acts destructive to cellulose. The treatment with hydrogen peroxide is therefore expected to either dissolve or bleach cellulose paper fibres. The coloured fibres observed are therefore likely not cellulose fibres. Magazin covers can be laminated and might be stained with acrylic colours. These colours, and any fibrous material shredded from such papers, would be more persistent to the treatment with $H_2O_2$. Especially the observed coloured fibres are therefore likely synthetic fibres.

The seawater and sediment samples obtained at Dangast contained moderately high coloured fibre loads of 8 and 6 mostly blue fibres as well. These fibres cannot be traced to a unique origin. Dangast beach has modest touristic activity, especially in the month of September, and bathing is limited due to the muddy consistence of beach sediments. On the other hand, Wilhelmshaven to the North of Dangast with the harbour area and the sewage treatment plant is a major source of anthropogenic discharge into the Jade Bay. As the North Sea water currents flow into the bay from the western side, passing Wilhelmshaven first on their journey through Jade Bay, and turn around towards the eastern coast in the South, it is expected that city discharge water passes Dangast beach and contributes to the microplas-

tic contamination.

In contrast to fibres, the number of 1-3 coloured particles in both sediment and water samples is low. The total numbers of uncoloured particles in the decanted solution above the sediment samples exceeds 1000 fragments. Even the seawater sample poured over zooplankton net filters without further extraction contained 451 particles. The seawater was collected in the shallows near the beach at a depth of 0.5m where surface water was allowed to flow freely into the canister. The observed particles are, nevertheless, indistinguishable in shape and colour from the sediment obtained in the nearby drift line. Following the arguments in Sec. 4.3, the particles floating on the surface are most likely natural sediment suspended by surface tension. These considerations suggest that the Jade Bay beach sediment and seawater samples do not contain particularly large synthetic particle counts, neither does the Varel freshwater sample drawn near the recycling plant contain particularly high microplastic *particle* contamination levels.

In summary, while the coloured fibre contamination is very large near the paper recycling plant, the Dangast beach samples do not show higher anthropogenic contamination loads than the sampling locations at the Baltic Sea coast.

### 4.5.2 Comparison to Baltic samples

One significant difference between the Jade samples and the Baltic samples was the salinity. With a salinity of 30.7 ‰ as measured in the lab, the Jade Bay water provides for a larger floatation capability of small grains and fibres. It is therefore expected that Jade Bay or North Sea samples have a larger mean load of fibres and particles near the surface, consistent with the observations. At this point, it is impossible to conclude unambiguously that the large fibre, foil, and plastic particle load is an effect of the industrial and city discharge into the Jade. Further studies will be required, and especially the salinity dependence of floating synthetic material has to be analysed in more detail before Baltic and North Sea samples can be compared. Nevertheless, it is striking that a large load of coloured fibres are found in the Jade samples, and it stresses the fact that fibres of anthropogenic origin are a major source of contamination, outnumbering plastic particles as the predominant contaminant for the marine food chain.

### 4.5.3 Comparison to previous studies in the Jade Bay

Previous studies by Dubaish & Liebezeit (2013) of seawater in the *Vareler Tief* at the outlet of the discharge pipeline of Varel's paper recycling plant found in excess of 1200 potential

plastic particles per liter of seawater. In their study, seawater-samples were drawn near the coast 20cm below the surface, vacuum-sucked through 1.2$\mu$m cellulose nitrate filters then treated with $H_2O_2$, and potential microplastic particles were counted on these filters with up to 80x magnification. While particles in these samples were too numerous to be counted, fibre counts near Varel and Dangast, in the same areas where our seawater and sediment samples were obtained, ranged approximately from 300 to 900 fibres/liter (Dubaish & Liebezeit 2013, see their Fig. 5). These very high fibre loads are not confirmed in our samples. With a total of 31 fibres in 10 liters or 3 fibres/liter in the seawater sample obtained at Dangast beach, very few fibres are observed. A higher total fibre load of 77 fibres in 4l is found in the freshwater sample in Varel, corresponding to $\sim 20$ fibres/l. Although this fibre load is likely caused by the paper recycling plant, it is more than one order of magnitude lower than the fibre loads observed in offshore locations by Dubaish & Liebezeit (2013). One reason for this discrepancy is the fact that we counted fibres above a length of $\sim 70\mu$m, while fibres as small as a few micron could be counted with up to 80x magnification by Dubaish & Liebezeit (2013). However, the authors found fibres with predominant length scales of 100$\mu$m to 1mm, identical to the length scale sampled here. The time of the day, tidal effects, as well as the time of the year might influence the fibre statistics. Further long-term studies are required to conclude on the mean fibre load in the Jade Bay.

In 10 liters of surface seawater, we detect 451 particles or 45 P/liter. Although most of those particles have the visual appearance of the ambient natural sediment, these particle numbers are still substantially lower than the amount of particles detected by Dubaish & Liebezeit. There are two technical differences to the analysis of our samples. First of all, Dubaish & Liebezeit might not have sampled from the beach, but at offshore locations, as in the case of the discharge pipeline site. Further offshore, wave activity is likely to be higher, and mechanical stirring of the sediment is expected to be larger than at the calm, shallow beach location in Dangast. This is particularly true in the plume of the pipeline, where the influx of 3500 $m^3$/day (according to Dubaish & Liebezeit 2013) causes strong mechanical forces on the underlying sediment. Secondly, particles down to a few micrometer are considered in their study with typical sizes below 100$\mu$m, while the mesh size of the zooplankton net filters limited our size fraction to $> 55\mu$m. One of the major differences therefore likely originates from sampling to a much smaller size regime (see also Norén 2008).

The same authors studied sediments on the North Sea islands of Spiekeroog, Kachelotplate, and a tidal flat in front of Nordland hosting a mussel bank. From these sediments, potential microplastics were extracted in zincchloride solution and the supernatant was decanted, which is directly comparable to the treatment of sediment samples presented in

this thesis. In these North Sea island sediments, Liebezeit & Dubaish (2012) detect between 300 and 900 particles/kg dry weight, and observe that higher particle numbers are correlated with finer-grained sediments. In the Dangast sediment samples presented here, more than 1000 particles per sample are observed, corresponding to approximately 1600 and 2000 particles/kg of dry sediment. Given the high content of clay underneath sediments in Dangast, these particles are very fine-grained in comparison to all other analysed sediments, suggesting that floatation in calciumchloride is more readily achieved for Jade sediment particles than for all other sampling locations. As discussed in Sec. 4.3, a correlation of floatation and size scale is expected if particles are suspended by surface tension. Furthermore, the surface tension in North Sea water at a measured salinity of 30.7 ‰ might be sufficient to suspend fine-grained silt for extended periods of time, which would explain the large particle numbers found in seawater samples at the pipeline discharge site. This problem has to be addressed further before final conclusions on the potential contamination with microplastics in the Jade Bay can be made.

## 4.6 Comparison of Baltic coast microplastic concentrations to other locations

For comparison with previous and forthcoming studies, the overall particle and fibre concentrations scaled to the weight of each sample are displayed in the two left panels of Fig. 29. Mean and standard deviations are derived from summing up pipetted and decanted number counts in aqueous solution (*ground + float*) for each location (Tables 8 to 14). The large particle counts in the left panel are caused by natural sediment contamination. Fibre number counts per kg sediment dry weight (DW) as shown in the second panel contain both organic fibres as well as anthropogenic influx. The mean fibre concentration in the Rostock area exceeds fibre counts in all other sampling locations by at least a factor of 4. The large mean fibre load and variation observed along the wider Rostock coastline is influenced by the high number counts in spring samples and the maximum fibre load of more than 300 fibres in the Warnemünde July sediment sample during peak tourist season. In the two right panels in Fig. 29, the mean and variation of coloured particles and fibres as the most likely candidates for microplastics are shown, and the coloured microplastic content in sea- and freshwater samples is summarised on a per liter basis in Fig. 30.

The maximum concentrations of 4-7 coloured particles/kg and 9-12 coloured fibres/kg dry sediment observed in Freest and Dangast indicate that the Oder and Jade river basins are severely contaminated by anthropogenic microplastic influx (see also Dubaish & Liebezeit

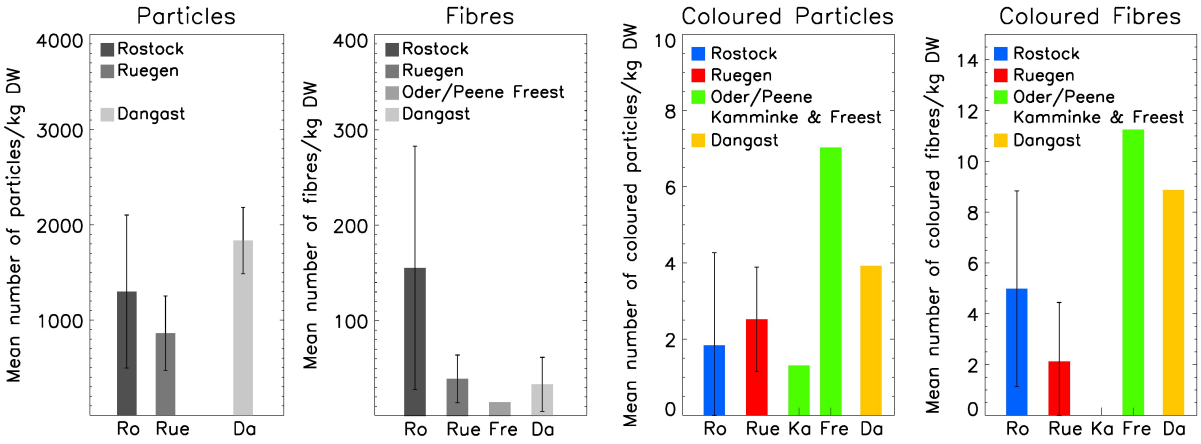


Figure 29: Combined measurements of all samples in the four sampling locations.
*Left two panels:* Mean number counts of particles and fibres per kg of dry weight sediment are shown with standard deviations where more than one measurement was available. For the Oder estuary, only the Freest sample is shown for comparison with all other Baltic coast sediment samples (particles in this sample could not be counted due to the high organic matter content).
*Right two panels:* Mean numbers of coloured particles and fibres per kg of dry weight sediment observed in each sampling area with standard deviations where more than one measurement was available. No coloured fibres were observed in the Oder estuary Kamminke sample.

2013). While Oder discharges are expected to transport urban and industrial runoff, the Jade Bay receives effluent from Varel's paper recycling plant as well as Wilhelmshaven's sewage treatment plant and overseas harbour. Estuaries with their specialised brackwater communities and river basins serving as ecological niches for juvenile fish populations can be considered particularly sensitive ecosystems. The severe contamination with microplastics in the Oder estuary and Jade Bay suggest that both leaching of toxins from microplastics into the marine coastal habitats and the entry into the food chain through juvenile fish and other plankton feeders are hazards to a healthy estuarine environment. The elevated concentrations of microplastics in sediment and water samples indicate that the water flow along both coastal areas does not efficiently remove microplastics from estuaries and river basins. If river basins serve as long-term repositories for microplastics, sewage treatment and industrial water treatment processes will have to be redesigned to reduce further microplastic influx into the marine environment.

In the following sections, the values obtained are compared to previous studies without the claim of completeness. Comparison studies are primarily selected on the basis that extraction and counting methods are comparable to the methods presented here. For scientific merit, only studies in the North Sea and Baltic areas are selected, as conditions in terms of ship traffic, touristic activity, waste treatment, and fisheries are assumed to be more com-

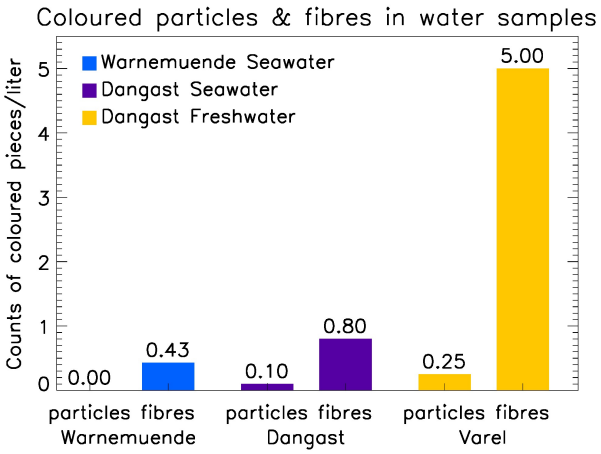


Figure 30: Numbers of coloured particles and fibres per liter in seawater and freshwater samples. The freshwater sample in Varel was drawn from the canal *Nordender Leke* near a paper recycling plant, while the Dangast seawater sample represents a beach location northward of Varel in the Jade Bay.

parable between the North Sea and the Baltic Sea than between the Baltic and the large oceans. In addition, both seas are too small for the built-up of gyres capturing plastics, although smaller eddies might contain plastic waste for the limited time of their existence (Omstedt et al. 2014). The microplastic concentrations reported in the text below are summarised in Fig. 31.

This is one of the first systematic studies of microplastic content in beach sediments at the Baltic Coast. The closest region probed for microplastics previously was sampled by boat between the Danish and the Norwegian coast. Norén & Naustvoll (2011) counted coloured or structured particles with sizes 10-500$\mu$m in seawater samples across a Skagerak transect. Their counting procedure using visual inspection and unnatural properties of particles was similar to the procedures established in this work. They discovered blue particles in 15 of their 17 seawater tows. FTIR analysis indicated that the particles were epoxybased paint flakes as used for ship hull sealing. With sizes of 30-70$\mu$m, the particles depicted in their Fig. 5 are similar in shape and appearance to the tiny blue fragments detected in most of our sediment and seawater samples. We therefore tentatively conclude that the tiny blue fragments might also be paint flakes with ship paintings being a likely origin.

A second study using floatation in saline solution counted microplastics in beach sediments at the North Sea island of Norderney (Dekiff et al. 2014). In their study, the means of visual selection of microplastics on the basis of colour and structure/morphology were particularly similar to the selection choices employed here. Dekiff et al. (2014) distinguish particles and fibres as colourless and intensely coloured, and found between 23 and 213 colourless

fibres/kg dry sediment with a mean of $114 \pm 48$ F/kg, which covers the same range and large variation observed in Baltic sediment samples (Fig. 29). For coloured fibres, Dekiff et al. (2014) found 4-25 F/kg with a mean of $16 \pm 4$ F/kg, which in their studies is indistinguishable from laboratory blank fibre counts. In our Baltic Sea beach samples, between 2 and 11 fibres/kg dry sediment are found, with a substantial number of samples well above the level of our blanks, where a maximum of 2 fibres per blank is observed. In three different beach locations on Norderney, Dekiff and colleagues found a total of 59 potential microplastic particles in 26 of their 36 samples, implying that 72% of the beach samples contained microplastic particles indentified on the basis of colour and structure. We observe coloured potential microplastic particles in 12 out of 23 samples, or slightly more than 50% of our samples. Numbers range from 1-7 coloured particles/kg dry sediment in our samples (Fig. 29). Dekiff et al. (2014) report 1-2 particles/kg with a maximum of 4 particles/kg detected, covering a similar range of particle concentrations as compared to the Baltic beach sediments. In their study, the authors were able to confirm 15 particles with gas chromatography as polymers with PP, PE, and PET being the dominant contributors, giving high confidence to the adopted selection procedures. The similarity of North Sea island and Baltic beach sediments is surprising in view of the fact that numbers as high as hundreds to thousands of microplastic particles were claimed in comparable island locations at the North Sea coast (Liebezeit & Dubaish 2012, see Sec. 4.5.3). While the island of Norderney might be particularly pristine in view of the westerly current and the moderate touristic activity, it is also one of the most comparable locations to the more remote Rostock and Rügen beaches investigated here.

Using colour as the major distinction criterion for microplastics, the resulting concentrations are lower limits of the true microplastic contamination in each sample. Claessens et al. (2011) analysed a large number of beach and subtidal harbour sediment samples along the Belgian coast with a lower size limit of 38$\mu$m. Detected microplastic particles and fibres were confirmed spectroscopically. Consistent with the observations presented above, they found that the majority of all synthetic pieces were fibres (59% fibres as compared to 25% granules). Polystyrene microspheres constituted as much as 12% of their micropieces and were exclusively found in harbour sediment. In beach sediments, Claessens et al. (2011) found on average $82 \pm 33$ fibres/kg and $6.3 \pm 2.5$ plastic fragments/kg with sizes $> 38\mu$m. A large variation of 43-132 fibres/kg is also observed in the fibre content, while concentrations for fragments range from 4 to 10 P/kg.

The amount and variation observed in total fibre and plastic fragment concentrations in

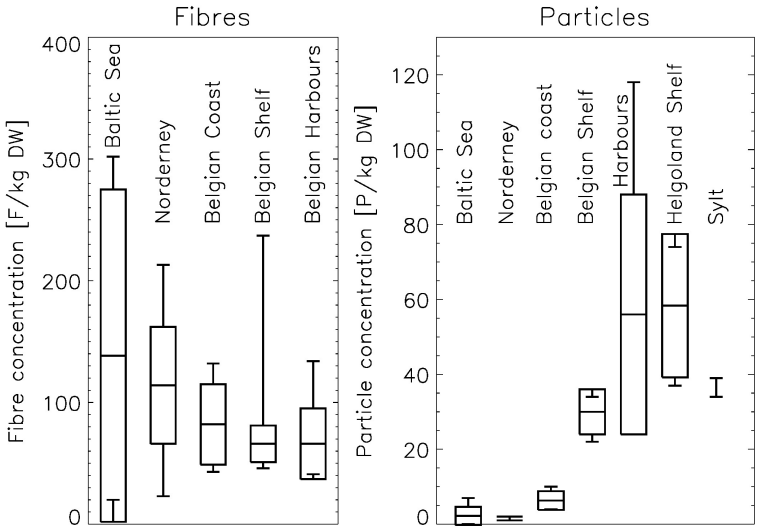


Figure 31: Comparison of potential microplastic concentrations in sediments measured in the North Sea and at beaches on the Baltic coast (this work). Total fibre concentrations including both coloured and uncoloured fibres are shown in the *left panel*. The *right panel* summarises the concentrations of particles confirmed to be microplastics either spectroscopically or on the basis of their colour. Boxes denote the mean (central line) and standard deviations, while the lines indicate the minimum and maximum value reported for each location. For Baltic Sea values, the mean and standard deviation are derived from all ten Baltic coast locations. Beach sediments were sampled at Norderney (Dekiff et al. 2014), Sylt (Lorenz 2014), and the Belgian coast (Claessens et al. 2011). The Belgian Continental Shelf, Belgian Harbours (Claessens et al. 2011), and the Helgoland Shelf (Lorenz 2014) report concentrations in sublitoral sediment. Norderney and Sylt contain only two values each, such that no standard deviation could be derived.

Belgian beach sediments are comparable to the total fibre and coloured particle concentrations found in Baltic and North Sea Jade Bay sediments. Claessens et al. (2011) compare the increase in microplastic concentrations derived from beach sediment cores on a timebase of 15 years (1993-2008) to the increase in annual global plastic production (see their Fig. 2), and conclude tentatively that a correlation between the global plastic growth rate and the deposited microplastics might be present. Microplastic concentrations are shown to have tripled in beach sediments from 55 to 156 pieces/kg (including fibres, granules, foil, and spheres) over just 15 years. The highest concentrations of microplastic particles are observed in three harbour locations with recreational or industrial activity. Here, particle numbers are with 24-118 P/kg one order of magnitude higher than in beach sediments (4-10 P/kg). Claessens et al. (2011) suggest that microplastics might be trapped in the harbours due to the enclosed geometry. On a larger scale, such trapping might affect the Jade Bay samples investigated here, leading to increased microplastic loads in the bay area, as discussed above. As in most of the Baltic coast samples, no clear correlation is found between human activities and microplastic content when beach sediments and offshore

sediments along the Belgian coast and continental shelf are compared. This suggests that microplastics are more uniformly distributed by natural forces with time than macroplastics, where accumulation on beaches near urban and recreational areas is more often observed, stressing the necessity to sample the spatial and temporal distribution of microplastics individually. Individual monitoring of microplastics is emphasised by a comparative study of the water surface, beach sediments, and the seafloor, where the accumulation of microplastics in seawater and on the seafloor is found to exceed the weight of macroplastics by a factor of 100 and 400, respectively (Van Cauwenberghe et al. 2013). The same authors estimate that a concentration of just 13 particles/kg dry sand correspond to a total microplastic load of $3.3 \times 10^6$ to $7.7 \times 10^7$ particles on a beach extent of just 100m. With particle concentrations only marginally lower in the Baltic coast samples presented here, beach sediments must be considered an important entry path of microplastics into the marine and coastal environment and food chain.

The size range of particles targeted in our study was 55$\mu$m-1mm, where the lower limit was fixed by the mesh size of the zooplankton net used for filtration and the upper limit emerged from the majority of sediment grains by weight. In seawater samples drawn with plankton net tows, mesh sizes frequently range from 333 to 450$\mu$m, such that most of the seawater findings are not directly comparable to sediment studies. Studying the amount of small plastic particles in seawater in 16 locations along the Swedish west coast, Norén (2008) found microplastic numbers to be steeply increasing with decreasing sampling size. A 1000-100,000 times higher concentration of particles plus fibres was found with 80$\mu$m mesh samples as compared to a 450$\mu$m mesh width.
With 80$\mu$m mesh, concentrations of 0.15-2.4 plastic particles/liter of seawater are detected, comparable to the concentrations of coloured particles and fibres found in seawater samples at both Warnemünde and Dangast/Jade Bay (Fig. 30). Three harbour sites were sampled by Norén (2008) for sediment, one industrial harbour and one commercial harbour near Stenungsund, and one small harbour near Tjuvkils. Stenungsund industrial harbour was sampled near a plastics production plant and yielded by far the highest microplastic concentrations in water and sediment. Two to five milkwhite or transparent plastic particles per 100ml sediment with sizes 1-7mm are found in the small harbour of Tjuvkils, while 332 microspheres with sizes 0.5-1mm were counted in Stenungsund industrial harbour per 100ml sediment. Concentrations hence range from 20 particles/kg (conversion factor 1.6 g sediment/1ml volume from our own wet sample experiments) to more than 2000 particles/kg sediment. Similarly high concentrations were reported in the studies of Jade Bay sediments

and seawater for particles down to a few micrometer in size near the paper recycling plant by Dubaish & Liebezeit (2013), as discussed in Sec. 4.5.3. Despite the inherent uncertainties with visual identification, the alarmingly high concentrations of microplastic particles, spheres, and fibres found near industrial discharge sites call not only for more consistent scientific monitoring of a representable range of locations in Europe, but also require the rethinking of production discharge practices in view of the EU Marine Strategy Framework Directive to reduce microplastic particle entry into the marine environment in the future.

## 4.7 Discussion of problems and biases

The observed difficulties in the distinction of microplastics from natural sediments and organic fibres have likely affected studies that were based on the visual inspection of samples under the microscope. At the same time, photo-induced bleaching and the production process cause substantially larger numbers of transparent and lightly coloured microplastics to be expected in the marine environment. A complete microplastic extraction with more accurate contamination rates requires micro-spectroscopy of transparent floating particles, as conducted in Lorenz (2014). In beach sediments from the island of Sylt and sublitoral sediments from the wider Helgoland shelf, Lorenz (2014) found 34-74 particles/kg dry sediment. In her study, imaging microscopic spectroscopy ($\mu$FT-IR) was applied to identify the materials of all particles extracted after zincchloride floatation. These values are about one order of magnitude larger than particle concentrations detected in the Baltic sediment samples by visual inspection alone. The comparison of these values suggests that only 10% of the particles might be detected with colour selection. However, this technology is costly and analysis is time-consuming, such that more practical solutions for plastic-sediment separation must be developped. Our experiments suggest that adding a centrifugation step after density separation in saline solutions improves the plastic-sediment separation efficiency. Centrifugation provides a simple means to separate truely low-density particles from suspended higher-density sediments with means available as standard laboratory equipment. For the distinction of organic and synthetic fibres, however, this method would not be sufficient. Here, adding a chitinase digestion step would allow a cleaner sample of transparent synthetic fibres to persist. With these concerns in mind, we focused the microplastics analysis on coloured particles and fibres. The reported concentrations per kg of dry weight sediment or per liter of seawater are therefore strict lower limits of the true microplastics contamination in Baltic Sea beach sediments and surface waters.

## 4.8 Future scientific goals

Standardised procedures to quantify the amount of microplastics in the marine environment will be beneficial for a wide variety of applications. From the methodical testing described in this thesis, an optimised minimal approach to extract microplastics from sediment samples is suggested in Appendix F. The further development of a more uncomplicated polymer identification system should be one of the major aims in methodical research. Recently, Langhals et al. (2014) demonstrated the power of polymer characterisation with the decline timescale in fluorescent lightcurves of irradiated plastic particles in the context of plastic recycling. If these methodologies could be employed to characterise marine microplastics, the time-consuming spectroscopy step would be avoided and polymer compositions could be deduced for a large number of samples.

The development of more automated imaging-spectroscopy methods to distinguish natural fibres from synthetic polymers is highly desirable especially given the high fibre loads found in several Baltic Sea coast sediment samples. The fact that anthropogenic fibres from laundry effluent are not exclusively made of synthetic polymers additionally complicates fibre counts. For natural organic fibres of animal origin and natural anthropogenic fibres such as cotton, wool or hemp, persistence times in the marine environment are unknown. A comparison of resilience time scales between natural and synthetic anthropogenic fibres would support the derivation of standardised synthetic fibre concentrations. Finally, as fibres do not correspond in shape to the natural prey pattern of plankton feeders, do they enter the food chain in substantial quantities? Before these open questions can be answered, it is indispensable that scientists agree on a standardised extraction and identification procedure for microplastic particles and fibres.

The results presented above revealed the difficulty to trace microplastic particles and fibres to their sources. High-spectral resolution IR spectroscopy harbours the potential to identify plastic origins on the basis of additives and chemical compositions. Available polymer databases contain the spectroscopic fingerprints of polymer materials produced worldwide, such that the regional production origins might also be traced. Several studies suggested a regional influx as the major source of microplastics in sediments, e.g. from sewage treatment plants, the plastics industry, or paper recycling, as discussed extensively above. Yet, secondary microplastics fragmented from macrodebris floating for extended periods of time in the marine gyres must contribute to local pollution in varying amounts depending on location and exposure to ocean streams. A correlation between the microplastic composition and the spatial dispersal through small and large ocean systems such as gyres and streams

could shed light on the lingering and breakup timescales, and hence the physical pathway from macrodebris to microplastics. Quantifying the global contribution to local microplastic pollution is urgently required to eliminate microplastics at the sources. For the same merrit, the fragmentation processes that lead to the increase of microplastic particles in the environment need to be understood. The combination between mechanical wave breakdown, ingestion, and UV dissociation cause a different disintegration pattern than might be observed for plastic materials on land. These fragmentation processes have to be quantified to understand the full extent of the microplastic tide and predict its long-term evolution.

Despite the demonstrated omnipresence of plastics in the marine environment, there is hope at the horizon. Concerning macroplastics, Ruanda was one of the first countries to entirely ban shopping and consumer good plastic bags because floating bags were identified as a health hazard for humans and nature. In Mauritania, plastic bag ingestion was found to be a major source of death in sheep and cattle, and bags were subsequently banned. Banghladesh was one of the first countries to ban plastic bags in 2002, after thin plastic bags were found to clogg the drainage system during a major floading event (Surfrider Foundation 2014). Today, a large number of African countries including Ehtiopia, Mali, Malawi, Mauritania, Ruanda, Uganda, and Tansania have either banned plastic bags, thin plastic bags, or non-biodegradable plastic bags (Earth Policy Institute 2014), with other places in the world slowly following. In terms of microplastics, primary sources are the easiest to identify and remove. New York state is the first state worldwide to prohibit *"the manufacture, distribution and sale of personal cosmetic products containing microbeads"* in the *"Microbead-Free Waters Act"* (New York State 2014). These are a few very first steps to reduce the influx of plastics into the environment, yet it can be hoped that they serve as examples of short-term measures to mitigate the long-term hazards of microplastics in the marine world.

# 5 Summary

**Plastic contamination in beach sediments at the German Baltic Sea coast**

The content of potential microplastics in sediments along the German Baltic coast was investigated. With the aim to characterise the entry pathes of plastics, the hypothesis was phrased that microplastic concentrations are expected to correlate with urban and harbour activity (Warnow, Oder outlets), touristic activity (Warnemünde, Rügen/Binz main beach areas), and should be minimal in beach sediments with low visitor numbers (Nienhagen, Rügen Dranske & Heidehof).

In the first methodical part of the thesis, the extraction efficiency of microplastics from natural sediment was tested using two common density separation methods: centrifugation and air-venting in high-density zincchloride and calciumchloride solutions. Air-venting in $CaCl_2$ proved an efficient, low-toxicity microplastic extraction method for both particles and fibres. A prerequisite to recover plastic particles even at densities as low as 0.9 g/ml (polyethylen) was that both the top-layer surface as well as the supernatant above the settled sediment were analysed for microplastic content.

One of the major findings recurrent throughout the presented analysis was that particles suspended on the surface of high-density saline solutions are visually indistinguishable from natural mineral grains. Sediment particles with diameters of less than $\sim 1$mm are shown to be suspended by surface tension against immediate sinking. Care thus has to be taken in experiments without spectroscopic material verification to prevent severe overcounting bias. A positive correlation is found between floating particle numbers and finer grain sizes in the sense that finer grains tend to be suspended by surface tension more easily while coarser grains sink more readily. Coloured particles and fibres provide the safest microplastics identification with visual inspection when spectroscopic verification is not available. From these conclusions, a microplastic extraction strategy is designed to minimise counting bias and allow a quantitative comparison of microplastic concentrations.

In the second part of the thesis, the spatial and temporal variation of microplastics in sediments along the German Baltic coast was investigated. While no systematic spatial trend is detected from west to east along the wider Rostock coastline, a maximum fibre load of more than 300 fibres/kg dry sediment is observed at Warnemünde beach in July, suggesting that touristic activity increases the presence of fibres in beach sediments and seawater. Warnemünde sediment samples were also found to contain coloured plastic fragments in all samples from March to July, indicating a high density of anthropogenic material in

beach sediments at the Rostock coast. Comparably high concentrations of microplastics and fibres are not observed at equally frequented beaches on Rügen island, indicating that local current patterns influence the persistence time of microplastics in beach sediments. River basins display large variations in anthropogenic influx when the Warnow, Oder, and Jade basins are compared. The location with the second highest microplastics load was the Oder/Peene outlet into the Baltic Sea, where large coloured particle concentrations are tentatively suggested to originate from urban and industrial discharge from cities along the Oder river banks. From the results presented in this study of beach sediments along the German Baltic coast, a detailed investigation of microplastic concentrations along the Warnow and Oder estuaries and the continuous monitoring in heavily contaminated areas such as Warnemünde beach is suggested to confirm the preliminary conclusions presented above.

The fact that maximum microplastic concentrations are observed in estuaries and on the most touristic beaches supports the hypothesis that microplastic monitoring has the potential to reveal the entry pathes of microplastics into the marine environment. Over the investigated timeframe of 5 months, systematic trends in the spatial and temporal variations of microplastics concentrations are not detected. Longer-term studies with larger sample sizes are required to conclusively distinguish the dominant anthropogenic entry pathways of microplastics into the Baltic Sea and coast.

The omnipresence of microplastics in almost all sediment samples analysed, and the increasing reports of microplastic detections in marine environments near coastlines and in the open ocean, emphasise the urgency to develop methods to decrease the microplastics influx into the marine ecosystem from the large variety of anthropogenic sources, including marine littering and fisheries not addressed here.

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## Appendix A: Results of Warnemünde test sample results

Table 5: **Warnemünde test samples: Number counts and remarks – page 1/3**

| Sample | extraction | fibers (blue) | particles | spheres | remarks |
|---|---|---|---|---|---|
| **Centrifugation $ZnCl_2$ 0.5-1mm 31.8g, analysis: collected from filter, transmitted & polarised light** | | | | | |
| P1 | centrifuge 1 pipetted | 29 | 7 | - | |
| | centrifuge 2 pipetted | 45 (4) | 46 | - | 1 foil/fibre/particle mix, plant roots? |
| | centrifuge 3 pipetted | 16 (2) | 1 | - | |
| | all | 90 (6) | 54 | - | 2.8 fib/g 1.7 P/g |
| | decanted | 37 | 20 | $>1$ | 2 dense agglom with globule |
| **Centrifugation $ZnCl_2$ solution-only reference tubes** | | | | | |
| P1 | pipetted | 37 | 11 | - | 4 foil, 7 fibres = glas surface? (wrong focus) |
| | decanted | 33 | 1 | - | 1-2 foil, 3 agglom. w/ particles/fibres, 2 nests |
| **Empty glass slides in polarised light** | | | | | |
| | empty | 17 | 11 | 0 | one light blue/green fibre (likely laboratory paper) |
| **Centrifugation $ZnCl_2$ $<$0.5mm 32g, analysis: transmitted light binocular** | | | | | |
| P1 | centrifuge 1 pipetted | 8 | 254 | ? | some round particles, 3 red particles |
| | centrifuge 2 pipetted | 41 | 215 | 31 | 2 fibres “clear synthetic” |
| | centrifuge 3 pipetted | $>5$ | 258 | 21 | 1 white agglomeration, 1 red particle |
| | all | 54 | 727 | 52 | 1.7 fibres/gramm 22.7 particles/gramm |
| | decanted | $>41$ | 136 | $\sim 18$ | calcites hamper detection of transparent P+F |
| **Centrifugation $ZnCl_2$ $<$0.5mm 32g, analysis: polarised light** | | | | | |
| P1 | centrifuge 1 pipetted | 31 | 18 | - | no spheres collected off filters |
| | centrifuge 2 pipetted | 34 | 3 | - | 4-5 dark blue fibres |
| | centrifuge 3 pipetted | 27 | 0 | - | no collectible particles, 1 blue fibre |
| | all | 92 | 21 | - | same sample as above $\Rightarrow$<br>large fibre contamination from glass fibre filter |
| | decanted | 28 | 7 | - | red polarising fibre, synthetic structure, transparent |

Table 5: **Warnemünde test samples: Number counts and remarks – page 2/3**

| Sample | extraction | fibers (blue) | particles | spheres | remarks |
|---|---|---|---|---|---|
| **Air-venting in $ZnCl_2$, coarse 0.5-1mm, weight 61.1g, analysis: binocular on filter** | | | | | |
| P2 | pipetted | 55 | $\sim$744 | 24 | spheres (pollen/calcites/synthetic?), glued structures |
| | decanted | | | | too many grains, many fibres, fibre “nests”, agglomerates |
| **Air-venting in $ZnCl_2$, fine $<$ 0.5mm, weight 217.5g, analysis: binocular on filter** | | | | | |
| P2 | pipetted | $>3$ | 6450 | ? | particle estimate see note below |
| | decanted | | | | view hampered by calcites, natural sticky structures |
| | | | | | count not possible at all |
| **Centrifugation in $CaCl_2$, coarse 0.5-1mm, weight 13.2g, analysis: binocular on filter** | | | | | |
| P3 | centrifuge 1-3 pipetted | 14 | 134 | 0 | round particles = pollen? (not counted as spheres) |
| | | | | | 1 sperical black particle (very small) |
| P3 | decanted | 28 | 168 | 0 | many “eye spheres” = pine pollen |
| **Air-venting after 3xCentrifugation in $CaCl_2$, coarse 0.5-1mm, weight 13.2g, binocular on filter** | | | | | |
| P3 | pipetted | 77 | $>>$ | ? | particles could not be counted, too much calcite |
| | decanted | 128 | $>>$ | ? | some foil, fibre nests, red/blue Ps, foil pieces, pollen? |
| P3 | reference decant | 15 | 89 | 1 | very clean, much smaller Ps on filter - good! |
| **Centrifugation in $CaCl_2$, fine $<$ 0.5mm, weight 40g, binocular on filter** | | | | | |
| P3 | centrifuge 1-3 pipetted | 21 | 118 | 1-2 | spheres at edge |
| | decanted | 59 | 460 | 1 | particle count estimated, 1 very green particle |
| **Air-venting after 3xCentrifugation in $CaCl_2$, fine $<$ 0.5mm, weight 40g, binocular on filter** | | | | | |
| P3 | pipetted | 64 | $>500$ | 1 | very round sphere at edge |
| | decanted | $\sim 120$ | $\sim 300$ | 2? | 1 foil, fibrenests, lots of sand |
| **Air-venting in $CaCl_2$, fine $<$0.5mm, weight 220g, binocular on filter** | | | | | |
| P3 | pipetted | 61 | $\sim 250$ | 2 | blue & red fibres and particles |
| | decanted | 121 | $>>$ | ? | mostly smaller particles |

Note P2 - sediment: The total particle number was estimated from the number of particles in one field, counted to be 150 particles/field of view with 3.2-fold magnification. The filter covers 43 fields, yielding a total of 6450 particles for the whole filter. Although this estimate is approximate, the order of magnitude should be correct, as the filter was homogeneously covered with fragments. Fibers and spheres were barely visible between the heavily coated filter surface covered in calcites and sediment pieces.

Table 5: **Warnemünde test samples: Number counts and remarks – page 3/3**

| Sample | extraction | fibers (blue) | particles | spheres | remarks |
|---|---|---|---|---|---|
| **Air-venting in $CaCl_2$, coarse 0.5-1mm, weight 14.3g, binocular on 55$\mu$m net** | | | | | |
| P4 | pipetted | 23 | 93 | 0 | blue fibre, one small blue particle |
| | | | | | zooplankton net rinsed with deionised water |
| | aqua ground | 38 | 14 | 0 | blue, red, green tiny particles |
| | aqua float | 9 | 8 | 0 | 4 possible plastic fragments |
| | total | 47 | 22 | 0 | tiny Ps stick to net, clear fibres are detected after rinsing |
| | decanted | 112 | 188 | 0 | fibre nests, 1 blue, 2 rainbow particles |
| | | | | | zooplankton net rinsed with deionised water |
| | aqua ground | 158 | 13 | 0 | one fibre net, some very long fibres |
| | aqua float | 21 | 26 | 0 | few fibres: most fibres sunken |
| | total | 179 | 39 | 0 | tiny particles $<< 100\mu$m remain on net, |
| | | | | | else net very clean, fibres easier to spot after rinsing |
| **Air-venting in $CaCl_2$, fine $<$0.5mm, weight 675g, binocular on 55$\mu$m net** | | | | | |
| P4 | pipetted | 52 | 408 | 0 | 4 fibre nests, 103 fibres counted in polarised light |
| | | | | | zooplankton net rinsed with deionised water |
| | aqua ground | 53 | 167 | 0 | some fibre nests remain intact |
| | aqua float | 19 | 105 | 0 | fibres and particles fuse into islands |
| | total | 72 | 272 | 0 | |
| | decanted | 137 | $>>$ | ? | 2 dense fibre nests, lots of sediment |
| | | | | | zooplankton net rinsed with deionised water |
| | aqua ground | 41 | $>>$ | ? | some apparent foil pieces, large amount of sediment |
| | aqua float | 46 | 66 | 0 | |
| | total | 87 | $>>$ | ? | most particles tend to sink, likely sediment |

## Appendix B: Laboratory air and reference samples

Table 6: **Laboratory air, water, and calciumchloride reference samples (F: fibres, P: particles, S: spheres)**

| Date | volume | extraction | P | F | S | comments |
|---|---|---|---|---|---|---|
| **Blind $CaCl_2$ sample – solution without sediment** | | | | | | |
| 18-08-14 | $\sim$ 1l | decanted | 3 | 8 | 2 | 2 dark fibres, 2 spheres of different sizes |
| | | aqua ground | 5 | 9 | 1 | dark fibres located on ground |
| | | aqua float | 3 | 11 | 0 | 2nd sphere not recovered, clear, thin fibres |
| 21-08-14 | $\sim$ 1l | decanted | 2 | ? | 0 | 4 large, 3 small fibre nests: broken glass fibre pre-filter? |
| | | aqua ground | 1 | 27 | 0 | 3 thick fibres only, else long, but very thin fibres |
| | | aqua float | 0 | 2 | 0 | 2 thick fibres on surface, numerous tiny organic fragments<br>residual organic despite thorough washing stuck to net,<br>this is likely common for scientific zoo plankton nets as well |
| 22-08-14 | $\sim$ 1l | decanted | 1 | 28 | 0 | disintegrating small fibre nests |
| | | aqua ground | 0 | 16 | 0 | only 2 thick, long fibres, else very thin fibres |
| | | aqua float | 1 | 9 | 0 | 1 petrol, 1 yellow thick fibre, else very fine fibres |
| 29-09-14 | 800 ml | pipetted | 1 | 0 | 0 | clean plankton net filter |
| | | aqua ground | 3 | 2 | 0 | likely residual organic “fibres” |
| | | aqua float | 2 | 4 | 0 | 1 blue fibre, 1 clear, long fibre $\rightarrow$ likely Synthetic (Polytex cloth?) |
| | | decanted | 2 | 3 | 0 | 1 blue, 1 black, 1 long & clear fibre |
| | | aqua ground | 1 | 5 | 0 | 1 black, long fibre |
| | | aqua float | 1 | 6 | 0 | 1 petrol-blue fibre, 1 clear, very long fibre |
| 30-09-14 | 1000 ml | pipetted | 4 | 1 | 0 | transparent particles look like sediment, likely residual contamination |
| | | aqua ground | 2 | 5 | 0 | frayed fibres, likely residual organic matter |
| | | aqua float | 2 | 3 | 0 | residual organic fragments on plankton net |
| | | decanted | 4 | 0 | 0 | particles = residual sediment fragments? |
| | | aqua ground | 1 | 6 | 0 | “foil” flakes from dried organic residuals on plankton net |
| | | aqua float | 1 | 1 | 0 | unsuspicious |
| **mean values** | | dry count | 3.4 | 4.0 (10.0) | 0.4 | fibre counts without (with) fibre nest samples! |
| | | aqua ground | 2.6 | 9.0 (14.0) | 0.2 | without (with) fibre nest count |
| | | aqua float | 2.0 | 7.2 | 0.0 | equally few fibres on all surface samples |
| **Laboratory water samples** | | | | | | |
| 18-09-14 | tap water | 10 l | 1 | 1 | 0 | 1 rose fibre, 1 transparent particle with a plastic structure |
| 18-09-14 | deionised water | 10 l | 1 | 13 | 0 | 1 red fibre, 13 tiny particles, residual sediment bits on filter |
| **Laboratory air 2h sample drawn through filter** | | | | | | |
| 27-06-14 | | | 89 | 27 | 0 | only tiny particles, would fall through 55$\mu$m net,<br>1 red, 1 blue tiny fragment, likely paper fibre mat, not used further<br>transparent fibres, one dark, one light blue, no fibre nests |

## Appendix C: Overview of all scientific samples

Table 7: **Sediment and seawater samples along the Baltic coast and in the Jade Bay – page 1/2**

| Location | GPS | date | position | Water Temp [$^o$C] | dry weight 0.5-1mm [g] | dry weight $< 0.5$mm [g] | $CaCl_2$ conc. [g/ml] |
|---|---|---|---|---|---|---|---|
| **Rostock March 2014** | | | | | | | |
| **Nienhagen** | 54.166184 11.962618 | 31-03-2014 | drift line | 7.4 | 67.0 | 889.8 | 1.29 |
| **Wilhelmshöhe** | 54.176462 12.026697 | 31-03-2014 | drift line | 8.0 | 70.0 | 866.5 | 1.34 |
| **Warnemünde** | 54.183315 12.083082 | 31-03-2014 | shallow water | 7.0 | 44.5 | 895.0 | 1.30 |
| **Markgrafenheide** | 54.193767 12.139279 | 31-03-2014 | shallow water | 6.4 | 357.8 | 96.8 | 1.35/1.33[a] |
| **Rostock April 2014** | | | | | | | |
| **Nienhagen** | 54.166184 11.962618 | 23-04-2014 | drift line | | 4.47 | 743.3 | 1.30 |
| **Wilhelmshöhe** | 54.176462 12.026697 | 23-04-2014 | drift line | | 5.91 | 787.4 | 1.30 |
| **Warnemünde** | no data taken | | | | | | |
| **Markgrafenheide** | 54.193767 12.139279 | 23-04-2014 | shallow water | | 242.8 | 479.0 | 1.30 |
| **Rostock May 2014** | | | | | | | |
| **Nienhagen** | 54.157514 11.913970 | 25-05-2014 | drift line | 15 | 132.3 | 582.9 | 1.34 |
| **Wilhelmshöhe** | 54.176462 12.026697 | 03-06-2014 | drift line | 14 | 117.5 | 552.7 | 1.30 |
| **Warnemünde** | 54.183315 12.083082 | 22-05-2014 | drift line | ? | 24.5 | 585.9 | 1.36/1.37 |
| **Markgrafenheide** | 54.193767 12.139279 | 22-05-2014 | drift line | ? | 156.1 | 455.0 | 1.32/1.31 |
| **Rostock July 2014** | | | | | | | |
| **Nienhagen** | 54.157514 11.913970 | 26-07-2014 | drift line | 20 | 105.8 | 653.5 | 1.35 |
| **Wilhelmshöhe** | 54.176462 12.026697 | 22-07-2014 | drift line | 20 | 6.93 | 730.2 | 1.34 |
| **Warnemünde** | 54.183315 12.083082 | 19-07-2014 | drift line | 20 | 5.08 | 738.8 | 1.30 |
| **Markgrafenheide** | 54.193767 12.139279 | 19-07-2014 | drift line | 20 | 27.50 | 710.1 | 1.30 |
| **Rostock Seawater sample** | | | | | | | |
| **Warnemünde** | 54.183315 12.083082 | 06-08-2014 | shallow water | 21 | – | 7l | – |

[a] First value of $CaCL_2$ densities for the larger 0.5-1mm size fraction, second value for the small $< 0.5$ mm size fraction, if different.

Table 7: **Sediment and seawater samples along the Baltic coast and in the Jade Bay – page 2/2**

| Location | GPS | date | position | Water Temp [$^o$C] | dry weight 0.5-1mm [g] | dry weight $< 0.5$mm [g] | $CaCl_2$ conc. [g/ml] |
|---|---|---|---|---|---|---|---|
| **Ruegen 2014** | | | | | | | |
| **Binz** | 54.402833 13.614562 | 22-06-2014 | drift line | 21 | – | 755.6 | 1.30 |
| | | | shallow water | 21 | – | 774.3 | 1.34 |
| **Breege** | 54.602267 13.389711 | 22-06-2014 | | 21 | – | 736.6 | 1.30 |
| **Heidehof** | 54.666044 13.267386 | 28-06-2014 | drift line | 20 | – | 704.7 | 1.35 |
| **Dranske** | 54.633245 13.220834 | 28-06-2014 | drift line | 19 | – | 610.0 | 1.35 |
| **Oder/Peene estuary August 2014** | | | | | | | |
| **Kamminke** | 53.866751 14.212367 | 23-08-2014 | drift line | 17 | – | 766.0 | 1.30 |
| **Freest** | 54.140276 13.728513 | 23-08-2014 | drift line[b] | 18 | – | 711.0 | 1.30 |
| **Jade Bay September 2014** | | | | | | | |
| **Varel/Nordender Leke** | 53.405249 8.132402 | 15-09-2014 | freshwater[c] | $\sim$20 | – | 4l | – |
| **Dangast/Jade Bay** | 53.451859 8.123518 | 15-09-2014 | seawater[d] | $\sim$18 | – | 10l | – |
| **Dangast** | | 15-09-2014 | sediment[e] | $\sim$18 | – | 765.4 | 1.35 |
| | | 15-09-2014 | sediment[f] | $\sim$18 | – | 676.6 | 1.30 |

[b] Shallow sand islands between silt and mud.
[c] Freshwater sample near paper recycling plant.
[d] Seawater sample near beach, few meters into mud shallows.
[e] Fine-grained sediment islands between silt/clay/mud.
[f] Second 500 ml fine-grained sediment sample taken few meters apart.

## Appendix D: Number counts and comments

Table 8: **March 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 1/3**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Nienhagen/Börgerende** | | | | | |
| 0.5-1 mm | pipetted | 7 | 5 | 0 | very clean, unsuspicious |
| | aqua ground | 7 | 5 | 0 | very thick fibres |
| | aqua float | 7 | 1 | 0 | very thick fibre, foil or organic fragments |
| | decanted | 20 | 62 | 0 | one thick fibre nest, one very long fibre |
| | aqua ground | 10 | 38 | 0 | thick, long fibres |
| | aqua float | 29 | 42 | 0 | 1 darkblue, 1 white thick fibre |
| $< 0.5$ mm | pipetted | 25 | 3 | 0 | unsuspicious |
| | aqua ground & float | 16 | 0 | 0 | very clean |
| | decanted | $>>$ | ? | ? | sediment scanned, no suspicious particles/fibres |
| | aqua ground & float | $>>$ | ? | ? | 1 long, dark fibre, numerous short, fine fibres |
| **Wilhelmshöhe** | | | | | |
| 0.5-1 mm | pipetted | 16 | 20 | 0 | unsuspicious |
| | aqua ground | 4 | 8 | 0 | unsuspicious |
| | aqua float | 1 | 2 | 0 | unsuspicious |
| | decanted | 29 | 57 | 0 | 2 thick fibre nests, 1 thick blue fibre |
| | aqua ground | 8 | 22 | 0 | 1 orange particle (unclear if sediment or synthetic) |
| | aqua float | 33 | 18 | 0 | most particles appear to be clear, fragmented organic |
| $< 0.5$ mm | pipetted | 14 | 17 | 0 | unsuspicious |
| | aqua ground | 4 | 4 | 0 | almost empty |
| | aqua float | 5 | 3 | 0 | mostly dissolved organic material |
| | decanted | $>>$ | 23 | 0 | sediment heaps |
| | aqua ground | $> 500$ | 37 | 0 | all fibres long & clear, else unsuspicious |
| | aqua float | $\sim$200 | 8 | 0 | 1 grey, 1 pink fibre |

Table 8: **March 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 2/2**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Warnemünde** | **shallow water** | | | | |
| 0.5-1 mm | pipetted | 41 | 35 | 0 | 1 foil fragment, 1 clear, sharp-edged fragment likely plastic,1 small blue plastic particle |
| | aqua ground | 5 | 4 | 0 | very clean |
| | aqua float | 34 | 8 | 0 | 1 small turquoise particle |
| | decanted | 36 | 21 | 0 | 1 foil fragment, 2 thick fibre nests, interlaced fibres |
| | aqua ground | 22 | 20 | 0 | 1 thick fibre nest, some very long $\sim 2$mm fibres |
| | aqua float | 9 | 2 | 0 | very clean |
| $<$0.5 mm | pipetted | 55 | 6 | 1 | 3 foil fragments, 1 violet fibre, surprisingly few fibres<br>1 turquoise & 2 blue plastic particles (blue mixed into organic!) |
| | aqua ground | 19 | 4 | 0 | unsuspicious, sphere not recovered |
| | aqua float | 27 | 5 | 0 | turquoise particle, 2 foil fragments, else unsuspicious |
| | decanted | $>>$ | 16 | 0 | no coloured fibres, few fibres compared to numerous sediment |
| | aqua ground | 301 | 25 | 0 | 1 darkblue fibre |
| | aqua float | $> 500$ | $> 25$ | 0 | unsuspicious sediment |
| **Markgrafenheide** | **shallow water** | | | | |
| 0.5-1 mm | pipetted | 5 | 3 | 0 | 1 fibre nest, very clean |
| | aqua ground | 0 | 0 | 0 | literally nothing |
| | aqua float | 1 | 8 | 0 | very clear fibres, hardly visible |
| | decanted | ? | $>$16 | 0 | 1 dark, 1 lightblue F, 6 black slag pieces, thick fibre nests $\rightarrow$ particles can't be counted |
| | aqua ground | 4 | 11 | 0 | plus many fine fibres, 1 nest looks like fibre glass |
| | aqua float | 79 | 28 | 0 | 1 long, blue fibre, plus fine fibres, many tiny fragments (dissolved organic?) |
| $<$0.5 mm | pipetted | 94 | 4 | 0 | unsuspicious, very few long fibres visible |
| | aqua ground | 87 | 3 | 0 | only 20 large sediments, else smallish, very few long yet many fine fibres |
| | aqua float | 73 | 8 | 0 | plus many fine fibres, 1 very long & thick, sediment mostly tiny |
| | decanted | $>$173 | ? | 0 | particles mixed with organic matter, difficult to count (esp. fibres) |
| | aqua ground | $\sim$800 | 19 | 0 | very small sediment fragments, many fine additional fibres |
| | aqua float | 23 | 11 | 0 | $\sim 560$ tiny fragments not counted, uncountable very fine fibres |

Table 9: **April 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 1/2**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Nienhagen/Börgerende** | | | | | |
| 0.5-1 mm | pipetted | 15 | 9 | 0 | one blue fibre, 1 thick fibre nest, particles sediment or mussel shell |
| | aqua ground & float | 0 | 80 | 0 | many fine fibres, only 1 very long clear, 1 dark thick fibre, 1 violet |
| | decanted | 169 | 7 | 0 | 1 thick, 3 fine fibre nests, particles = sediment... |
| | aqua ground & float | – | 34 | 0 | 1 long, thick clear fibre, 1 yellow-occre particle (synthetic or sediment?) |
| | | | | 0 | sediment not re-counted |
| $< 0.5$ mm | pipetted | 26 | 7 | 0 | foil or chitin fragments, very clean |
| | aqua ground & float | 8 | 6 | 0 | 1 foil fragment, very clean |
| | decanted | ? | ? | ? | sediment humps mixed with fibres, 1 turquoise particle |
| | aqua ground | $> 2000$ | $\sim$110 | 0 | 2000-3000 fine & coarse sediment particles<br>numerous long fibres near ground (darkblue, blue, turquoise) |
| | aqua float | 170 | 0 | 4 | 2 green, 1 violet, 1 darkblue & yellow-orange particle, 1 foil fragment<br>floating fibre-sediment mix, but no long fibres visible |
| **Wilhelmshöhe** | | | | | |
| 0.5-1 mm | pipetted | 27 | 34 | 0 | several thick fibres, one fibre nest |
| | aqua ground | 7 | 8 | 0 | sediment |
| | aqua float | 4 | 11 | 0 | almost empty |
| | decanted | 103 | 38 | 0 | 1 royal blue P, blue & petrol fibres, some very clear fibres likely synthetic |
| | aqua ground | 54 | 19 | 0 | 1 fibre nest, sediment |
| | aqua float | 1 | 4 | 0 | almost empty |
| $< 0.5$ mm | pipetted | 48 | 3 | 0 | one red fibre, else almost no fibres at all |
| | aqua ground | 29 | 4 | 0 | 1 thick, blue fibre |
| | aqua float | 30 | 22 | 0 | 3-4 foil fragments, 3 thick fibres, 4 knotted fibres |
| | decanted | $>>$ | 52 | 0 | numerous long fibres, some lightly coloured<br>one turquoise particle, several rose-coloured clear sediments |
| | aqua ground | $>$700 | 36 | 0 | 2 darkblue, only long, thick fibres counted |
| | aqua float | $>$1 | $>$5 | 0 | 1 darkblue, 3 clear thick fibres, 2 turquoise tiny particles |

Table 9: **April 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 2/2**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Warnemünde – could not be taken** | | | | | |
| **Markgrafenheide in shallow water** | | | | | |
| 0.5-1 mm | pipetted | 27 | 16 | 0 | thick & small fibre nest, dark blue, turquoise & white fibres, one bright blue plastic particle ($\sim 100\mu$m) |
| | aqua ground | ? | 13 | 0 | 1 long, thick, dark fibre, Ps not recounted |
| | aqua float | ? | 9 | 0 | unsuspicious, Ps not recounted |
| | decanted | 124 | 66 | 0 | 7 fibre nests, one bright green plastic particle |
| | aqua ground | ? | 33 | 0 | 1 green particle, thick fibres counted, Ps not recounted |
| | aqua float | ? | 17 | 0 | 1 foil fragment, thick fibres counted, Ps not recounted |
| $<$0.5 mm | pipetted | 20 | 20 | 2 | lightblue & petrol fibre |
| | aqua ground & float | ? | 11 | 0 | blue, petrol, occre fibre, 1 very thick fibre, no suspicious particles |
| | decanted | ? | ? | ? | white-green & turquoise particle, too much organic material to count |
| | aqua ground & float | ? | 41 | 0 | thick fibres counted, Ps not counted<br>3 coloured fibres, 1 very thick & clear fibre |

Table 10: **May 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 1/3**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Nienhagen/Börgerende** | | | | | |
| 0.5-1 mm | pipetted | 6 | 4 | 1 | one dark fibre, fibres invisible between canola pollen, clear sphere, 2-3 foil pieces |
| | aqua ground | 1 | 4 | 0 | one smallish fragment |
| | aqua float | 3 | 0 | 1 | particles very clear (syn or sediment?), plus two smallish fragments |
| | decanted | ? | $> 16$ | ? | $\sim 2000$ canola pollen, two blue fibres, several foil-like, clear fragments |
| | aqua ground | 0 | 4 | 0 | 26 flat, brittle fragments (foil?) |
| | aqua float | 6 | 6 | 2 | plus 9 smaller fragments |
| $< 0.5$ mm | pipetted | $> 19$ | $> 1$ | 4 | sample with most globules!, one dark fibre |
| | aqua ground | 6 | 19 | 3 | one dark fibre, 8 likely foil pieces (very clear $- >$ synthetic) |
| | aqua float | 5 | 4 | 1 | darkblue fibre, plus numerous tiny clear fragments likely plastic |
| | decanted | $>>$ | 1 | 1 | plus 2 very clear, smallish globules |
| | aqua ground | 206 | 8 | 1 | 3 blue fibres, plus numerous small fragments |
| | aqua float | $> 200$ | 3 | 0 | darkblue fibre, many clear floating fragments $\rightarrow$ foil? agricultural origin? |
| **Wilhelmshöhe** | | | | | |
| 0.5-1 mm | pipetted | 12 | 2 | 0 | darkblue & clear fibre, one particle with inlets (synthetic or sediment?) |
| | aqua ground | 8 | 0 | 0 | empty |
| | aqua float | 5 | 1 | 0 | just one dark fibre, clear fibre not recovered |
| | decanted | $>8$ | $>3$ | 0 | 2 clear, long, 1 dark long fibre |
| | aqua ground | 9 | 3 | 0 | many fine fibre nests mixed with small particles (not counted) |
| | aqua float | 9 | ? | 0 | fibres in mix of fibre nests not visible |
| $< 0.5$ mm | pipetted | 41 | 8 | 0 | sediment |
| | aqua ground | 18 | 5 | 0 | one darkblue, thick fibre |
| | aqua float | 19 | 9 | 0 | one darkgrey, thick fibre |
| | decanted | $>>200$ | ? | ? | sediment mixed with thick, dense nests of fine, beige fibres |
| | aqua ground | $>420$ | 9 | 0 | one blue fibre, milky-white particle (plastic?), several green glass bits<br>large grains counted, numerous tiny grains in fine fibre mix<br>only long, separated fibres counted, small fibres too many & mixed! |
| | aqua float | $>17$ | 2 | 0 | 2 long fibres clearly distinct from fine mix<br>fine fibre nests on surface also contain particles $\leq 200\mu$m (not counted) |

Table 10: **May 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 2/3**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Warnemünde** | **drift line** | | | | |
| 0.5-1 mm | pipetted | 158 | 7 | 0 | mostly sediment, a few chitin shell pieces |
| | aqua ground | 55 | 5 | 0 | long, clear fibres, one white-yellow particle (plastic?) |
| | aqua float | 80 | 3 | 0 | + numerous fine fibres, 3 flat/clear particles may be plastic |
| | decanted | 36 | 21 | 0 | 1 red plastic, dissolved in $H_2O_2$ (likely nylon/polyamide), 1 thick fibre/sediment nest |
| | aqua ground | 22 | 11 | 0 | plus numerous fine fibres |
| | aqua float | 18 | 15 | 0 | |
| $< 0.5$ mm | pipetted | 36 | $> 3$ | 0 | fibres mixed with sediment, few foil/organic pieces |
| | aqua ground | 11 | 0 | 0 | |
| | aqua float | 18 | 2 | 0 | scarcely long fibres, plus clear, very fine fibres (not counted) |
| | decanted | >400 | ? | ? | plus numerous tiny fragments, likely sediment, fibres not visible in sediment mix, some black pieces (slag/coal?) |
| | aqua ground | 165 | 14 | 0 | plus numerous tiny fragments/fine fibres (ground & float) |
| | aqua float | >200 | 32 | 0 | one dark-petrol particle ca. 50$\mu$m (tiny! likely plastic) |
| **Warnemünde** | **shallow water** | | | | |
| 0.5-1 mm | pipetted | 6 | 0 | 0 | no longer fibres visible on zooplankton net |
| | aqua ground | 6 | 1 | 0 | empty |
| | aqua float | 1 | 2 | 0 | fine fibres, unsuspicious |
| | decanted | 22 | 3 | 0 | one lightgrey fibre |
| | aqua ground | 13 | 9 | 0 | long, thick, clear fibres (invisible on net) |
| | aqua float | 10 | 7 | 0 | equally clear fibres in float as on ground |
| $< 0.5$ mm | pipetted | 23 | 2 | 0 | one black fibre |
| | aqua ground | 12 | 2 | 0 | sediment, one transparent particle could be plastics |
| | aqua float | 8 | 5 | 0 | unsuspicious |
| | decanted | 420 | ? | ? | fibres indistinguishable in mix, one red particle (likely quartz) |
| | aqua ground | 192 | 18 | 0 | |
| | aqua float | 187 | 8 | 0 | |

Table 10: **May 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 3/3**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Markgrafenheide** | **drift line** | | | | |
| 0.5-1 mm | pipetted | 92 | 5 | 0 | one darkblue fibre, else unsuspicious |
| | aqua ground | 66 | 4 | 0 | |
| | aqua float | 26 | 7 | 0 | darkblue fibre floats |
| | decanted | $> 7$ | $> 2$ | 0 | sediment, organic with scelletons/shells, ants, ... not counted |
| | aqua ground | 6 | 8 | 1 | lightyellow globule yields slightly to lanzette |
| | aqua float | 2 | 20 | 0 | fibres difficult to count in organic mix |
| $< 0.5$ mm | pipetted | 2 | 6 | 0 | sediment, numerous insect shell pieces (not counted) |
| | aqua ground | 2 | 4 | 0 | |
| | aqua float | 0 | 1 | 0 | nothing except for exosceleton pieces |
| | decanted | 33 | ? | ? | sediment, exosceletons not counted, fibres not visible in mix |
| | aqua ground | 21 | 24 | 0 | one darkblue fibre, large amount of fine fibres/particles |
| | aqua float | 0 | 8 | 0 | fibres in floating particle islands difficult to recognise |
| **Markgrafenheide** | **shallow water** | | | | |
| 0.5-1 mm | pipetted | 0 | 7 | 0 | one long, darkgreen fibre, else net very clean |
| | aqua ground | 0 | 2 | 0 | |
| | aqua float | 0 | 16 | 0 | dark fibre, two thin fibre nests (glasfibre filter contamination?) |
| | decanted | 64 | 8 | 0 | tiny turquoise plastic, many tiny fragments (not counted) |
| | aqua ground | 58 | 5 | 0 | plus numerous tiny particles |
| | aqua float | 8 | 13 | 0 | small “foil” fragments with white pollen (not canola, else similar to Nienhagen) |
| $< 0.5$ mm | pipetted | 24 | 0 | 0 | no fibres visible on net |
| | aqua ground | 5 | 1 | 0 | |
| | aqua float | 23 | 11 | 0 | sediment, sample very unsuspicious |
| | decanted | 278 | ? | ? | fibres uncountable in sediment, plus numerous small fragments |
| | aqua ground | 291 | 9 | 0 | long fibres, plus many short fibres (glasfibre nests?) |
| | aqua float | 17 | 9 | 0 | large amounts of tiny fibres/fragments (worse than Nienhagen/Wilhelmshöhe!) $\rightarrow$ comparably “dirty” sample |

Table 11: **July 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 1/3**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Nienhagen/Börgerende** | | | | | |
| 0.5-1 mm | pipetted | 30 | 9 | 1 | one dark fibre, one thick, one very long |
| | aqua ground | 11 | 3 | 0 | one petrol fibre |
| | aqua float | 20 | 8 | 1 | one very red fibre |
| | decanted | 61 | 5 | 0 | sediment |
| | aqua ground | 64 | 13 | 0 | some thick fibres |
| | aqua float | 3 | 5 | 0 | 4 pieces like foil (foil or organic?) |
| $< 0.5$ mm | pipetted | 23 | 2 | 0 | very clean net |
| | aqua ground | 19 | 2 | 0 | unsuspicious |
| | aqua float | 7 | 3 | 0 | very small particles, almost nothing on surface |
| | decanted | $>>$400 | ? | ? | some fine fibre nests, else unsuspicious sediment |
| | aqua ground | $>$960 | 22 | 4 | white, crumbling globuli (NOT transparent, salt?), orange-rose particle extremely many tiny fragments/thin fibres, 9 green bottle bits |
| | aqua float | $>$300 | $>$10 | 1 | white sphere, one green glass, numerous tiny fragment and fibre islands |
| **Wilhelmshöhe** | | | | | |
| 0.5-1 mm | pipetted | 0 | 0 | 0 | no visible particles or fibres |
| | aqua ground | 0 | 3 | 0 | empty |
| | aqua float | 0 | 3 | 0 | one foil piece |
| | decanted | 9 | 5 | 0 | one green glass piece |
| | aqua ground | 9 | 9 | 0 | 2 long, thick fibres (likely synthetic), 1 foil piece (organic or synthetic?) |
| | aqua float | 0 | 6 | 0 | one foil/organic piece |
| $< 0.5$ mm | pipetted | 11 | 1 | 0 | unsuspicious |
| | aqua ground | 7 | 6 | 0 | unsuspicious |
| | aqua float | 9 | 10 | 0 | lengthy fibres |
| | decanted | $>>$ | ? | ? | too many particles, bottle fragment |
| | aqua ground | $\approx$1000 | 3 | 0 | three very long fibres, 4 white spheres (crumbling) |
| | aqua float | $>$400 | 3 | 0 | three very long fibres, small islands w/ tiny fragments/thin fibres |

Table 11: **July 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 2/3**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Warnemünde** | **drift line** | | | | |
| 0.5-1 mm | pipetted | 6 | 3 | 0 | $1\times$thick + $1\times$small fibre nest, with 2 petrol fibres |
| | aqua ground | 3 | 36 | 0 | very clear fibres, long& thick, not visible on net |
| | aqua float | 3 | 12 | 0 | foil piece, thick nest persists |
| | decanted | 18 | ? | 0 | 3 thick nests, one dark, long fibre |
| | aqua ground | 9 | >190 | 0 | dissolved nests, one persists |
| | aqua float | 32 | 26 | 0 | 2-3 small nests, particles likely dissolved organic material |
| $< 0.5$ mm | pipetted | 45 | 4 | 0 | sediment, practically no fibres visible |
| | aqua ground | 25 | 3 | 0 | unsuspicious |
| | aqua float | 14 | 12 | 0 | one small nest with 3 long fibres |
| | decanted | >400 | ? | ? | sediment islands, particles not countable, one small red particle (plastic?) |
| | aqua ground | 733 | 83 | 0 | one green plastic, one red fibre, 1 foil |
| | aqua float | 100 | >34 | 0 | one tiny bright blue plastic |
| | | | | | numerous tiny Ps with thin fibres, small foil segments not counted |
| **Warnemünde** | **shallow water** | | | | |
| 0.5-1 mm | pipetted | 11 | 2 | 0 | unsuspicious |
| | aqua ground | 3 | 8 | 0 | clear, thick fibres, no fine/short fibres at all |
| | aqua float | 8 | 6 | 0 | unsuspicious |
| | decanted | 8 | 5 | 0 | unsuspicious |
| | aqua ground | 5 | 5 | 0 | 2 long, thick fibres, no fine fibres |
| | aqua float | 16 | 1 | 0 | plastic foil or organic particles |
| $< 0.5$ mm | pipetted | 337 | 1 | 1 | one milky-dirty and one rose particle (likely synthetic), no fibres at all |
| | aqua ground | 134 | 3 | 0 | still extremely few fibres, particles: sediment |
| | aqua float | 213 | 5 | 0 | one thick, two very long fibres (likely synthetic) |
| | | | | | floating particles sink when pushed down |
| | decanted | >>500 | ? | ? | turquoise plastic, 2 green glass/bottle |
| | aqua ground | 840 | 8 | 0 | particles estimated, long, thick fibres, 3 green glass |
| | aqua float | 270 | 14 | 0 | turquoise plastic, 2 glass, no thin fibres |

Table 11: **July 2014 Rostock samples (P: particles, F: fibres, S: spheres) – page 3/3**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Warnemünde seawater sample (7 liter) – 06-08-2014** | | | | | |
| | plankton net | 60 | 3 | 0 | 2 black fibres, disintegrating organic & diatoms not counted |
| | aqua ground | 49 | 17 | 0 | 1 very thick, black fibre, several transparent thick fibres, 1 thick foil piece (synthetic?), 1 blue particles, 3 tiny glass pieces particles are very small compared to sediment samples |
| | aqua float | 20 | 6 | 0 | 1 blue fibre, 1 white plastic droplet |
| **Markgrafenheide** | **drift line** | | | | |
| 0.5-1 mm | pipetted | 2 | 2 | 0 | net contains nothing except for small bits of digested organic material |
| | aqua ground | 2 | 6 | 0 | long, thick, clear fibres, flat fibre may be organic |
| | aqua float | 0 | 0 | 0 | literally empty |
| | decanted | 32 | 33 | 0 | petrol, grey, red fibres, insect scelettons |
| | aqua ground | 21 | 20 | 0 | unsuspicious, sediment |
| | aqua float | 2 | 3 | 0 | surface empty except for 2 exosceletton pieces |
| $< 0.5$ mm | pipetted | 15 | 3 | 0 | sediment |
| | aqua ground | 8 | 5 | 0 | extremely unsuspicious |
| | aqua float | 11 | 4 | 0 | unsuspicious |
| | decanted | $>>400$ | ? | ? | 2 blue fibres, dense sediment islands |
| | aqua ground | $>680$ | 34 | 0 | darkblue fibre, some very long, thick, clear fibres (likely synthetic) |
| | aqua float | 98 | 7 | 0 | small particles with fine fibres on ground and in islands |

Table 12: **June 2014 Rügen samples (P: particles, F: fibres, S: spheres) – page 1/2**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Heidehof** | | | | | |
| | pipetted | 12 | 2 | 0 | very clean, 1 red, clear particle 70$\mu$m (glas or plastic) |
| | aqua ground | 6 | 2 | 0 | 1 foil fragment, very clean |
| | aqua float | 9 | 1 | 0 | 2 smaller foil fragments, else virtually nothing |
| | decanted | 115 | ? | 0 | 1 brown glas, tiny green glas bits, some organic matter mixed with fibres |
| | aqua ground | 110 | 6 | 0 | tiny green & brown glas pieces, organic matter, 1 red fibre |
| | aqua float | 110 | 8 | 0 | smaller particles only, some transparent might be plastics |
| **Dranske** | | | | | |
| | pipetted | 109 | 3 | 0 | coarse sediment, unsuspicious |
| | aqua ground | 93 | 5 | 0 | sediment only, almost no fibres, no thin fibres at all |
| | aqua float | 16 | 1 | 0 | very unsuspicious |
| | decanted | 180 | ? | 0 | small & large sediments, fibres mixed with organic |
| | aqua ground | 304 | 7 | 0 | 1 dark blue fibre |
| | aqua float | 100 | 12 | 0 | 1 light turquoise & 1 dark turquoise plastic particle<br>numerous fine fibres float on the surface, similar to Rostock samples |
| **Breege** | | | | | |
| | pipetted | 7 | 1 | 0 | 1 dark grey fibre, nothing else |
| | aqua ground | 6 | 23 | 0 | very clear, long fibres, 2 light blue, some knots |
| | aqua float | 2 | 11 | 0 | 1 tiny blue plastic particle (not seen on filter) |
| | decanted | $>$500 | ? | ? | unsuspicious |
| | aqua ground | $\sim$450 | 14 | 0 | 1 petrol fibre, 1 dark red structure, 1 turquoise flake<br>numerous fine fibres on the ground |
| | aqua float | $\sim$300 | 6 | 0 | fibres several mm long, few coarse black slag pieces |

Table 12: **June 2014 Rügen samples (P: particles, F: fibres, S: spheres) – page 2/2**

| Location/size | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Binz drift line** | | | | | |
| | pipetted | 98 | 2 | 0 | unsuspicious |
| | aqua ground | 38 | 1 | 0 | unsuspicious |
| | aqua float | 55 | 3 | 0 | very few fibres, no fine fibres at all |
| | decanted | $>$800 | ? | ? | 1 foil fragment with synthetic appearance |
| | aqua ground | $\sim$750 | 8 | 0 | surprisingly few fibres, no fine fibres |
| | aqua float | $\sim$100 | 1 | 0 | 1 long fibre, 1 tiny turquoise flake |
| **Binz in shallow water** | | | | | |
| | pipetted | 167 | 0 | 0 | 1 tiny blue particle |
| | aqua ground | 79 | 3 | 0 | unsuspicious |
| | aqua float | 92 | 5 | 0 | 1 petrol fibre |
| | decanted | $>$700 | ? | ? | many fine fibres, no long fibres visible |
| | aqua ground | $\sim$720 | 2 | 0 | no long fibres, fine fibres of natural origin? |
| | aqua float | $\sim$450 | 2 | 0 | uncountable fine, short fibres, no long fibres at all |

Table 13: **August 2014 Oder/Peene estuary bodden & Baltic Sea coast samples – page 1/1**

| Location | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Kamminke** | pipetted | >400 | ? | ? | few fibres, clean sediment |
| Bodden | aqua ground | 260 | 0 | 0 | literally no fine or long fibres in this sample |
| | aqua float | 240 | 2 | 0 | sediment, unsuspicous |
| | decanted | >300 | ? | ? | at least 9 green glass pieces |
| | aqua ground | ∼300 | 5 | 0 | unsuspicious, except for glass |
| | aqua float | ∼200 | 4 | 0 | plus many micro particles & tiny glass bits |
| | | | | | 8 irridiscent foil pieces (not found in other samples), 1 tiny blueish particle likely plastics |
| | | | | | tiny particles are consistent with the silt-like nature of the sediment |
| **Freest** | pipetted | 0 | ? | 0 | 2 tiny green glass bits, else empty net |
| Baltic Sea coast | aqua ground | 0 | 9 | 0 | 3 green glass bits (too small for regular count) |
| | aqua float | 0 | 1 | 0 | literally nothing |
| | decanted 1 | ? | ? | ? | too much fine sediment to count/estimate |
| | | | | | >48 green glass bits, 1 petrol, 2 blue, 1 orange-occer fibres |
| | aqua ground | ? | 61 | ? | very long & thick fibres, irridiscent foil |
| | | | | | some long fibres attached to organic material, not clear if organic or synthetic |
| | | | | | 69 green glass bits detected |
| | aqua float | ? | 37 | ? | 1 blue floating plastic particle, clear fibres: organic vs synthetic? |
| | decanted 2 | ? | >3 | ? | numerous fine-grained sediment, organic & fibrous material |
| | | | | | 78 green glass pieces |
| | aqua ground | ? | >46 | ? | fibres difficult to spot in clumps, 1 blue tiny particle |
| | aqua float | ? | 20 | ? | 1 small clear apricot rod likely plastic |
| | | | | | some very clear floating particles might be plastic |
| | decanted 3 | ? | ? | ? | >40 green glass pieces, insect shields/skins |
| | aqua ground | ? | >42 | ? | very long, clear fibres (organic or plastics?), 1 red, 1 rose synthetic fibre |
| | | | | | > 44 green glass bits, 1 green & 1 turquoise plastic piece |
| | aqua float | ? | >45 | ? | long, thick fibres likely synthetic, 1 petrol long & thick fibre |
| | | | | | long fibres mixed in shell/shield material − > attached organic or sticky synthetic? |
| | | | | | 3 green glass bits caught in floating sediment |
| | decanted 4 | ? | ? | ? | 9 green glass bits, 1 floating amber |
| | aqua ground | ? | >34 | ? | 6 green glass, 1 petrol fibre, long, clear fibres mixed with organic |
| | aqua float | ? | >7 | ? | 2 green glass in floating organic islands, very few long fibres on surface |

Table 14: **September 2014 Jade Bay water & sediment samples (P: particles, F: fibres, S: spheres) - page 1/2**

| Location | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Varel/Nordender Leke** | Filter dry counts[a] | | | | |
| **Freshwater 4l** | zoo 1 | – | >7 | – | numerous organic particles prohibit count, visual appearance of all Ps = organic material<br>6 coloured fibres (4 blue, 1 black, 1 violet), 1 very long clear fibre |
| | zoo 2 | – | >6 | – | rich organic mix, again 6 coloured fibres (5 blue, 1 dark green) |
| | rest | 2 | >3 | – | rinsed canister water, 3 blue fibres, 2 clear, structured particles (plastic?) |
| | **total** | 2 | >16 | 0 | 15 coloured fibres in dry count |
| | Counts in deionised water after rinsing | | | | |
| | zoo 1 aqua ground | 2 | 13 | 0 | 8 long, clear F, 1 blue, 1 green, 3 violet fibres<br>1 yellow & 1 clear, flat particle, both potentially plastics |
| | zoo 1 aqua float | 14 | 16 | 0 | 1 petrol fibre, particles very clear (plastics?),<br>10 foil fragments consistent with paper laminate, but could also be organic material |
| | zoo 2 aqua ground | 0 | 24 | 0 | all visible particles organic (not counted), 4 blue fibres, 18 foil fragments |
| | zoo 2 aqua float | 6 | 16 | 0 | 2 blue fibres, 8 foil fragments |
| | aqua ground | 2 | 13 | 0 | 8 long, clear F, 1 blue, 1 green, 3 violet fibres<br>1 yellow & 1 clear, flat particle, both potentially plastics |
| | aqua float | 14 | 16 | 0 | 1 petrol fibre, particles very clear (plastics?),<br>10 foil fragments consistent with paper laminate, but could also be organic material |
| | rest aqua ground | 1 | 7 | 0 | long, clear fibres, particle likely rest sediment in canister |
| | rest aqua float | 2 | 1 | 0 | almost nothing on surface |
| | **total** | 25 | 77 | 0 | 18 coloured fibres (2 dry count blue fibres not recovered) |
| **Dangast** | Filter dry counts[b] | | | | |
| **Seawater 10l** | zoo 1 | 53 | 5 | 0 | 2 blue fibres |
| | zoo 2 | 228 | 9 | 0 | 4 blue, 1 lightblue fibres, 1 violet plastic particle |
| | zoo 3 | 88 | 3 | 0 | all clear fibres, up to 8mm length |
| | **total** | 369 | 17 | 0 | 7 blue fibres, 1 violet plastic particle |

[a] The freshwater sample was filtered onto 2 zooplankton net filters (zoo 1 & zoo 2) because of filter clogging by dense organic material. The residual material rinsed from the canister is denoted “rest”.

[b] The seawater sample was filtered over 3 zooplankton net filters (zoo 1 - zoo 3) because of clogging.

Table 14: **September 2014 Jade Bay water & sediment samples (P: particles, F: fibres, S: spheres) - page 2/2**

| Location | extraction | P | F | S | comments |
|---|---|---|---|---|---|
| **Dangast** | Counts in deionised water after rinsing | | | | |
| **Seawater 10l** | zoo 1 aqua ground | 19 | 7 | 0 | 2 blue fibres, many clear fragments |
| | zoo 1 aqua float | 38 | 2 | 0 | clear fragments could be chitin shields or plastics |
| | zoo 2 aqua ground | 246 | 10 | 0 | 4 blue fibres (lightblue missing), numerous clear fragments |
| | zoo 2 aqua float | 68 | 1 | 0 | numerous clear fragments & chitin pieces |
| | zoo 3 aqua ground | 73 | 10 | 0 | very long fibres ($\leq$1.5cm), 1 dark blue fibre knot |
| | zoo 3 aqua float | 7 | 1 | 0 | numerous irridiscent chitin & foil/skin pieces (not counted) |
| | **total** | 451 | 31 | 0 | 7 blue fibres (1 not recovered from dry count) |
| **Dangast** | | | | | |
| **Sediment 1** | pipetted | 18 | 0 | 0 | very clear particles, some might be plastics |
| | aqua ground | 12 | 3 | 0 | 1 dark red plastic particle, almost no fibres |
| | aqua float | 4 | 4 | 0 | 3 clear & 1 blue-green P, structures suggest plastic particles |
| | decanted | $>$2000 | ? | ? | fine-grained sediment, 1 green plastic particle, 1 long, white fibre |
| | aqua ground | $\sim$900 | 4 | 0 | some clear particles structured as plastics |
| | aqua float | $\sim$300 | 0 | 0 | unsuspicious, no long fibres at all |
| | centrifuge | 0 | 1 | 0 | no particles remain on surface after centrifugation |
| **Sediment 2** | pipetted | 26 | 5 | 0 | 1 dark-grey fibre |
| | aqua ground | 15 | 5 | 0 | unsuspicious |
| | aqua float | 23 | 14 | 0 | 2 (thick) fibre nests, 1 foil fragments |
| | decanted | $\sim$700 | ? | ? | 2 long, dark blue fibres |
| | aqua ground | $>$970 | 9 | 0 | 1 black, 1 very long dark blue fibre |
| | | | | | many tiny particles, silt-like sediment |
| | aqua float | $>$400 | 8 | 0 | 1 black, 3 dark blue fibres, plus many very fine fibres (organic) |
| | | | | | lots of organic material impede particle & fibre counts |
| | centrifuge | | | | none of the fibres remain on surface, no particles |
| | | | | | 4 long fibres in the water column, 2 clear, 1 dark & 1 light blue |

## Appendix E: Selection of potential microplastic particles and fibres

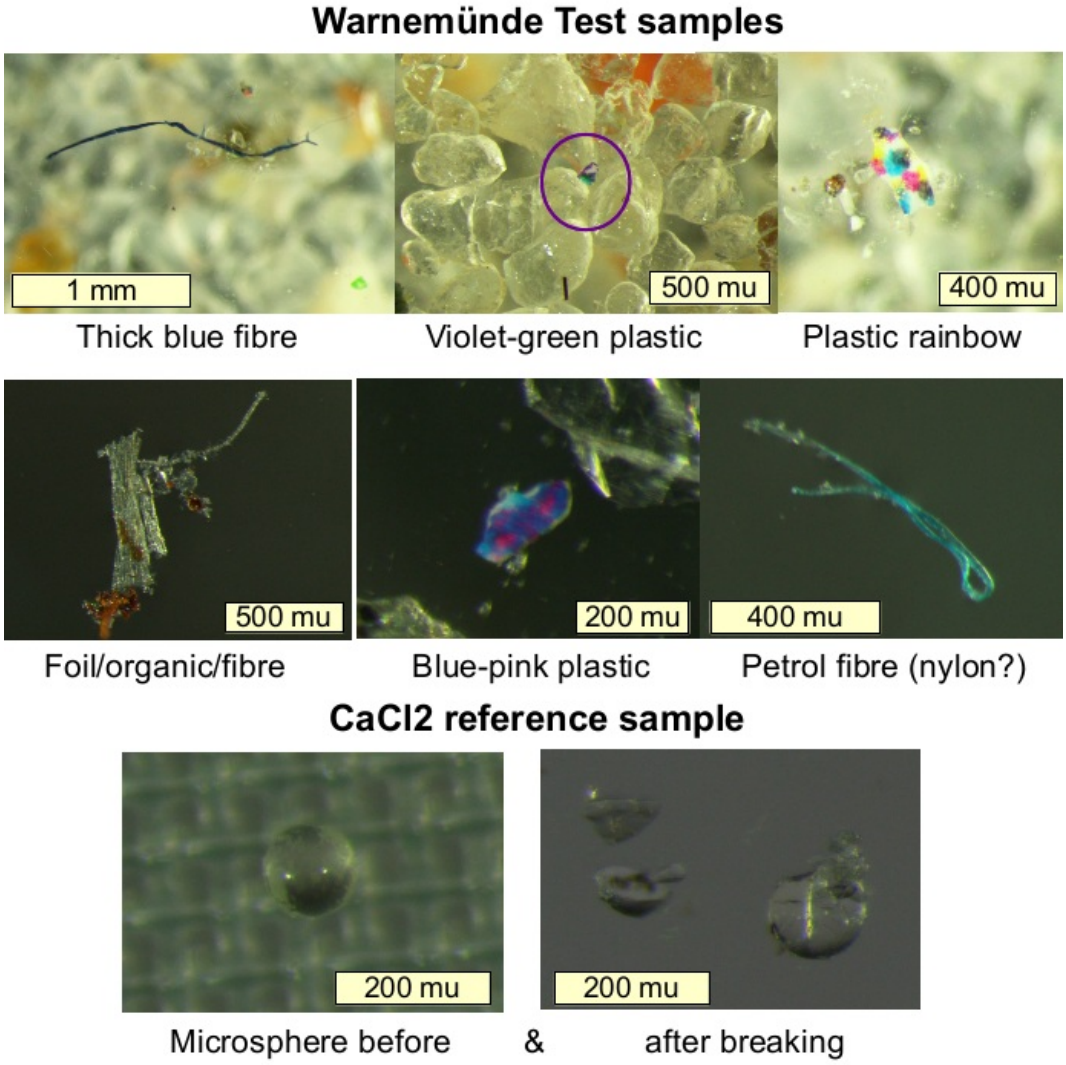



Figure 32: *Top rows:* Particularly conspicious microplastic particles and fibres observed in the Warnemünde test samples. *Bottom row:* Microsphere detected in one of the reference samples containing only calciumchloride solution. The sphere displays no internal structure, the two bright dots are reflections from the halogen lamp. Note the thick outer shell visible after breaking, from which a gel-like liquid emerges.

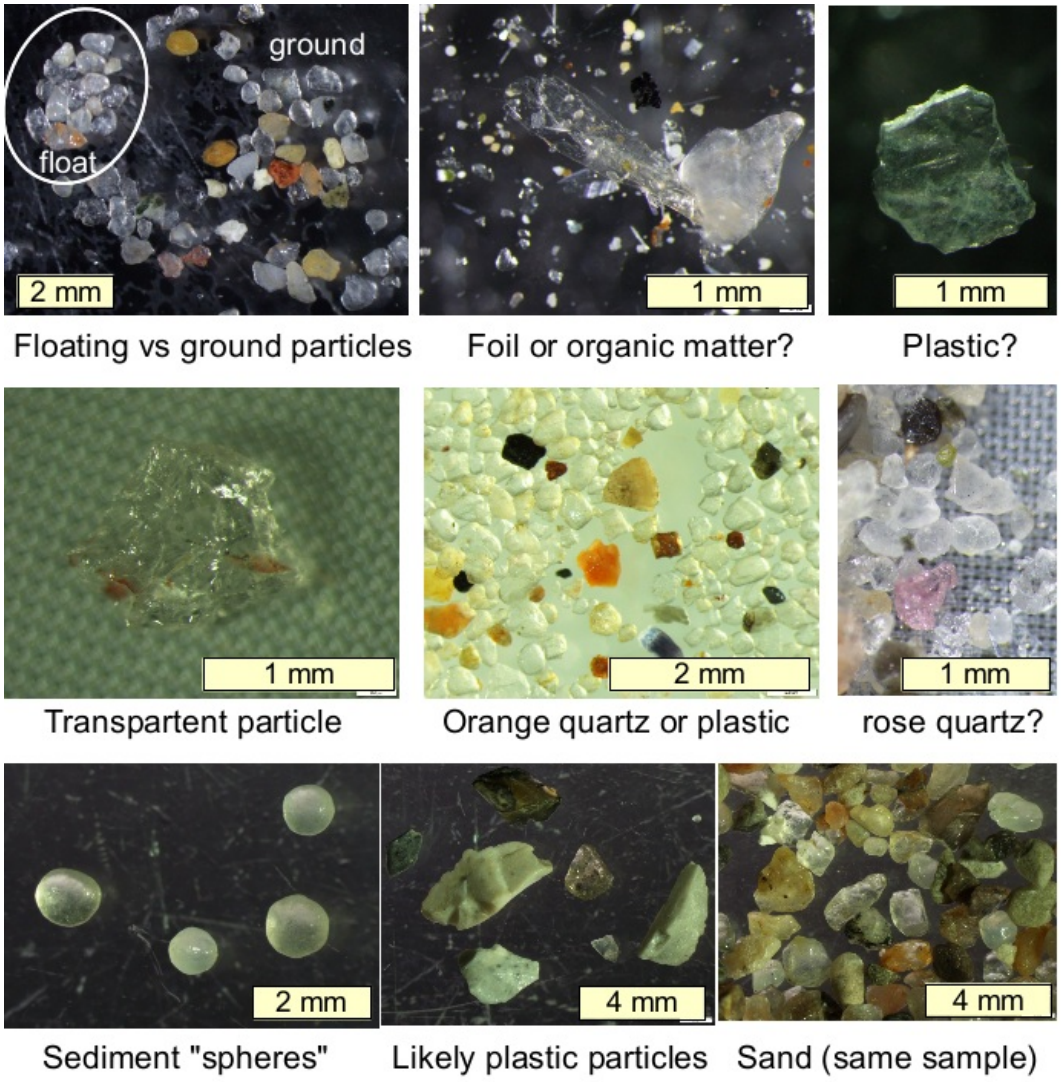



Figure 33: Illustration of the similarities between natural sediment grains and microplastic particles. The *top left panel* shows particles floating on the surface in comparison to sediment immediately sunken to the ground in deionised water. Thin foil fragments can be of either organic or synthetic origin (*top middle*). Note that the two transparent particles (*top right and middle left*) have distinct surface structures compared to the majority of sediment grains, while the shape and structure of the rose-coloured and orange particle are indistinguishable from the surrounding sediment. The *bottom left panel* displays sediment spheres without the characteristic perfectly round and transparent appearence of microplastic spheres.

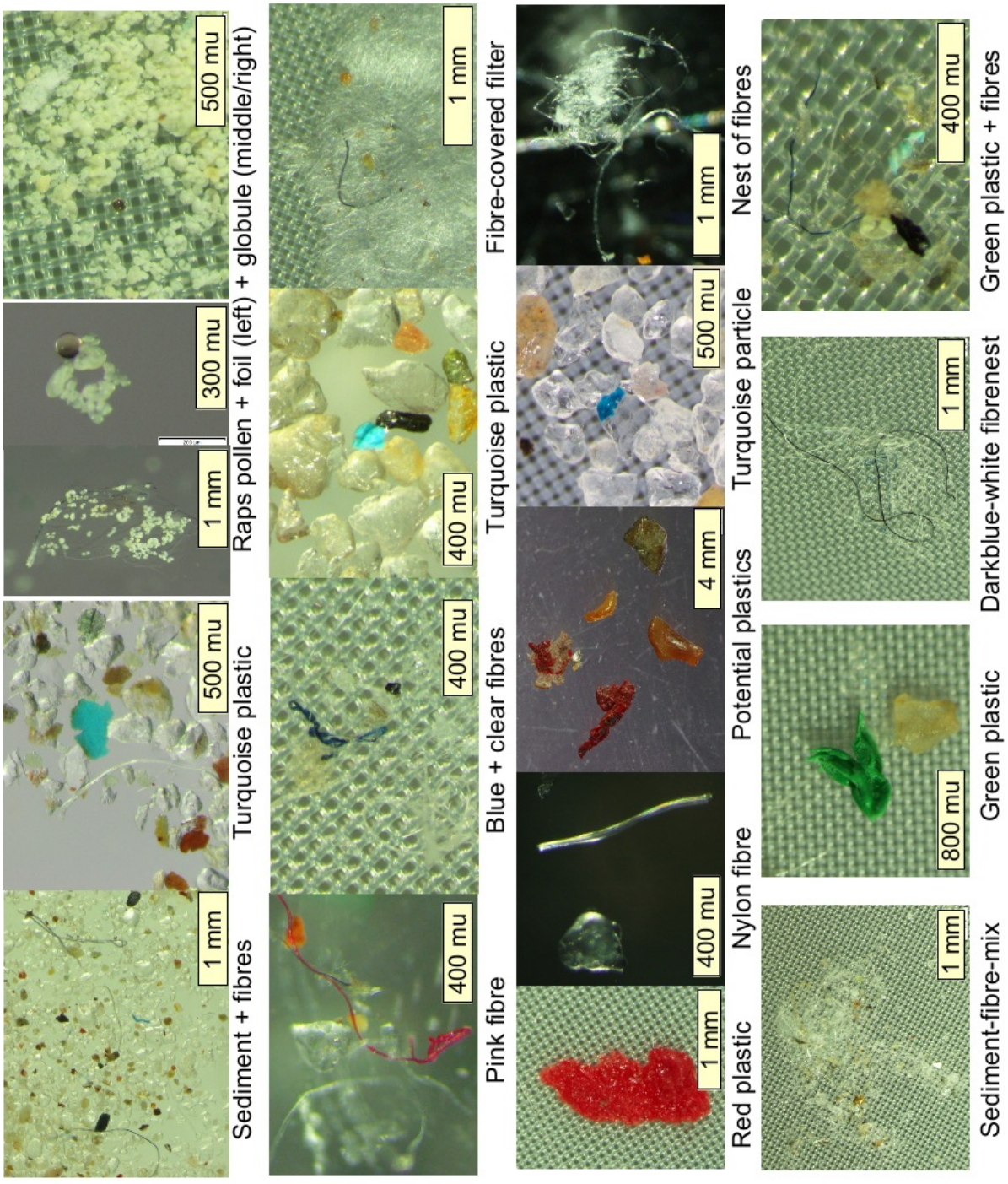
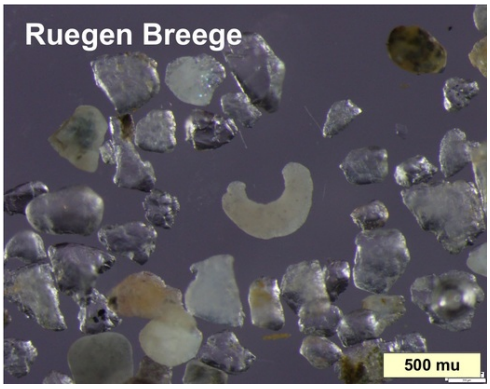
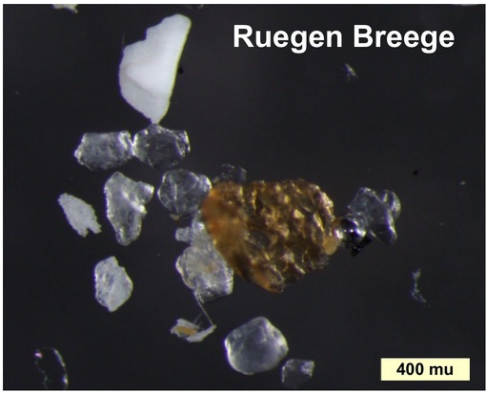


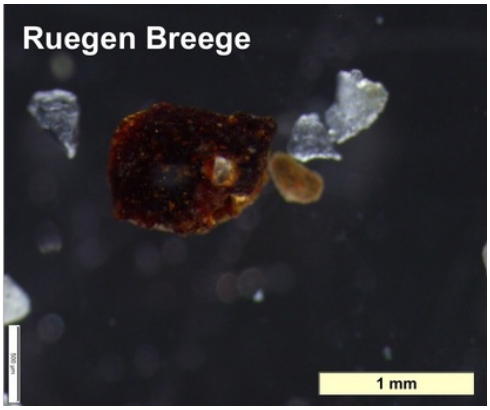

Figure 34: Selection of particularly conspicious particles and fibres detected in sediments at the four Rostock locations.



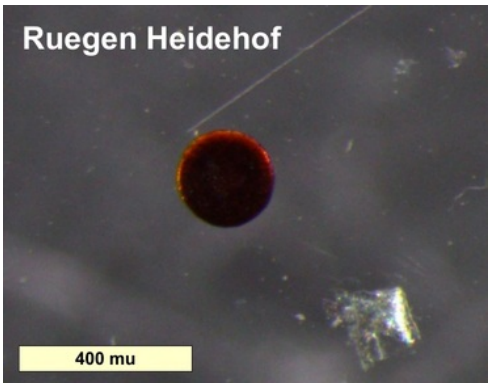

(a) White plastic ring



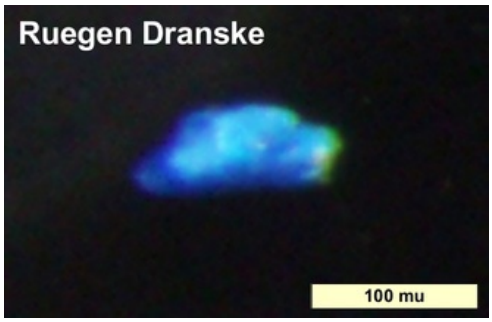

(b) Occer particle




(c) Darkbrown fragment

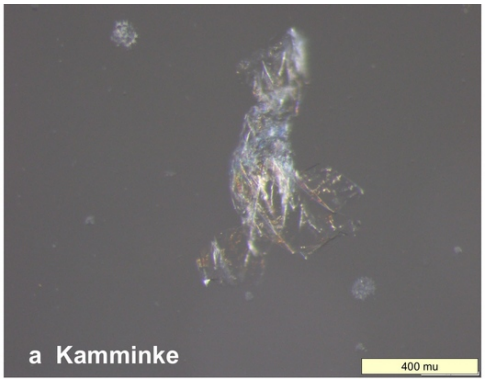



(d) Brown disc (glass?)

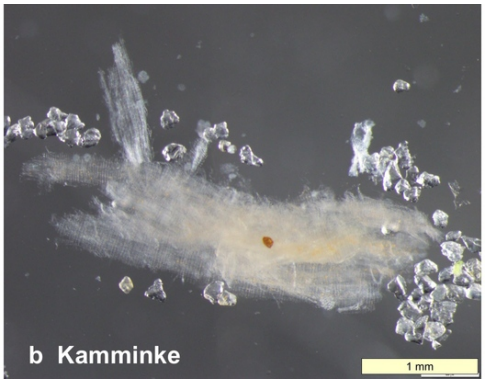



(e) Turquoise fragment

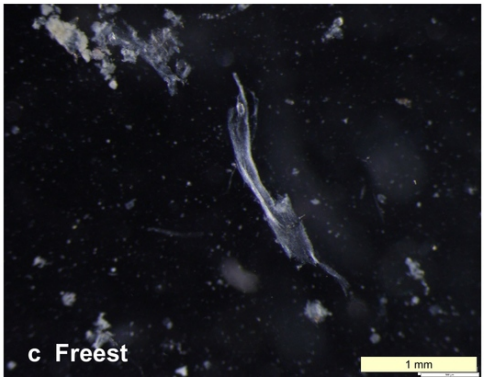
Figure 35: Conspicious particles found in beach sediments on the island of Rügen. Note the unusual surface structure of the occer and darkbrown fragments.



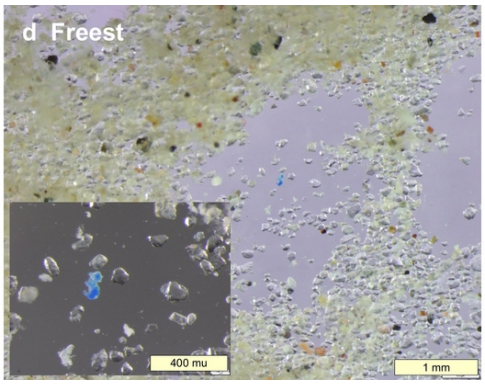

(a) Foil fragment



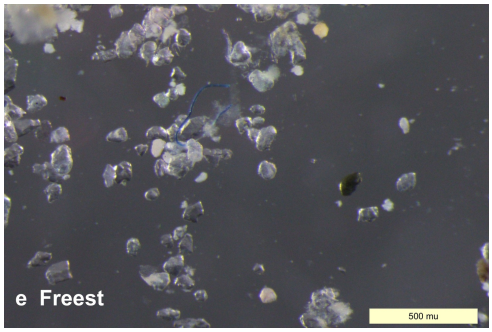

(b) Cigarette filter or sanitary pad



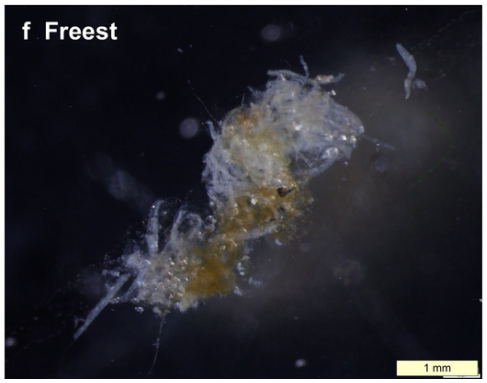

(c) Foil or organic skin fragment



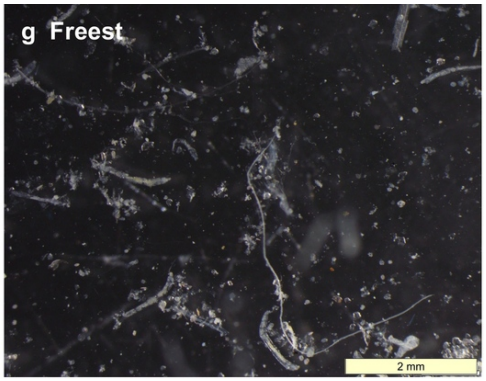

(d) Blue plastic particle



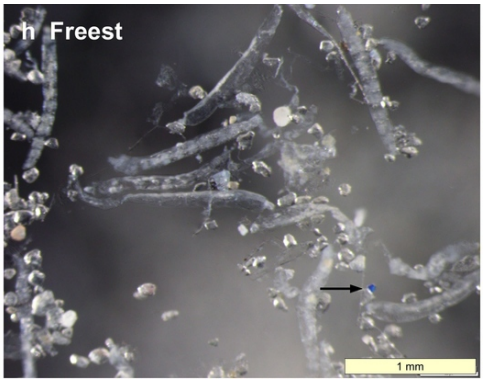

(e) Blue synthetic fibre




(f) Organic matter with coloured fibre nest

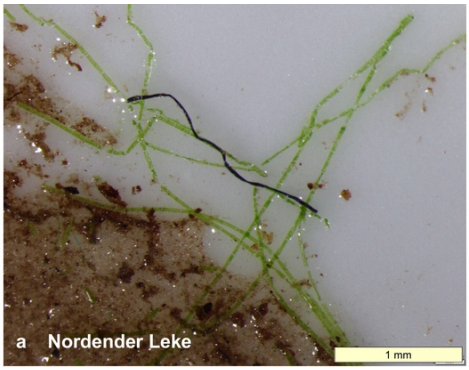



(g) Very long fibre, synthetic or organic

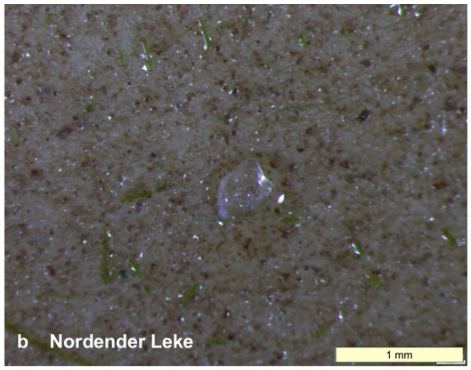



(h) Blue plastic particle & organic matter

Figure 36: Selection of potential microplastics found in the Oder/Peene outlet.

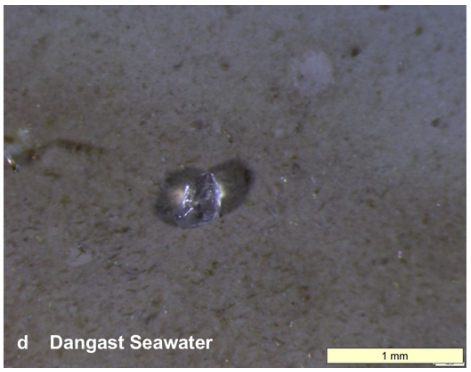



(a) darkblue fibre (freshwater sample)

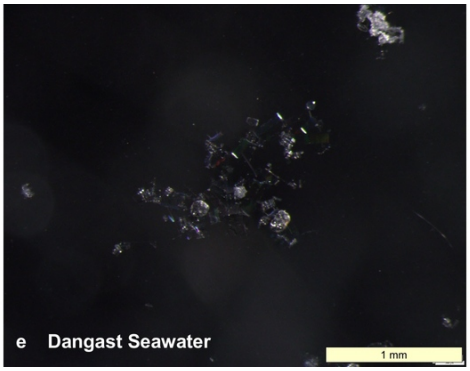



(b) plastic particle in freshwater sample

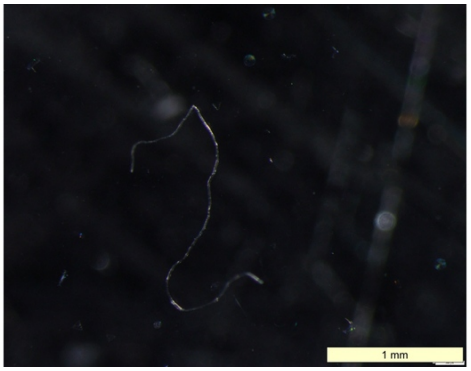

(c) darkblue fibre (seawater sample)



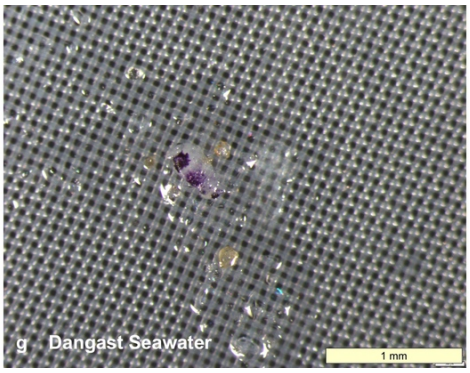

(d) clear particle likely plastics



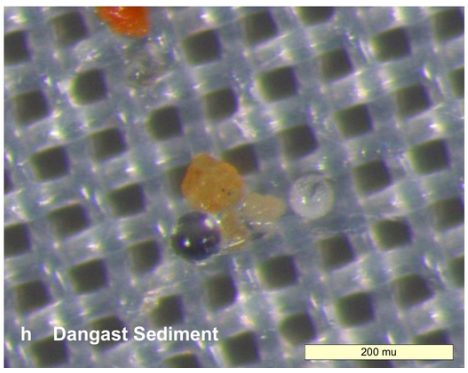

(e) plastic or sediment particles




(f) long fibre in seawater sample




(g) violet particle in organic matter




(h) two microspheres

Figure 37: Selected synthetic particles and fibres detected in the Jade Bay. Freshwater samples obtained in the rivulet *Nordender Leke* opposite the paper recycling plant in Varel: a) and b). Note the large amount of organic material in this freshwater sample. Dangast beach seawater samples: c) to g), Dangast sediment sample: h) two microspheres with different sizes and colouring.

## Appendix F: Technical recommendations: An improved methodology

Although a diversity of methods were developped over the past decade to extract microplastics from sediment and seawater samples, there is no standardised procedure ensuring a reliable extraction and identification of microplastics from natural environments. In the framework of the European marine strategy framework directive (MSFD), but also in view of the fact that microplastics are omnipresent in the marine environment and need to be monitored in order to find both quantitative arguments for policy makers as well as solutions for the growing so-called “plastic soup” problem, time and cost efficient monitoring techniques need to be developped. In this thesis, the attempt was made to use basic laboratory equipment and accessible chemistry to extract and quantify the amount of microplastic particles and fibres in sediments.

We find increasing amounts of floating particles in aqueous and saline solution with decreasing grain size. As those particles are visually indistinct from natural sediment, they are likely suspended by surface tension due to their light overall weight. The high level of contamination of presumed plastic samples with natural sediment is problematic in sediment, coastal and shallow water samples, but is not expected to influence zooplankton tows obtained in the open sea. Methods suggested here include centrifugation after extraction to separate higher and lower density material, and if possible spectroscopic analysis of a subsample of extracted particles. After counting the artificially enriched sediment samples (Sec. 3.2), centrifugation was used to separate natural sediment from plastic fragments in the floating islands of the aqueous solution. Centrifugation (800 rotations/minute) of the surface solution in the petri dish increases the detection rates especially of transparent microplastic particles, as suspended sediment particles sink to the bottom of the tube. As for all density-separation extraction methods, however, this method is also limited to the detection of particles lighter than both the sediment and the employed extraction medium.

For transparent and white fibres, a more extensive detection method has to be developped. Digestion with natural enzymes or dissolution with hydrogen peroxide, as used to minimise the content of organic matter in the Baltic sediment samples, can efficiently separate distinct organic materials, such as proteins, chitin (with chitinase as detergence agent), and byssus fibres. On the other hand, especially chitinase digestion requires substantial time frames (one week per sample, following the procedures outlined in Lorenz 2014). Ultimately, FTIR-microscope spectroscopy and similar methods are the principal way to uniquely identify polymer particles and fibres in sediment and water samples. The timeconsuming nature of this method, and the fact that costly laboratory equipment is required, unfortunately impede

the use of spectra for large amounts of sediment as well as for every individual particle and fibre found.
Based on our method testing, we suggest here a minimal approach to extracting microplastics as readily conducted in a standard biological or chemical laboratory. The following steps – combining several of the technical approaches employed in the literature previously – are recommended for an efficient microplastic detection process:

**0. Preparation & choice of equipment**

Employment of glass equippment whereever possible is a prerequisite to minimise biases/losses by sticking of plastic particles to the surface.

**1. Polymer extraction**

Air-venting sediment in high-density saline solutions, possibly with a preceding floatation step (see Claessens et al. 2013), proved an efficient way to handle large samples. Refilling steps should be kept to a minimum (Imhof et al. 2012).

**2. Top-layer extraction & Filtration**

Extracting the surface of the solution, preferably via separating funnels, or by pipetting as a less efficient alternative, to capture low-density particles and fibres in the top layer, onto stainless steel mesh or zooplankton net with a pre-defined lower size limit. Comparative studies should be investigated prior to setting the lower size boundary to facilitate the quantitative comparison.

The distinction of natural sediment and organic fibres from synthetic polymers proved more difficult when membrane filters were used. The use of filters that do not allow rinsing of the captured material is therefore not recommended.

**3. Water column extraction & Filtration**

The supernatant should be decanted and analysed separately to include higher-density particles and fibres, and filtered in the same way as the extracted surface solution.

**4. Counting procedure**

The combination of two different counting procedures yielded the highest microplastic recovery rates.

**i) Dry count** of particles and fibres on the zooplankton mesh.

**ii) Wet count** after rinsing of captured material from mesh filters into aqueous solution for recounting of particles and fibres. Fibre number counts were substantially facilitated in aqueous solution, and settled and floating material provided clues on the composition of particles.

**5. Centrifugation**

Centrifugation of the extracted surface fraction to separate suspended high-density from low-density particles is suggested to further distinguish natural minerals from microplastics.

**6. Visual inspection**

Distinction of plastic particles on the basis of colour and structure proved the most secure means to visually select microplastics from natural sediment samples, especially when a complete spectroscopic analysis is not feasible.

**7. Spectroscopic confirmation**

Spectroscopic confirmation of at least a subsample of extracted microplastics, including both coloured and transparent particles and fibres, is highly desireable to obtain realistic microplastic densities from sediment samples (see also Lorenz 2014, and refernces therein).

This procedure further expands the suggestions given in Hidalgo-Ruz et al. (2012), and further systematic testing would be beneficial to confirm the recovery rates of transparent synthetic particles and fibres from natural sediment samples.

## Acknowledgement

First of all, I wish to express my sincere thanks to Prof. Hendrik Schubert for offering me the opportunity to spend a delightful summer at the Baltic coast working on ocean plastics – it was a courageous endavour (on both sides) and I enjoyed every single day!

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My very personal thanks go to my friends and family for their continuous support, and to Eckhard Sutorius for being there at all times.

## Declaration of Academic Honesty

I hereby declare that I have prepared the presented Master thesis *"The detection of microplastics in beach sediments. Extraction methods, biases, and results from samples along the German Baltic coast"* independently and on my own account, using exclusively the cited sources, including publicly available web sources, and auxiliary means as completely described in the text and reference lists.

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Location, Date Author Signature

## Eidesstattliche Erklärung

Ich erkläre, dass ich die vorliegende Masterarbeit zum Thema *"The detection of microplastics in beach sediments. Extraction methods, biases, and results from samples along the German Baltic coast"* selbständig und ohne fremde Hilfe angefertigt und nur die angegebenen Quellen und Hilfsmittel genutzt habe.

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Ort, Datum Unterschrift der Autorin